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Chemical and Microbiological Studies on Some Agricultural Waste of Sesame (*sesamum indicum*) Plant

Aziza, A. Salah-Eldin

Special Food and Nutrition Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

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ABSTRACT

This study investigated the chemical composition, antioxidant and antimicrobial activity of some sesame plant parts such as stems, leaves and fruit covers. The results found that sesame leaves were the highest of moisture (6.85g/100g) and protein (18.03 g/100g), while the moisture of the sesame fruit covers was (6.25g/100g) and protein was (6.65 g/100g). The results showed that the sesame stems had a lowest content of protein and fat. From the fractionation of phenols and flavonoids compound of sesame plant parts found that the major components of flavonoids were Naringin (120.54mg/100g) and Chlorogenic and Caffeine (2.95 and 4.96mg/100g respectively) as phenolic compounds in sesame leaves. The dried leaves showed significant vitamin A (10.01 IU), vitamin E (0.34mg/g), vitamin C (8.65 mg/g) and vitamin D (10.27 IU) but sesame stems contain vitamins A and E (12.51 and 0.32 respectively) Sesame plant parts play an important role as antioxidant and antimicrobial agent that can be attributed to the presence of phenolic and flavonoid compounds. The study on utilization of sesame leaves in cake was undertaken to upgrade the nutritional quality and assess the acceptability cake were prepared by substitution of wheat flour with sesame leaves powder at concentrations of 0% (control), 2.5%, 5%, and 7.5%.and evaluated in terms of their physical characteristics and sensory properties. Cake substituted with sesame leaves showed a significant increase in protein from13.34g/100g in control to 17.93g/100g in cake with a replacement of 7.5% sesame leaves powder, also, zinc significantly increased from 2.11 (ppm) in control to 7.50 (ppm) in cake with a replacement of 7.5% sesame leaves powder. So, the incorporation of sesame leaves into the cake significantly enhances its nutritional value, as these leaves possess a higher nutritional content.

1. Introduction

Sesame, known as (Sesamum indicum), is one of the oldest items farmed since ancient times, and one of the oldest seasonings utilized. It's been used for centuries as a food, medicinal, and cosmetic ingredient. Sesame seed is one of the world's most important oil seed corporations, with a lipid content of 50% and a protein content of 20%. Sesame seeds have been discovered to have health-promoting properties in numerous scientific researches (Rounizi et al., 2021). Sesame plants are farmed for their seeds, which are one of the world's most important oil crops, in tropical and subtropical locations. Sesame-specific lignans, such as sesamin and sesamolin, as well as the chemical composition and functional qualities of the seeds have been extensively researched (Andargie et al., 2021). In Japan, a new health food supplement has been introduced, consisting of a dried powder made from the young leaves of sesame plants that achieve a height of 30–70 cm, roughly 40–60 days post-planting. Fuji et al. (2018) found that these young sesame leaves possess three iridoids and eight polyphenols, with acteoside showing significant antioxidant properties that increase as the leaves develop. There is a notable scarcity of information regarding sesame leaves, particularly when compared to the extensive knowledge available about sesame seeds,

*Corresponding Author
Email: azizaali161@gmail.com

as the primary focus of sesame cultivation is on seed production, rendering the leaves largely as agricultural byproducts. In various African regions, however, sesame leaves are utilized as a vegetable due to their significant nutritional content, including essential amino acids (Liu et al., 2012). In 2020, Egypt produced 38 thousand tons of sesame from an area of 29 thousand hectares (FAO, 2020). A typical sesame leaves, with those located in the middle and lower sections approximately one-third of the plant's height being suitable for consumption at the end of the flowering period. These leaves represent a vital vegetable resource, particularly for impoverished populations in low-income countries, as they provide essential nutrients necessary for human health. The World Health Organization (WHO/FAO, 2002) has previously indicated that inadequate vegetable consumption increases the risk of micronutrient deficiencies. Furthermore, adequate intake of fruits and vegetables is associated with a reduced risk of cardiovascular diseases and cancer, which are the leading causes of premature mortality globally, accounting for 25.5 million deaths in 2013 (Aune et al., 2017). The WHO also estimates that approximately 14% of deaths from gastrointestinal cancers, 11% from ischemic heart disease, and 9% from strokes worldwide can be attributed to insufficient fruit and vegetable consumption. Therefore, utilizing various parts of plants, including stems, leaves, and fruit covers, as sources of nutrients and bioactive compounds plays a crucial role in enhancing food and nutrition security. In this study, the chemical composition of sesame leaves were identified, stem and fruit cover and evaluated antioxidant (DPPH) and antibacterial activity and the possibility of using sesame leaves to increase the nutritional value of some food products from cheap sources.

2. Materials and Methods

Materials

The sesame (*sesamum indicum*) of 2022 was sourced from the Field Crops Research Institute at the Agricultural Research Center in Giza, Egypt. The collection included leaves, stems, and fruit covers, gathered promptly after harvest when the plants reached a height of approximately 120-125 cm, 120 days post-planting. The samples underwent washing with water, followed by a drying process at room temperature for two weeks, after which they were ground into a fine powder. All chemicals, standards, and solvents utilized in this study were procured from Sigma-Aldrich, USA.

Methods

Preparation of crude extracts

A total of 10 grams of powdered sesame leaves, stems, and fruits cover were placed in a flask containing 500ml of ethanol. The mixture was subjected to agitation for 5 hours at a temperature of 25°C for extraction. Subsequently, the extracts underwent maceration overnight for 24 hours. The ethanolic layer, which contained the extract, was collected. The extraction process was repeated on the remaining precipitate using 150 milliliters of ethanol, and all extracts were filtered through a Buchner funnel and Whatman No. 1 filter paper. The two fractions of extracts were mixed and concentrated using a rotary evaporator set at 40°C. The extracts were then stored at -20°C until required for various tests.

Chemical composition

The moisture, protein, crude fiber, fat, and ash contents of the extracts. The moisture, protein, crude fiber, fat, and ash contents of the extracts were analyzed in triplicate, following the methodology outlined in A.O.A.C. (2012), ash contents of the extracts were analyzed in triplicate, following the methodology outlined in A.O.A.C. (2012), while carbohydrates were calculated by difference.

The mineral content, iron (Fe), calcium (Ca), and zinc (Zn), was assessed using an atomic absorption spectrophotometer (model 3300, Perkin-Elmer, Beaconsfield, UK). The vitamins A, E, C, and D were quantified according to the procedure established by Batifoulier et al. (2005) utilizing high-performance liquid chromatography (HPLC) with an Agilent 1200 system from Germany, which included a variable wavelength detector. Furthermore, the HPLC system was outfitted with an autosampler, a degasser for the quaternary pump, and a column compartment. The experiments were performed on a C18 reverse phase stainless steel column (BDS 5 µm, Labio, Czech Republic) with dimensions of 4×250 mm (internal diameter).

Determination of phenolic and flavonoid compounds

Phenolic and flavonoid compounds were separated using High-Performance Liquid Chromatography (HPLC) following the method established by Mattila et al. (2002). Various treatments were introduced into an Agilent HPLC (Series 1200) equipped with a 5HC-C18 column measuring 250 x 4.6 mm, along with an ultraviolet detector calibrated to 280 nm for phenolic acids and 330 nm for flavonoid compounds. Gradient separation was performed using methanol and acetonitrile as the mobile phase at a flow rate of 1 mL/min, with the column temperature maintained at 35°C. The identification of the fractionated phenolic and flavonoid compounds was achieved by comparing their retention times with those of the automatic areas.

Determination of antioxidant activities

The radical scavenging activity of methanolic extracts DPPH (2,2-diphenyl-1-picrylhydrazyl) was assessed using the methodology established by Blois, 1958. 2 ml solution of DPPH (0.004%) was mixed with 1 ml of the methanolic extracts. The mixture was allowed to incubate for 30 minutes in a dark at room temperature. The absorbance was measured at 517 nm using a Carry Bio100 UV-visible spectrophotometer from Varian, Australia. The synthetic antioxidant DHMC (7, 8-Dihydroxy-4-methyl coumarin) at a concentration of 100 µM served as a standard for evaluating radical scavenging activity. The percentage of inhibition for the radical scavenging activity was calculated using the following formula:

% inhibition = (Absorbance of control - Absorbance of test sample) / Absorbance of control × 100.

Determination of antibacterial activity

Microorganisms and Culture Conditions

Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Streptococcus pneumoniae 33018, and Salmonella typhimurium ATCC 20231 were utilized as the test bacterial strains. All strains were procured from the Microbial Resource Centre of the Faculty of Agriculture, Ain Shams University (Ogunsola and Fasola, 2014). The strains had been previously preserved at 4°C in sterile Luria Bertani (LB) broth (Sigma, Egypt) that was supplemented with 20% sterile glycerol (Sigma, Egypt). The bacteria were subsequently thawed and introduced into LB broth at an inoculum concentration of 2%.

Microbiological analysis

Total bacterial count and total mold and yeast of cake at zero time and (5 and 10 days) after baking were determined according to the method described by (APHA 1992).

Preparation of media

Nutrient agar was prepared by dissolving 2 grams of nutrient agar in 1 liter of distilled water, which was then heated in a water bath at 100°C. Following this, the solution was autoclaved at 121°C for duration of 15 minutes. After autoclaving, the medium was cooled to 45°C before being poured into petri dishes for subsequent bacterial plating. For the sensitivity test, bacteria were inoculated onto the prepared Nutrient Agar and then transferred into sterile dishes at 45°C. The plates were permitted to be colonized by the pathogenic organism. Using an 8mm flamed cork borer, wells were created in the seeded plates. Various concentrations of plant extracts were introduced into these wells and left to incubate for 24 hours. The diameters of the inhibition zones were measured in millimeters. The Minimum Inhibition Concentration (MIC) for each microorganism was determined using the micro-dilution method as described by Eloff (1998), identifying the lowest concentration of extract that effectively inhibited the growth of the tested pathogenic microorganisms.

Cake preparation

In the formulation, wheat flour was substituted with sesame leaves powder at concentrations of 0% (control), 2.5%, 5%, and 7.5%. The cake recipe included (from local market) 120g of wheat flour (72% extraction), 100g of sugar, 100g of whole egg, 14g of milk powder, 25g of shortening, 0.5g of baking powder, 1.0g of vanillin, and 2.3g of salt. The ingredients were combined using the creaming method during the mixing process. The baking temperature was initially set at 180°C, which was then lowered to 160°C after 30 minutes for an additional 10 minutes (Sharoba et al., 2013).

Physical properties of cake

Volume (cm3) and specific volume were determined according to (Türker et al., 2016). Cakes were weighed in grams (g) by laboratory balance. The volume (cm3) of cakes was estimated by Alfalfa seeds displacement. Specific volume was determined using the following equation:

Specific volume = Volume (cm3) / Weight (g)

Sensory evaluation

The cake's sensory evaluation was carried out using a taste panel as described by Sudha et al. (2007). A numerical hedonic scale from 1 to 10 was employed for the sensory assessment, where 1 indicates very poor quality and 10 represents excellent quality. Ten panelists from food technology research institute were asked to evaluate the cake based on attributes such as color, surface texture, crumb color, aroma, flavor, and mouth feel.

Statistical analysis

The experimental data were subjected to an analysis of variance for a completely random design using a (SAS 2000). Duncan's multiple range tests were used to determine the difference among means at the level of ≤ 0.05.

3. Results and Discussion

Results obtained from Table 1 showed that the sesame leaves, stems and fruit covers contained crude protein (18.03, 5.34 and 6.65g/100g) respectively, meanwhile sesame . leaves contained higher percentages of ash (14.34g/100g), and total carbohydrates (43.28g/100g) while sesame stem contains a higher amount of carbohydrate (69.81g/100g).

Although the protein content in sesame waste is relatively high, it is significantly lower than the protein content in the seed (22.32g/100 g dry weight) as mentioned by (Kadhim and Shakir, 2019). Sesame is characterized by a low content of moisture 5.7%, which is a great advantage that increases the storage period (Nzikou et al., 2009). The sesame waste was significantly higher in crude fiber compared with seeds (4.8g/100g) as mentioned by (Adeola, 2010). High fiber content has been reported to be beneficial in preventing constipation and diverticulosis, bind to and remove toxic materials from the body, and have high water holding capacity thereby making stooling easy and bulky (Jansen, 2004). It has also been reported that dietary fiber improves glucose tolerance, is beneficial in treating maturity onset of diabetes, and has health promoting-potential (Larrauri et al., 1996).

Sesame seeds are distinguished by their elevated content of moisture (7.34%), protein (40.90%), crude fiber (7.82%), and ash (7.49%). In contrast, the crude fat content is notably higher in whole sesame seeds at 41.20% (Mbabie et al., 2010). These findings contrast with those of sesame leaves, which exhibit moisture levels of 6.85%, protein content 18.03%, crude fiber 17.27%, ash 14.34%, and a significantly lower crude fat content 0.23 g/100g. Our research demonstrates that different parts of the sesame plant are rich in carbohydrates, underscoring the importance of these plant elements, as carbohydrates are crucial for providing energy to the body. Each gram of carbohydrates produces 4 calories. The body converts carbohydrates into glucose, which serves as the primary energy source for both the brain and muscles. Carbohydrates are recognized as one of the three essential macronutrients required by the body in significant amounts, alongside proteins and fats. Proteins also yield 4 calories per gram, whereas fats deliver a greater energy value of 9 calories per gram. (Awuchi and Amagwula, 2021).

Table 1. chemical composition of sesame plant parts (g/100g dry weight)

	Sesame leaves	Sesame stems	Sesame fruit covers
Moisture	6.85±0.21[a]	6.39±0.91[a]	6.25±0.26[a]
crude protein	18.03±0.53[a]	5.34±0.11[c]	6.65±0.93[b]
Fat	0.23±0.02[a]	0.01±0.003[b]	0.24±0.02[a]
Fiber	17.27±3.65[b]	20.85±5.23[a]	21.53±5.18[a]
Ash	14.34±1.33[a]	7.60±1.02[b]	6.15±1.08[c]
Total carbohydrate	43.28±5.38[c]	69.81±3.43[a]	59.18±5.22[b]

Mean ± Standard deviation triplicate determination.
Mean with the same alphabet in a row are not significantly different ($p \geq 0.05$).

Vitamins and Minerals content in sesame plant parts

The content of vitamins and minerals in sesame parts is presented in Table 2. Vitamins are essential for adequate nutrition; however, while sesame seeds are rich in various important nutrients, they are relatively low in vitamin content compared to other seeds. The vitamin A levels in sesame leaves are 10.01 IU, while the stems contain 12.51 IU, both of which are considered relatively high compared to the fruit covers, which has only 2.81 IU. Additionally, sesame leaves provide 8.65 mg/g of vitamin C. The analysis indicates that the vitamin E content is 0.34, 0.32, and 0.05 in the sesame leaves, stems, and fruit covers, respectively. Vitamin E, a natural fat-soluble antioxidant, is produced exclusively by plants such as sesame and functions as a scavenger of reactive oxygen species (ROS) (Liebler, 1993). Sesame leaves contain some minerals, with 23 percent of the daily suggested intake of calcium and 9 percent of the daily suggested intake of iron in a 100 g serving. The sesame leaves contain iron, zinc and calcium (7.74, 12.24 and 20.32 ppm respectively) so we must be focused on utilize of it in food because of mineral-nutrient malnutrition is a global health problem (Tulchinsky, 2010). The Sesame stem contains (8.38ppm) of iron, (14.37ppm) of zinc and (22.92ppm) of calcium while sesame fruit covers contain (3.54, 8.27 and 12.43) of iron, zinc and calcium respectively. Sesame waste is a very good source of calcium, as it contains a high percentage of it, almost equal to the percentage of calcium in the seeds (Pathak et al., 2014) and these calls for optimizing the use of agricultural waste of sesame.

Table 2. Vitamins and minerals content in sesame tissues

	Sesame leaves	Sesame stems	Sesame fruit covers
Vitamin A (IU)	10.01±1.65[b]	12.51±2.87[c]	2.81±0.94[a]
Vitamin E(mg/g)	0.34±0.07[c]	0.32±0.54[b]	0.05±0.01[a]
Vitamin C (mg/g)	8.65±1.38	-	-
Vitamin D (IU)	10.27±1.31	-	-
Iron (Fe) ppm	7.74±1.43[b]	8.38±2.13[c]	3.54±0.97[a]
Zinc (Zn) ppm	12.24±2.83[b]	14.37±3.15[c]	8.27±2.46[a]
Calcium (Ca) ppm	20.32±5.11[b]	22.92±5.90[c]	12.43±2.02[a]

Mean± Standard deviation triplicate determination.
Mean with the same alphabet in a row are not significantly different ($p \geq 0.05$).

Fractionation of phenolic and flavonoid Compounds in sesame plant parts

Fractionation of phenolic and flavonoid Compounds in sesame plant parts were recorded in Table 3. Flavonoids (mg/100g) were distributed in the sesame plant parts with relatively high content in sesame stem. However, some total flavonoids such as Rutin, Naringin, and others exhibited the highest relative content in sesame fruit covers. Fuji et al., (2018) reported that acteoside content in dried mature sesame leaves represented 12.9 %. Accordingly, they suggested that it might represent the major bioactive compound in the sesame leaves. Kaemp.3 -(2-p-comaroyl) glucose was (3.47mg/100g in sesame leaves and 3.36mg/100g in sesame fruit covers) but the sesame stems was characterized by the absence of Kaemp.3-(2-p-comaroyl) glucose. Quercetin is a natural flavonoid present in sesame parts in a high amount especially in the sesame stems (26.30mg/100g) but in small amounts in the sesame leaves (4.76mg/100g) and in the sesame fruit covers (4.04mg/100g), It has been used most effectively for colorectal cancer (Darband et al., 2018). Kaempferol is found in significant quantities in sesame stems, measuring 4.54mg per 100g, in contrast to, its lower concentrations in sesame leaves at 0.43mg/100g and sesame fruit covers at 0.85mg per 100g. This compound can potentially to lower cancer risk by enhancing the body's antioxidant defenses against free radicals associated with cancer development (Chen and Chen, 2013). Flavonoids are the third largest category of natural products, extensively found throughout the plant kingdom, and represent the most varied class of polyphenolic secondary metabolites. They play crucial roles in plant interactions with their environment and are integral to various self-defense mechanisms against pathogens, ultraviolet (UV) radiation, and abiotic stresses (Dias et al., 2021). One hundred thirteen flavonoids, mainly flavones (Apigenin), flavonols (Kaempferol and Quercetin) and Flavanones (Naringenin), were chemically and structurally identified in sesame plant parts (Laoué et al., 2022).The derivatives of polyphenol contents which estimated with the HPLC method, the sesame stems proved to be the richest source of these compounds (Catechein 7.57 and Chlorogenic 12.36). Moreover, low polyphenol content was detected for the sesame fruit covers (Catechein 3.27, Chlorogenic 3.01 and Caffeine 5.21) (Table 3). Also, the sesame leaves proved to be the richest source of these compounds (Pyrogallol 0.35, Gallic 0.36 and Benzoic 1.26). Although the sesame stems contain the highest percentage of polyphenols, it does not contain any of the Pyrogallol, Benzoic and Caffeine. Polyphenols have been extensively studied for their health benefits related to cardiovascular diseases (Bondonno et al., 2017) and cancer (Qadir et al., 2016). Phenolic acid act as antioxidant and antidiabetic agents (Kumar and Goel, 2019). Diabetes is identified as an oxidative stress disorder, a consequence of an imbalance between the formation of free radicals and an individual's ability to oxidize them. Oxidative stress is vastly associated with the damage to organs through ROS which is poorly neutralized by antioxidants and leads to the inflammation and a variety of metabolic disorders. Antioxidants hinder the free radicals activity by numerous mechanisms and phenolic compounds, particularly phenolic acids (possess a high antioxidant and free radical scavenging potential) work against oxidative stress and its impediments by inhibiting the ROS-producing enzymes (Wu et al., 2014). Also, polyphenol play an important role as an antimicrobial agent while Benzoic, Caffeic, Vanillic and Caffeine inhibit the growth to the greatest extent of Staphylococcus aureus developing in a food product. In addition, the antibacterial properties of hydroxybenzoic acids decrease as the number of -OH groups increases (Stojković et al., 2013).

DPPH scavenging assay of extracts of sesame plant parts

Results in Table 4 showed that both leaves and stems of sesame have exerted high antioxidant power at different concentrations. The DPPH test demonstrated that 100 mg/mL of the ethanol extracts from the sesame leaves significantly increased the percentage of scavenger activity by 67% and 78%, respectively as shown in Table 4. On the other hand, extracts from the stems of sesame have exerted a scavenger activity by 56% and 50% respectively. A possible mechanism responsible for the antioxidant effect of sesame waste in this study may be the presence of vitamin E which can directly scavenge the free radicals. Vitamin E, which is the main lipid-soluble antioxidant present in foods, prevents the lipid peroxidation process in biological lipid-protein complexes (Fellenberg and Speisky, 2006). It was known that phenolic and flavonoid compounds are potential free radical scavengers; hence, a close correlation was observed between the amount of phytochemical compounds and their antioxidant activity (Palanichamy et al., 2018). Although no previous study has directly investigated the antioxidant and antimicrobial effects of sesame waste, many studies have reported the antioxidant and antimicrobial effects of sesame seeds (Ahmed et al., 2009 and Bankole et al., 2007).

Table 3. Fractionation of phenolic and flavonoid Compounds in sesame plant parts

	Sesame leaves	Sesame stems	Sesame fruit covers		Sesame leaves	Sesame stems	Sesame fruit covers
Flavonoid compounds (mg/100g)				Phenolic compounds (mg/100g)			
Rutin	5.64	-	7.01	Pyrogallol	0.35	-	0.23
Naringin	120.54	519.78	124.44	Gallic	0.36	0.21	0.12
Rosmarinic	2.43	7.82	1.68	3-OH Tyrosol	0.17	0.60	0.15
Apigenin-7-glucose	3.12	9.72	2.15	Catechol	1.71	5.21	1.54
Quercetin	4.76	26.30	4.04	4-Amino-benzoic	0.16	0.57	0.21
Naringenin	2.02	3.83	1.66	Catechein	3.22	7.57	3.27
Kaemp.3-(2-p-comaroyl) glucose	3.47	-	3.36	Chlorogenic	2.95	12.36	3.01
Kampferol	0.43	4.54	0.85	Benzoic	1.26	-	1.01
Apigenin	0.25	0.42	0.15	Caffeic	0.52	2.00	0.55
				Vanillic	1.64	4.00	1.00
				Caffeine	4.96	-	5.21
				Ellagic	-	-	10.02
				Coumarin	-	-	0.99
				Ferulic	-	17.60	-

Table 4. DPPH scavenging assay of extracts of sesame plant parts

Concentrations	Sesame leaves	Sesame stems	Sesame fruit covers
Ethanol extracts			
100mg/ml	50.43 ± 3.54^{b}	56.42 ± 5.23^{c}	45.34 ± 3.48^{a}
200mg/ml	76.62 ± 8.13^{c}	65.29 ± 6.08^{b}	50.56 ± 5.22^{a}
300mg/ml	80.19 ± 6.03^{c}	77.04 ± 6.21^{b}	55.12 ± 4.01^{a}
400mg/ml	80.34 ± 8.48^{c}	76.23 ± 7.80^{b}	56.58 ± 4.18^{a}
Aqueous extracts			
100mg/ml	23.93 ± 4.21^{b}	12.23 ± 2.45^{a}	24.11 ± 2.02^{c}
200mg/ml	33.49 ± 5.85^{c}	25.22 ± 3.40^{a}	29.79 ± 3.12^{b}
300mg/ml	39.10 ± 4.62^{c}	23.06 ± 3.11^{a}	31.82 ± 5.20^{b}
400mg/ml	51.16 ± 5.33^{c}	30.05 ± 4.52^{a}	48.21 ± 6.33^{b}

Mean ± Standard deviation triplicate determination.
Mean with the same alphabet in a row are not significantly different ($p \geq 0.05$).

Assessment of the antimicrobial activity of sesame plant parts against some pathogenic microbial strains

The diametric zones of inhibition was measured as shown in Table 5. *Escherichia coli* had the highest diameter of inhibition followed by *Staphylococcus aureus* and *Streptococcus pneumonia* while *Salmonella typhimurium* recorded the lowest diameter of inhibition. Aqueous extracts of the sesame stems didn't effect on *Salmonella typhimurium*. Also, aqueous extracts derived from sesame stems did not exhibit any inhibitory effect on *Escherichia coli* at concentrations of 100 mg/ml and 200 mg/ml. The ethanolic extracts of *sesame indicum* leaves had a very strong antimicrobial effect on *Escherichia coli* and were mildly effective against *Salmonella typhimurium* and at (100, 200, 300 and 400mg/ml while it didn't have effect on *Staphylococcus aureus* using the aqueous extracts at concentration 100 and 200 mg/ml. the aqueous extract of the sesame fruit covers didn't have antimicrobial effect on the organisms screened only at concentration 400mg/ml had mild effect on Escherichia coli. A mild inhibition was also observed using 200mg/ml of the aqueous extract of sesame leaves on *Streptococcus pneumonia*. Syed et al., (2015) found that the Sesame leaves extracts have inhibitory effects on *Streptococcus pneumoniae*, *Candida albicans*, *Staphylococcus aureus*, and symbiotic fungus of leafcutter ants. Also, the sesame leaves, stem and fruit cover extracts showed inhibitory activity against *M. phaseolina* and *F. oxysporum*. Sesamol, which is a major lignan of sesame supposed to be involved in the inhibition of the growth of invading pathogens. Also, Bankole, et al., (2007) found that, the methanolic extracts of some sesame species leaves have antibacterial effect against *Staphylococcus aureus* and *Candida albicans* except the growth of *Streptococcus pneumoniae*. The ethanolic extract didn't have inhibitory effects against the *Staphylococcus aureus* but had both antibacterial and antifungal activities against both *Streptococcus aureus* and *Candida albicans* respectively. The aqueous extract showed antimicrobial effects on all the tested microorganisms.

Table 5. Assessment of the antimicrobial activity of sesame plant parts extract against some pathogenic microbial strains

	Extracts concentration	Staphylococcus aureus	Escherichia coli	Streptococcus pneumonia	Salmonella typhimurium
		Inhibition zone (mm)			
Sesame leaves Ethanol extract	100mg/ml	21.10±2.73	32.09±3.27	15.34±2.61	9.37±2.54
	200 mg/ml	19.16±1.92	32.89±4.56	17.91±2.94	9.34±2.41
	300 mg/ml	18.87±1.67	39.04±4.95	18.30±2.68	10.12±3.12
	400 mg/ml	18.04±2.11	39.43±5.02	22.10±3.11	11.07±2.85
Sesame leaves Aqueous extract	100 mg/ml	-	13.10±2.41	-	-
	200 mg/ml	-	15.23±2.94	-	-
	300 mg/ml	13.12±1.02	16.22±1.32	7.33±1.63	-
	400 mg/ml	15.50±1.33	17.33±1.63	8.07±1.33	8.17±1.32
Sesame stems Ethanol extract	100 mg/ml	11.63±1.37	22.16±2.77	-	6.69±0.74
	200 mg/ml	13.25±0.69	23.62±2.15	-	7.40±0.63
	300 mg/ml	15.27±2.15	22.03±2.19	12.64±2.13	7.21±1.04
	400 mg/ml	15.93±1.20	24.64±2.86	13.22±2.82	10.36±1.73
Sesame stems Aqueous extract	100 mg/ml	-	-	-	-
	200 mg/ml	-	-	-	-
	300 mg/ml	-	9.22±1.68	-	-
	400 mg/ml	11.53±1.93	11.25±2.14	6.67±0.42	-
Sesame fruit covers Ethanol extract	100 mg/ml	6.22±0.89	15.31±2.79	8.34±1.03	-
	200 mg/ml	7.86±1.02	17.21±3.22	9.11±1.28	-
	300 mg/ml	8.34±1.10	18.92±3.12	12.43±2.11	6.54±1.14
	400 mg/ml	10.33±1.90	18.23±2.19	12.63±1.99	10.27±2.05
Sesame fruit covers Aqueous extract	100 mg/ml	-	-	-	-
	200 mg/ml	-	-	-	-
	300 mg/ml	-	-	-	-
	400 mg/ml	-	12.91±2.58	-	8.24±1.13

Physical properties of cake

The physical features of the substituted cake samples with the sesame leaves powder (2.5, 5, and 7.5%) are shown in Table 6. The control sample had the highest height (5.32cm) but the cake fortified with 5% sesame leaves powder showed the lowest height (4.94 cm). The control sample and the sample containing (7.5%) sesame leaves exhibited the highest weight (434.23g and 427.56g respectively) and this may be due to increasing of fiber in the sesame leaves. Our results are dissimilar to those of Mashkour et al., (2022) who found that the volume of sponge cakes fortified with green tea powder significantly decreased. The volume of the cake decreased from 1102.23cm^3 in control to1029.30 cm^3 in cake fortified with 7.5% sesame leaves powder and this may be due to the deceasing of gluten which plays an important role in increasing the volume of bakery products by trapping carbon dioxide produced during backing (Akubor and Ishiwu 2013).

Sensory evaluation of substituted with the sesame leaves powder

The results in Table 7 showed that sesame leaves powder have an effect on all sensory properties of the cake when compared to the control. Furthermore, sesame leaves powder will be accelerated as human nutrition, gaining more value as a waste product. The control sample outperformed the treated cakes in all sensory attributes. After the control cake, the cake containing 2.5% sesame leaves powder had the second highest overall acceptability score (8.05), while cake containing 7.5% sesame leaves powder had the lowest (6.02). The taste score decreased significantly as the amount of sesame leaves powder increased by 7.5%, which could be due to the presence of phenolic compounds with a bitter taste. Because the sesame leaves powder had a dark green color, the acceptability of crumb cake color fortified with the sesame leaves powder 7.5% was significantly decreased (5.95) in comparison with control (9.51).

Table 6. Physical properties of cake substituted with the sesame leaves powder

	Control	2.5%	5%	7.5%
Volume (cm^3)	1102.23^a±100.23	1093.83^b±146.39	1034.19^d±123.41	1029.30^c±123.71
Weight(g)	434.23^b±90.34	418.95^a±75.03	424.04^a±66.70	427.56^a±92.16
Specific volume(cm3/gm)	2.53^a±0.35	2.61^a±0.83	2.43^a±0.23	2.47^a±0.45
Height(cm)	5.32^c±1.36	5.10^{ab}±1.02	4.94^a±1.15	5.22^{ab}±1.34

Mean ± Standard deviation triplicate determination.
Mean with the same alphabet in a row are not significantly different (p ≥ 0.05).

Table 7. Sensory evaluation of substituted with the sesame leaves powder

	Control	2.5%	5%	7.5%
Taste	8.25 ±1.21^a	8.25 ±0.38^{ab}	8.65 ±1.28^b	7.14 ±2.31^a
Odor	9.28 ±0.82^c	8.18 ±1.02^a	8.45 ±1.71^b	8.12 ±1.13^a
Mouth feel	9.43±0.86^c	7.30 ±1.66^b	7.76 ±0.86^b	6.03 ±2.44^a
Crust color	8.10 ±0.97^c	8.17 ±1.82^c	7.32 ±1.21^b	5.42 ±1.94^a
Crumb color	9.51 ±1.03^d	7.94 ±1.24^b	8.63 ±0.69^c	5.95 ±2.33^a
Surface character	8.93 ±1.35^c	7.55 ±2.16^b	7.91 ±1.18^b	6.33 ±1.02^a
Overall	9.33 ±0.37^d	8.05 ±1.11^c	7.42 ±1.93^b	6.02 ±1.87^a

Mean ± Standard deviation triplicate determination.
Mean with the same alphabet in a row are not significantly different (p ≥ 0.05).

Protein, calcium, and zinc of substituted cake with the sesame leaves powder

As shown in Table 8 the protein, calcium and zinc content of cake samples were significantly ($P < 0.05$) affected by the replacement of wheat flour with sesame leaves powder. Protein content was significantly increased in 2.5%, 5% and 7.5% sesame leaves powder (15.73, 15.21 and 17.93 respectively). Proteins are important biomolecules that play major roles in maintaining human health, and due to their vital functional properties, they are key ingredients in many food systems (Dos Santos-Silva, et al., 2024). So, the use of plant proteins is considered essential when animal-derived proteins fail to satisfy the requirements of the global population special for poor population. Leafy green vegetables can provide substantial amounts of vitamins, minerals, proteins, and fibers, as well as antioxidant phytonutrients that play a pivotal role in the prevention or mitigation of obesity, cardiovascular disease and other diseases including cancer (Aslam, et al., 2020). The data presented in Table 8 indicate that the protein, calcium, and zinc levels in the cake samples were significantly influenced ($P < 0.05$) by substituting wheat flour with sesame leaves powder. Notably, the protein content exhibited a significant increase with the addition of 2.5%, 5%, and 7.5% sesame leaves powder 15.73, 15.21, and 17.93, respectively. Proteins are crucial biomolecules that are essential for maintaining human health, and their significant functional properties make them vital components in numerous food systems (Dos Santos-Silva et al., 2024). Consequently, incorporating plant-based proteins is deemed necessary, particularly when animal-derived proteins do not meet the nutritional needs of the global population, especially among impoverished communities and children who are particularly affected by malnutrition. Leafy green vegetables are capable of providing considerable quantities of vitamins, minerals, proteins, and fibers, along with antioxidant phytonutrients that are instrumental in preventing or alleviating obesity, cardiovascular diseases, and other health issues, including cancer (Aslam et al., 2020). It was observed that the calcium non significantly decreased with the increased of sesame leaves powder in a replacement of 2.5% (35.26) but significantly decreased with the increased of sesame leaves powder in a replacement of 7.5% (32.05). Calcium enhances the process of inhibition on the surface of the brain, regulates the imbalance between excitement and inhibition, reduces inflammation, reduces swelling, resist allergy and detoxification, and is negatively correlated with hypertension (Fan, et al., 2006). Zinc was significantly increased (6.18, 6.91 and 7.50 respectively) in comparison with control (2.11). The higher zinc content of the cake is due to the higher percentage of zinc in sesame leaves compared to wheat flour, which achieves the goal of increasing the nutritional value of the cake. Zinc an essential trace mineral, is required for the metabolic activity of the body's enzymes, and is considered essential for cell division and the synthesis of DNA and protein. These enzymes are involved with the metabolism of protein, carbohydrate and fat (Fan et al., 2006).

Table 8. Protein, calcium, and zinc of substituted cake with the sesame leaves powder

	Control	2.5%	5%	7.5%
Protein (g/100g)	13.34±1.82[c]	15.73±1.23[b]	15.21±1.22[b]	17.93±2.14[a]
Calcium (ppm)	35.23±3.05[a]	35.26±3.02[a]	34.48±3.54[b]	32.05±3.63[c]
Zinc (ppm)	2.11±0.73[d]	6.18±1.27[c]	6.91±0.93[b]	7.50±1.44[a]

Mean ± Standard deviation triplicate determination.
Mean with the same alphabet in a row are not significantly different ($p \geq 0.05$).

Total bacterial and Yeast and mold count (cfu/g) of substituted cake with the sesame leaves powder

Data in Table 9 showed that the different microorganisms (bacteria, mold and yeast) present in cake prepared from sesame leaves powder with stored room temperature (20±5˚C) at zero time,5 and 10 days. The microbiological count is thought to be an appropriate indicator for cake production's shelf life (Maio et al., 2020). The findings showed that cake produced using sesame leaves powder in place of wheat flour had the lowest amount of total bacteria. Increasing of sesame leaves powder (2.5, 5 and 7.5%) caused a decrease in yeast and mold count during storage period. Moreover, the yeast and mold count in control sample was increased throughout the storage period in control cake. Studies have indicated a direct relationship between antioxidant and antimicrobial properties of materials. Studies have demonstrated a direct relationship between antioxidants and antimicrobial properties of substances, so that as the amount of antioxidants in food increases so do antimicrobial properties (Nukabadi et al., 2020). The sesame leaves powder is considered a good source of crude fiber, ash, and carbohydrates. Crude fibers provide multiple health benefits, such as their ability to lower serum LDL cholesterol levels, enhance glucose resistance and insulin levels, decrease hyperlipidemia and hypertension, contribute to gastrointestinal health, and inhibit certain cancers such as colon cancer (Thomas *et al.,* 2020).The sesame leaves powder is considered a good source of crude fiber, ash, and carbohydrates. Crude fibers provide multiple health benefits, such as their ability to lower serum LDL cholesterol levels, enhance glucose resistance and insulin levels, decrease hyperlipidemia and hypertension, contribute to gastrointestinal health, and inhibit certain cancers such as colon cancer (Thomas et al., 2020).

Table 9. Total bacterial and Yeast and mold count (cfu/ g) of substituted cake with the sesame leaves powder during storage at room temperatures

	Control	2.5%	5%	7.5%
Total bacterial count				
Zero Time	0	0	0	0
5 Days	3×10^2	4×10^3	4×10^3	2×10
10 Days	7×10^5	6×10^4	6×10^3	4×10^2
Yeast and mold count				
Zero Time	0	0	0	0
5 Days	8×10	2×10^2	3×10^2	6×10
10 Days	6×10^3	5×10^3	4×10^2	3×10^2

4. Conclusion

The sesame wastes (*Sesamum indicum*) contain dietary fiber, protein, vitamins (A, E and C), and minerals (iron, calcium and zinc). Moreover, it contains phenolic and flavonoid compounds. Therefore, according to the results of this study on the antioxidant and antibacterial of sesame waste, we recommended using it to produce foods with high nutritional value from cheap source. So addition of sesame leaves to cake increase its content from protein and zinc. Sensory evaluation of control and substituted cake with sesame leaves (taste, color, odor and overall acceptability) showed non-significant decreased with increasing of sesame leaves powder which makes it acceptable, especially for children who suffer from malnutrition in poor communities.

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