*Egypt. J. of Appl. Sci., 40 (5-6) 2025* *16-38*

# ASSESS THE PHENOLIC CONTENT AND BIOACTIVITY OF MORINGA LEAF EXTRACTS

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## ABSTRACT

The utilization of new natural antioxidants and antimicrobials in meals, particularly beef and chicken, is urgently needed because some synthetic antioxidants and antimicrobials have harmful, mutagenic, and carcinogenic consequences.

In this study, the antioxidant activity of moringa leaf ethanol extracts at 50%, 70%, 90%, and 100% was investigated. Based on its antioxidant activity, the 70% ethanol extract was chosen to enhance the chemical, physical, and microbiological characteristics and prolong the shelf life of beef meat.

According to their dry weight, moringa leaves have the following chemical composition: 6.9% moisture, 24.1% crude protein, 7.19% crude fat, 15.23% total ash, 6.29% fiber and 40.32% carbohydrates.

The results indicated that the highest DPPH percentage (91.59%) was found in 70% ethanolic extract which was marginally higher than that of the 100% ethanolic extract (91.47%). Both antioxidant activities were comparable to those of ascorbic acid.

Moringa leaf ethanol extract (MO 1%) produced the highest total phenol (64.497 ± 0.465 mg GAE/g extract or 6.450 ± 0.465 g GAE/100 g extract) followed by 0.5% moringa extract (MO 0.5%, 42.027 ± 0.826 mg GAE/g).

Moringa leaves ethanol extract (MO 1%) yielded the highest amount of flavonoids (30.86 ± 0.248 mg QE/g extract), followed by MO 0.5%, (24.067 ± 0.179 mg QE/g).

The HPLC analysis of moringa leaf extract showed the existence of 20 phenolic compounds that fall between <LOQ and 28.12%. By comparing the retention duration of authentic standards, the four recognized components of chlorogenic acid, gallic acid, naringenin, and vanillin—have been determined to be the most abundant chemicals in moringa extracts, (with variations in their amount). The lower percentages of other chemicals ranged from 2.81% to less than 0.2%.

Five out of the twenty compounds namely Coumaric acid, Cinnamic acid, Daidzein, Catechin and Hesperetin exhibited <LOQ with

146, 148, 254, 290 and 302 MW (molecular weight). Additionally, leaves are abundant in flavonoids, which include rutin, kaempferol, and quercetin. These compounds are well-known for their anti-inflammatory, anti-allergic, anti-cancer, and anti-spasmodic effects.
**Key Words:** Antioxidants, Antimicrobials, Beef, Chicken, Moringa.

# INTRODUCTION

The use of naturally occurring plant-based preservatives as food antimicrobials is gaining popularity (**Davidson and Naidu, 2000)**. This is a result of both decreased customer preference because of the lingering effects of chemical food preservatives and consumer safety, as well as increased resistance in pathogenic strains against these chemicals (**Yadav and Singh, 2004).** Because they contain phytochemicals, several herbs and spices have been shown by studies to be effective preservatives (**Ogunka–Nnoka and Mepba, 2008**). Due to the presence of chemical components with antioxidant qualities, the usage and consumption of diets made with spices and herbs have become more acceptable to both the scientific community and consumers (**Ibrahim *et al.,* 2010**). Assessing a plant materials antimicrobial activity against food-borne bacteria are a crucial step in screening it for a preservation role (**Alozie and Sonye, 2015**). The creation of improved food production, preservation, and storage methods is the food industries main issue in order to offer wholesome, nutrient-dense food for human wellbeing. It is generally acknowledged that in the upcoming years, green foods will be significantly accessible to customers globally, **(Lugani *et al.,* 2021**). However, moringa, including its leaf extract, is gaining recognition for its nutritional value and potential health benefits, making it a likely candidate to contribute to the increasing availability of green foods. Still, there are a lot of different opinions about how safe and effective these plant-based products are. The main causes of worry with herbal products are the lack of defined dosages and uneven quality control (**Murro *et al.,* 2003**). Microbial growth and lipid oxidation are the primary causes of meat decomposition and a reduction in its shelf life (**Luong *et al.,* 2020 and Zehi *et al.,* 2020).** Legal authorities often report microbial contamination of meat and meat products (**EFSA and ECDC, 2019**). One of the top priorities for the meat industry's research and development division in recent years has been the creation of new processed meat with functional qualities and no chemical preservatives in order to control the spoiling of these products and meet the demands of green marketing and consumerism (**Alirezalu *et al.,* 2021 and Pereira *et al.,* 2019).** Consequently, this research is an attempt to: Making extracts from moringa and examine the antibacterial properties of moringa extracts against strains of pathogenic microorganisms and the antioxidant capacity of DPPH-synthesized.

# MATERIALS AND METHODS

Moringa leaves were obtained from National Research Institute, Giza, Egypt.

**Technological methods**

**Preparation of moringa leaf extracts:**

The collected moringa leaves were first cleared of any foreign objects, thoroughly cleaned with water to get rid of any dust and then allowed to dry for five days at room temperature in the shade. A household blender [IKA, WERKE, (USA)] was used to grind the dried leaves into a coarse powder, which was then placed in a Ziploc bag with a label and stored in the refrigerator at 4 °C until needed. Overnight at ambient temperature in a shaker, 10 g of dry ground samples were extracted using 100 ml of ethanol at different concentrations (50%, 70%, 90%, and 100% ethanol). The residues were extracted again under the same circumstances after the moringa extracts were filtered via filter paper (Whatman No. 1). A rotary evaporation machine (BÜCHI Rotavapor R-124, Germany) was used to evaporate the combined filtrate at temperatures lower than 40ºC. Following the evaporation of organic solvents, all extracts were dried at 40ºC in a hot air oven before being ground into powder.

**Measurements methods:**

**Analytical methods:**

**Analysis of chemicals:**

According to the official procedures of the **AOAC (2016)**, the moisture content, crude fat, crude protein, total ash, and crude fiber of moringa leaves were measured.

**Assay for Total Phenolic Content (TPC):**

The Folin–Ciocalteau colorimetric method was used to measure the total phenolic content (TPC) **(Singleton and Rossi, 1965; Singleton *et al.*, 1999)**. The Folin-Ciocalteau method was used to measure TPC. Three milliliters of 10% Folin-Ciocalteau solution were combined with five microliters (0.05 milliliters) of plant extract and 0.8 milliliters of 7.5% sodium bicarbonate. The reaction solution was incubated at room temperature for half an hour. The mixture absorbance was measured using a Milton Roy (Spectronic 1201) spectrophotometer at 765 nm. Gallic acid equivalents (GAE) per gram of extract were used to express the TPC.

**Assay for Total Flavonoid Content (TFC):**

**Chang *et al.* (2002)** method was used to quantify the total flavonoid content (TFC). Briefly, 3.90 mL of distilled water, 0.1 mL of

extract, and 0.3 mL of sodium nitrite (5%) solution were combined and left to react for 5 minutes. After that, 0.3 mL of 10% aluminum chloride solution was added. The mixture was left to continue reacting for six minutes,. Then, 2 mL of sodium hydroxide (1 $mM^{-1}$) was added to the mixed solution. Then, each sample received 2.4 milliliters of distilled water. The Milton Roy (Spectronic 1201) spectrophotometer was used to measure the absorbance at 510 nm in comparison to a sample blank that had not undergone any reaction. The extracts TFC was given as milligrams of quercetin equivalents (QE) per gram of extract.

**HPLC-based phenolic and flavonoid compound fractionation:**

**Phenolic compound identification and fractionation:**

HPLC analysis was used to identify the phenolic components in moringa leaf extracts using **Dragovic-Uzelac *et al.*, (2005)** method utilizing Agilent's Series 1200 HPLC (USA). Elution was carried out using a mobile phase that included water: acetic acid (98:2 v/v as solvent A) and methanol: acetonitrile (50:50 v/v as solvent B). The concentration of B was increased from 5% to 30% through 25 minutes. 280 nm at 1.0 ml/min was the setting for the ultraviolet (UV) detector. The concentration of phenolic compounds was determined using retention time and peak area using Hewlett Packard software for data analysis.

**Identification and fractionation of molecules containing flavonoids:**

As described by **Mattila *et al.*, (2000)**, flavonoid compounds were identified using HPLC (Agilent, Series 1200, USA). The all parameters were the same as those previously applied to phenolic compounds. However, the ultraviolet (UV) detector was set at 330 nm.

**Antioxidant activity of treatments**

**Antioxidant Assay:**

Using the DPPH free radical scavenging test, the antioxidant activity of the extract was assessed in triplicate at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University. Average values were taken into account.

**Activity of DPPH Radical Scavenging:**

A freshly made methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (0.004%w/v) was made and kept at $10^{o}C$ in the dark. The test compound's methanol solution was made. Three milliliters of DPPH solution were mixed with a 40 µL aliquot of the methanol solution. A UV-visible spectrophotometer was used to record absorbance data right away (Milton Roy, Spectronic 1201). Continuous measurements of the absorbance drop at 515 nm were made, with data being taken every minute until the absorbance stabilized (16 min). Measurements were also made of the absorbance of the reference molecule ascorbic acid and the DPPH radical without antioxidant (control). Three replicates of each determination were made, and the results were averaged.

The following equation was used to determine the DPPH radical's inhibition percentage (PI):

PI = [{(AC-AT)/ AC} x 100]. **(Gomha and Others, 2018)**

where AT is the sample's absorbance plus DPPH at t = 16 minutes and AC is the control's absorbance at t = 0 minutes.

Graphical plots of the dose response curve were used to estimate the 50% inhibitory concentration (IC50), or the concentration needed to decrease the activity of the DPPH radical by 50%.

**3.2.3.6 Antimicrobial activity:**

The well diffusion method was used to measure the antimicrobial activity. According to **Hulikere and Joshi (2019)**, the well diffusion method was used to measure the diameter of the inhibition zone in order to examine the effects of various treatments. Four distinct human pathogenic bacteria were taken into consideration for antimicrobial research: two gram-positive strains of bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* RCMB 015 (1) NRRL B-543), two gram-negative strains of bacteria (*Escherichia coli* ATCC 25922 and *Proteus vulgaris* RCMB 004 (1) ATCC 13315), and two fungal strains (*Aspergillus fumigatus*, RCMB 002008) and a yeast (*Candida albicans*, RCMB 005003 (1) ATCC 10231). The fungal strains *Aspergillus fumigatus*, RCMB 002008, and *Candida albicans*, RCMB 005003 (1) ATCC 10231, were kept on chloramphenicol glucose yeast extract agar, whereas bacterial cultures were cultivated on nutritional agar. 15 milliliters of sterile agar (at 45 to 50 degrees Celsius) were inoculated with one milliliter of the above incubated culture (the average count for all strains was 1.0 to 7.0 × $10^6$ cfu/ml). Sterile petri dishes were aseptically filled with the inoculated agar. The agar was given time to set. Moringa leaf extracts were added to each well of the petri dishes that had been infected with bacteria. The bacterial and fungal strains were controlled with gentamycin and ketoconazole, respectively. The zone of inhibition (ZOI) was measured in millimeters after all the dishes were incubated for 24 hours at 37°C for bacterial strains and four days at 25ºC for mould strains.

**Statistical Analysis:**

According to **Snedecor and Cochran (1994)**, data were subjected to the Least Significant Difference (LSD) approach and Analysis of Variance (ANOVA) in order to check for a difference in means ($p < 0.05$).

## RESULTS AND DISCUSSION

**Proximate composition of moringa leaf:**

Results in Fig (1) illustrated *moringa oleifera* leaf powder's approximate composition (percentage of dry weight). From the obtained results , it could be noticed that moringa leaf powder (on dry weight basis) contained 6.90 % moisture, 24.10% crude protein, 7.19% crude fat, 15.23% ash, 6.26 % fiber and 40.32% carbohydrates.

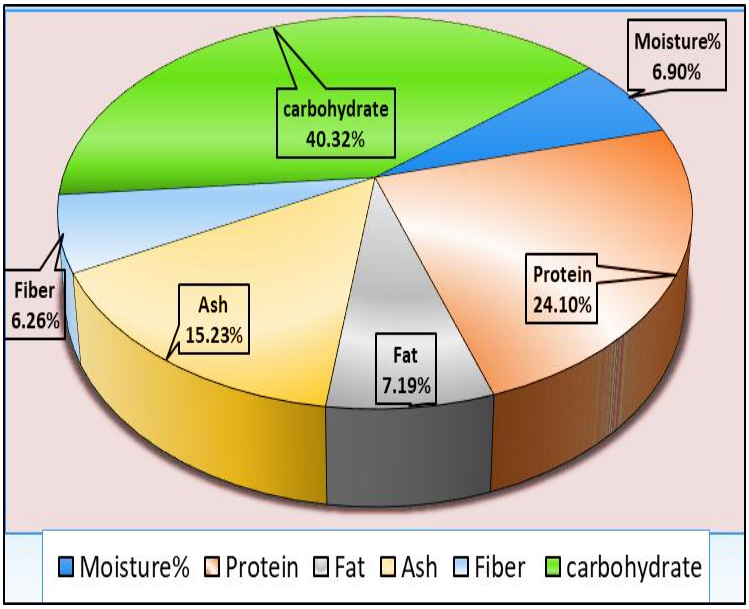


**Fig. 1:** Chemical composition of *moringa oleifera* leaf powder (% dry weight basis)

**Phenolic compounds in moringa leaves extract:**

The main phenolic components in the ethanolic extract were identified using HPLC analysis in order to comprehend the phenolic nature of the moringa extract. Fig 2 and Table 1 list the main phenolic compounds together with their molecular formula (MF), retention time (RT), and area concentration (percentage). They are listed by their order of molecular weight (MW).

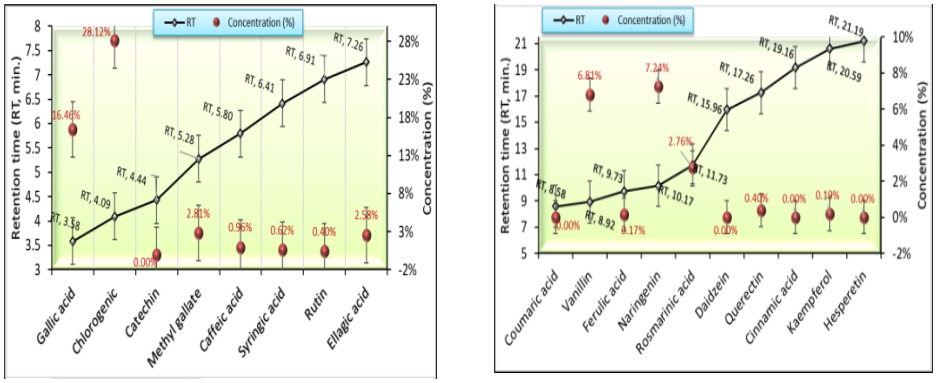


**Fig. 2:** Number and percentage of phenolic compounds found in ethanolic extract of moringa leaf.

**Table 1. HPLC phenolic profile of Moringa extracts**

| Compound | MW (g/mol) | MF | RT | Area (%) |
|---|---|---|---|---|
| **Phenolic Acid (PA)** | | | | |
| Coumaric acid | 146 | C9H6O2 | 8.584 | <LOQ |
| Cinnamic acid | 148 | C9H8O2 | 19.159 | <LOQ |
| Gallic acid | 170 | C7H6O5 | 3.584 | 16.4559 |
| Caffeic acid | 180 | C9H8O4 | 5.796 | 0.9606 |
| Methyl gallate | 184 | C8H8O5 | 5.275 | 2.8104 |
| Ferulic acid | 194 | C10H10O4 | 9.733 | 0.1692 |
| Syringic acid | 198 | C9H10O5 | 6.412 | 0.6225 |
| Ellagic acid | 302 | C14H6O8 | 7.26 | 2.5771 |
| Chlorogenic | 354 | C16H18O9 | 4.092 | 28.1229 |
| Rosmarinic acid | 360.3 | C18H16O8 | 11.734 | 2.7576 |
| total PA | | | | 54.4762 |
| **Phenolic aldehyde (P ald)** | | | | |
| Vanillin | 152 | C8H8O3 | 8.918 | 6.8094 |
| total Pald | | | | 6.8094 |
| **Flavonoid (F)** | | | | |
| Daidzein | 254 | C15H10O4 | 15.961 | <LOQ |
| Naringenin | 272 | C15H12O5 | 10.168 | 7.2396 |
| Kaempferol | 286 | C15H10O6 | 20.588 | 0.1934 |
| Catechin | 290 | C15H14O6 | 4.435 | <LOQ |
| Hesperetin | 302 | C16H14O6 | 21.193 | <LOQ |
| Querectin | 302 | C15H10O7 | 17.262 | 0.3988 |
| Rutin | 610 | C27H30O16 | 6.908 | 0.3987 |
| total F | | | | 8.2305 |
| **Others (ND)** | | | | |
| ND | ND | ND | 7.505 | 6.05 |
| ND | ND | ND | 8.005 | 24.44 |
| total ND | | | | 30.48 |
| Total identified compounds | | | | 100 |

ND: not detected, MW: molecular weight, MF: molecular formula, RT: retention time, LOQ: Limit of Quantification

As shown in this study, phenolic chemicals are abundant in moringa leaves (Table 1 and Figs 4, 5, 6). The HPLC analysis of moringa leaves extract recorded at 280 nm revealed the presence of 20 phenolic compounds that were found in the range of <LOQ to 28.12%. Five out of the twenty compounds namely Coumaric acid, Cinnamic acid, Daidzein, Catechin and Hesperetin exhibited <LOQ with 146, 148, 254, 290 and 302 MW (molecular weight) respectively.

The four known components: Chlorogenic, Gallic acid, Naringenin and Vanillin have been identified as the highest compounds found in moringa extracts (with differences in their content) by contrasting it with the persistence period of real standards (Table 1 and Figs. 2, 3, & 4). Other compounds were present in lower percentages ranging from 2.81% to <0.18%).

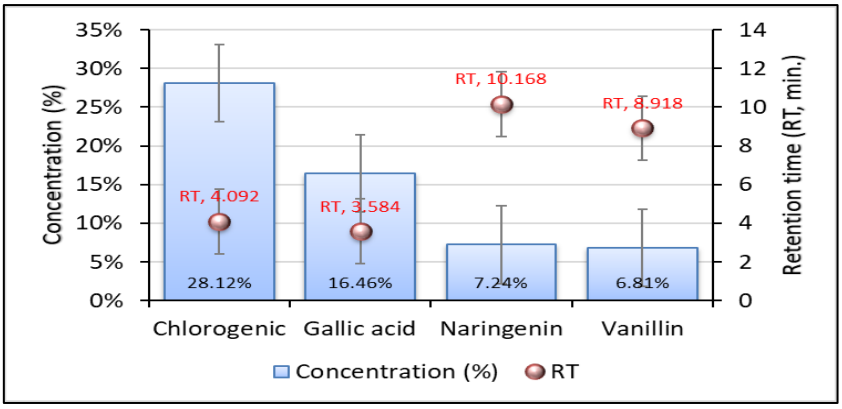


**Fig.3:** Content of the highest detected phenolic compounds in moringa extracts along with retention time (RT)

These chemicals' existence raises the possibility that the extracts have bioactive and antioxidant qualities, which could support moringa's therapeutic and medical benefits. Gallic and chlorogenic acids, which have antifungal, antiviral, and antioxidant properties, are the most potent phenolic components found in *Moringa oleifera* leaves **(Mohamed *et al.*, 2021)**. This could mitigate the effects of oxidative damage **(Zucca *et al.*, 2013)**. Additionally, leaves contain a wealth of flavonoids, including kaempferol, quercetin, and rutin, which are well-known for their anti-inflammatory, anti-allergic, anti-cancer, and anti-spasmodic effects **(Anwar *et al.*, 2007).**

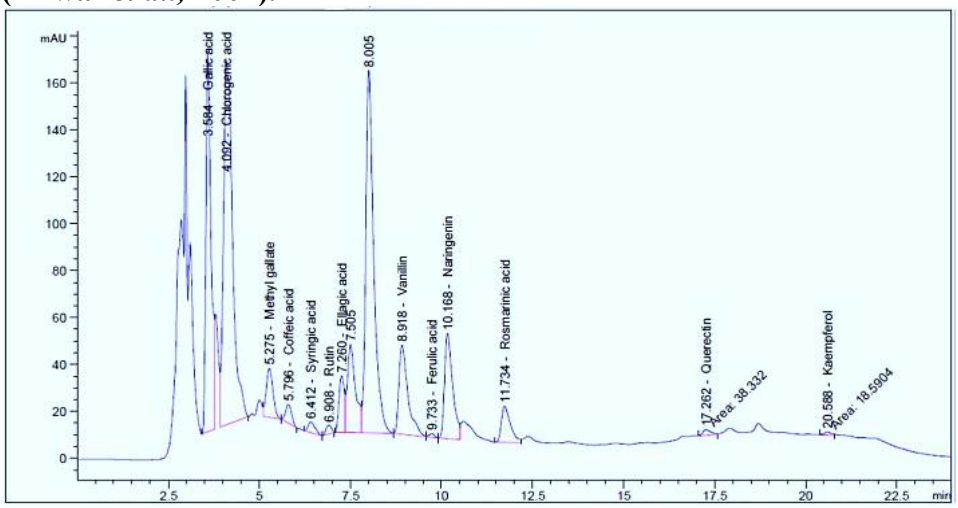


**Fig. 4:** Identified components of moringa leaves by HPLC.

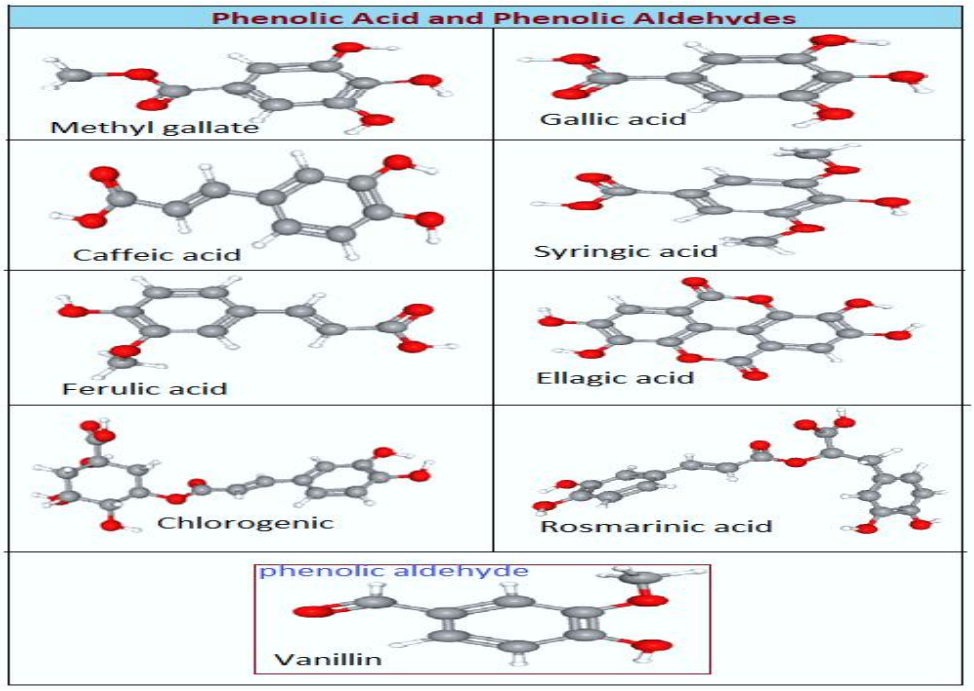


**Fig.5:** Chemical structure of nine phenolic acids and aldehyde compounds found in the studied extract of moringa leaves.

***Identified*** **the amount of flavonoids (TFC), total phenol content (TPC), and antioxidant activity (DPPH) in treatments with moringa and nanochitosan:**

**Calculating Total Phenol:**

Phenol is one of the main secondary metabolites in plants that have antioxidant qualities. The total phenol content was ascertained using the Folin-Ciocâlteau reagent. The phosphomolybdic acid and phosphotungstic acid in the Folin-Ciocâlteau reagent will be reduced by the polyphenol compounds in the sample to a blue molybdenum-tungsten complex. The absorbance of this compound was measured at 765 nm using a spectrophotometer. Because more phenolic ions will transform the heteropoly acid (phosphomolybdate-phosphotungstate) into the molybdenum-tungsten complex, the intensity of the ensuing blue colour rises with the concentration of phenolic chemicals **(Singleton and Rossi, 1965).**

According to **Bancuta *et al.*, (2016)**, the sample's phenolic compounds react with the Folin-Ciocâlteau reagent, which is composed of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$), to form a combination of blue oxides (W8O23 and Mo8O23) (Fig 7).

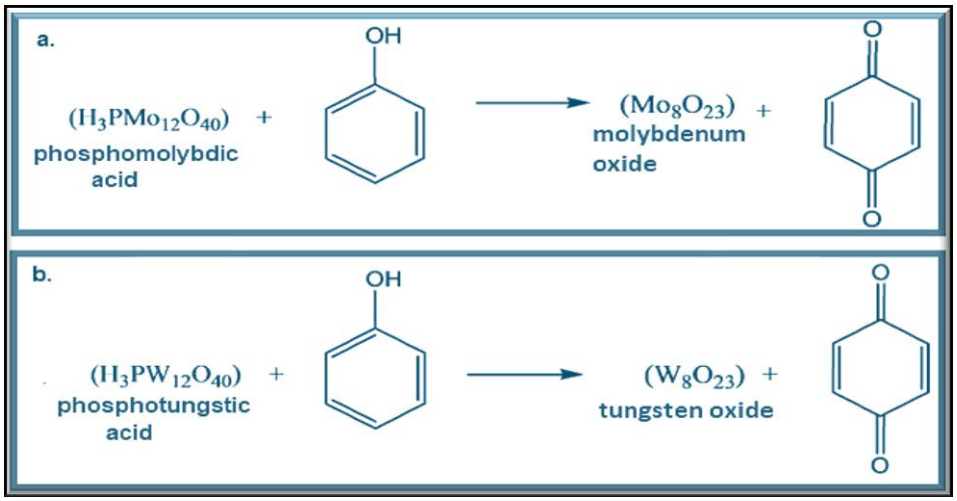


**Fig. 7:** Particular reactions between phenolic chemicals and the Folin-Ciocâlteau reagent

Gallic acid was measured as total phenol reference. Figure 8 displays the findings from calculating the absorbance in the gallic acid standard curve yields the equation y=0.0025x+0.045 with a correlation coefficient $R^2$ = 0.9965. Gallic acid is used as a measuring reference since it is a polyphenol component found in almost all plants. The phenolic content of these organic acids is stable and pure **(Singleton and Rossi, 1965)**. An analysis of the total phenol content was carried out in order to determine the antioxidant capacity of the ethanol extract of the investigated treatments of moringa leaves as a defense against free radicals.

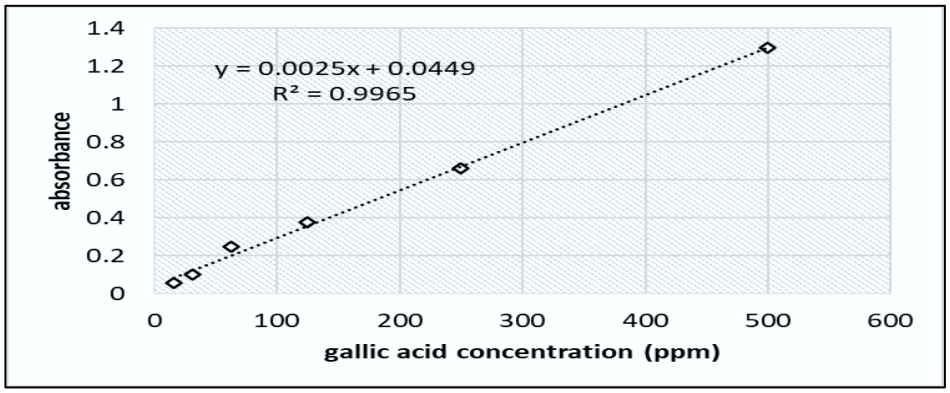


**Fig. 8.** The correlation between the gallic acid reference standard's absorbance and concentration

Results in Table 2 and Fig 9 show that, the highest yield (64.497 ± 0.465 mg GAE/g extract or 6.450± 0.465 g GAE/100g extract) was obtained from the ethanol extract (1%) of moringa leaves' total phenol content followed by moringa extract (0.5%, 42.027 ± 0.826 mg GAE/g) in descending order.

**Table 2. Identified total phenol (TPC) and flavonoids (TFC) contents of the extracts of moringa treatments**

| Treatment | Phenolics content | Flavonoids content |
|---|---|---|
| Moringa Extract (0.5%) | 42.027 ± 0.826 | 24.067 ± 0.179 |
| Moringa Extract (1%) | 64.497 ± 0.465 | 30.86 ± 0.248 |
| LSD | 1.465 | 0.969 |

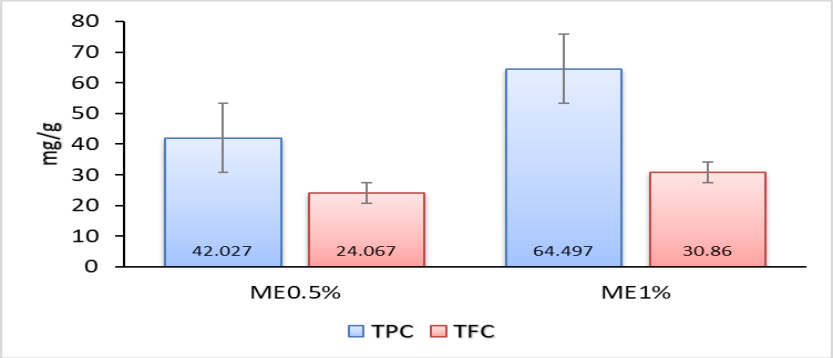


**Fig. 9:** Identified of total phenol content (TPC) and flavonoids content (TFC) of moringa treatments

While this yield was lower than **Vongsak *et al.*, (2013)**, who obtained a yield of 13.23 g CAE/100g extract in 70% ethanol solvent, it was comparable to the results of **Shanmugavel *et al.*, (2018) and Fachriyah *et al.*, (2020)**. This discrepancy in yield is most likely caused by variations in moringa cultivars and growing environments (**Şanli and Karadoğan, 2017**).

**Determination of Total Flavonoids**

As shown in Fig.10, the $AlCl_3$ colorimetric method was used to measure the total flavonoid content of the studied ethanol extracts of moringa leaves. Creating a stable acid combination with C-4 keto groups is the fundamental principle behind the $AlCl_3$ colorimetric approach. The $AlCl_3$ reagent then reacts with C-3 or C-5 hydroxyl groups from flavones and flavonols **(Pallab *et al.*, 2013)**. Furthermore, a persistent acid complex is produced when $AlCl_3$ joins with orthodihydroxy categories on flavonoid A or B rings **(Markham, 1982)**.

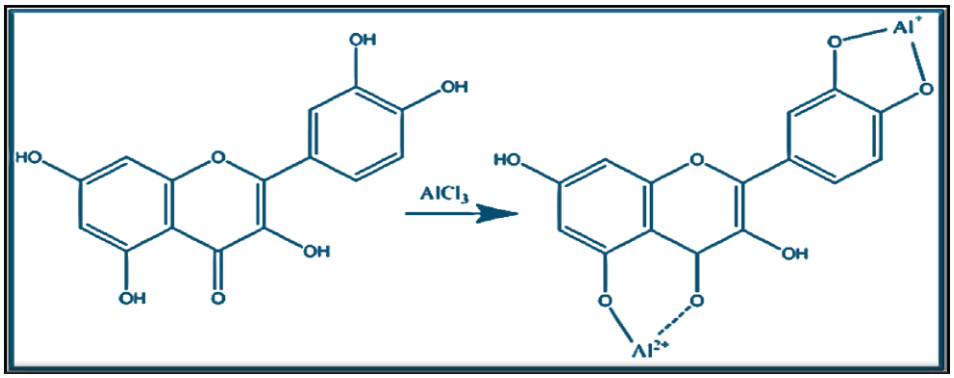


**Fig.10:** The chemical reaction of aluminum chloride method for flavonoids assay.

Quercetin was used as a standard solution at different concentrations to determine the levels of total flavonoids. Fig. 11 shows the absorption results of the usual quercetin determined at 510 nm.

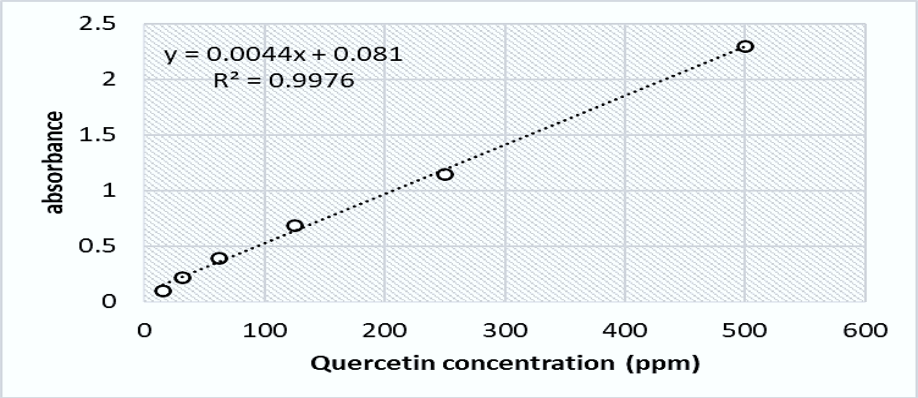


**Fig. 11:** The correlation between the quercetin reference standard's absorbance and concentration.

Results in Table 2 and Fig. 9 showed that, the total flavonoid assessment of moringa leaf ethanol extract (1%) produced the maximum yield (30.86 ± 0.248 mg QE/g extract) followed by moringa extract (0.5%, 24.067 ± 0.179 mg QE/g).

This result is higher than the findings of **Fachriyah *et al.*, (2020),** who found that a total flavonoid of 10.477 mg QE/g extract was obtained. **Shanmugavel *et al.*, (2018)**, found that a total flavonoid of 22.16 mg QE/g of extract was obtained from moringa extracts.

**Antioxidant activity of the extract of moringa leaves by different ethanol concentrations:**

Solvent rates had a significant ($p \leq 0.05$) impact on the antioxidant properties of moringa leaf extracts. The DPPH radical scavenging activity of moringa leaf extracts at varying ethanolic concentrations ranged from 88.13 to 91.59%.

The DPPH [%] assay for moringa ethanolic extracts is shown in Fig 12. The findings showed that moringa ethanolic extract 70% had the highest DPPH percentage, which was marginally higher than that of moringa ethanolic extract 100% (DPPH for 70% and 100% extracts was 91.59% and 91.47%, respectively), with both antioxidant effects having comparable to ascorbic acid. The reults in Fig. 12 were evaluated using a one-way ANOVA test, and the statistical significance ($p < 0.01$) shows how the antioxidant activity of the four extracts differed from one another.

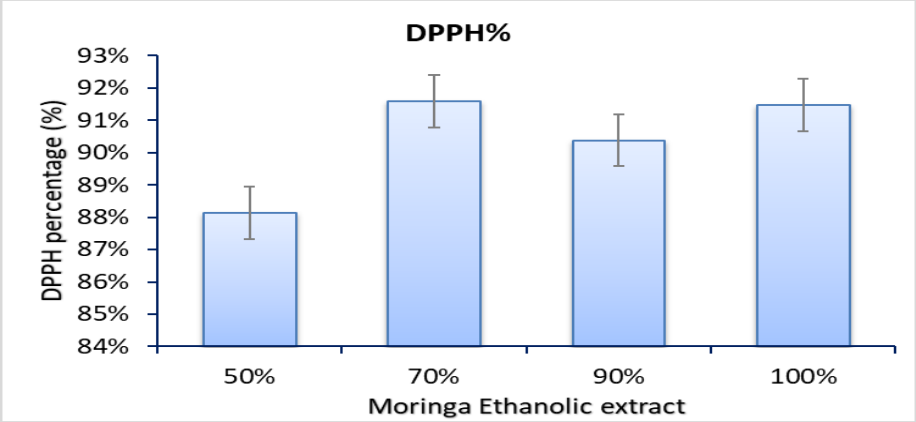


**Fig. 12:** Ethanolic extracts of moringa leaves exhibit antioxidant activity. The mean ± SD is used to express the data. Groups were compared using a one-way ANOVA test and an LSD test (** $p < 0.01$).

Numerous research has shown that ethanolic extracts of moringa leaves have higher antioxidant activity, as measured by the DPPH assay, which is consistent with the present investigation. For instance, ethanolic extracts of moringa leaves exhibit higher DPPH radical scavenging activity than methanolic extracts, according to **Oluewu *et al.* (2024)** who study of methanolic and ethanolic extracts from five different nations. **Herman-Lara *et al.*, (2024)** reported that the antioxidant activity is

correlated with the total phenolic content, and the 70% ethanolic extracts of moringa exhibited strong antioxidant values.

According to these findings, the best solvents for removing the chemicals that give moringa leaves their antioxidant activity are the extracts listed above.

The higher levels of total phenolic and total flavonoids in the 70% ethanol extract may be the cause of its increased antioxidant activity. Many fruits, vegetables, and solvents have been used to study the relationship between antioxidant activity and total phenolic compounds **(Bartolomé *et al.*, 2004; Kedage *et al.*, 2007; Jayaprakasha *et al.*, 2008 and Radovanovic *et al.*, 2009)**. Total phenolic compounds and the DPPH radical-scavenging activity of the methanol-extracted samples were found to be significantly correlated, whereas TPC and the DPPH radical-scavenging of the ethanol-extracted samples were found to be excellently correlated. Moringa leaves extracted with 50% ethanol showed the lowest antioxidant activity (88.13%).

**Treatments with an ethanol extract of moringa leaves that exhibit antioxidant activity:**

The DPPH antioxidant activity assay method was used to examine the free radical scavenging potentials of various extracts from moringa leaves and ascorbic acid (ASA) at varying doses. The results are shown in Table 3 and Figs. 13-15.

**Table 3 The DPPH method's radical scavenging activity of several extracts at varying concentrations.**

| Sample conc. (µg/ml) | ASA | MO 0.5% | MO 1% |
|---|---|---|---|
| 1000 | 98.65 ± 0.21 | 89.72 ± 1.65 | 96.18 ± 1.27 |
| 500 | 96.37 ± 0.45 | 86.29 ± 1.33 | 92.04 ± 0.96 |
| 250 | 94.25 ± 0.73 | 80.46 ± 0.98 | 87.95 ± 1.32 |
| 125 | 90.89 ± 0.28 | 72.31 ± 0.75 | 82.13 ± 1.45 |
| 62.5 | 86.42 ± 0.71 | 65.19 ± 1.34 | 77.93 ± 0.98 |
| 31.25 | 79.35 ± 0.97 | 58.26 ± 1.32 | 69.52 ± 0.74 |
| 15.6 | 65.09 ± 1.43 | 49.37 ± 1.05 | 60.45 ± 0.91 |
| 7.8 | 44.21 ± 2.87 | 39.85 ± 0.87 | 51.79 ± 1.35 |
| 3.9 | 31.39 ± 0.62 | 31.42 ± 0.34 | 46.23 ± 1.46 |
| 2 | 22.18 ± 0.93 | 23.17 ± 0.65 | 34.97 ± 0.68 |
| 1 | 14.48 ± 0.56 | 18.03 ± 0.31 | 27.51 ± 0.73 |
| 0.5 | 9.37 ± 0.45 | 10.75 ± 0.43 | 19.56 ± 0.28 |
| IC50 (µg/ml) | 8.88 ±0.6 | 17.07 ±1.05 | 6.17 ±0.21 |

ASA: ascorbic acid, MO: moringa leaf extract.

At 1000 µg/ml, ascorbic acid showed 98.65% free radical scavenging. A lower IC50 value (6.17±0.21) indicated that moringa

extract 1% (MO1%) had the highest antioxidant activities. On the contrary, the lowest antioxidant activity was observed in MO 0.5% extract (IC50=17.07 ±1.05) at all the tested concentrations. These results are comparable to those of **Punia *et al.*, (2020)**, who found that moringa leaves had an IC50 of 1.87 ± 0.03. Due to its capacity to donate electrons quickly, DPPH, a nitrogen-centered radical with a distinctive absorbance at 517 nm, will typically react with antioxidants to form 1,1,-diphenyl-2-picryl hydrazine **(Huang *et al.*, 2005)**.

The level of discolouration reveals the antioxidant extracts' capacity for scavenging. Free radicals are known to cause unsaturated lipids in meals to autooxidize **(Shimada *et al.*, 1992)**. Antioxidants, on the other hand, are thought to stop the free radical chain of oxidation and transfer electrons from the phenolic hydroxyl groups, creating a stable end product that doesn't start or spread additional lipid oxidation **(Suzuki *et al.*, 2002)**. Ascorbic acid, limonoids, carotenoids, and flavonoids are among the chemical components that may differ in the antioxidant potential of various extracts (Figs. 13).

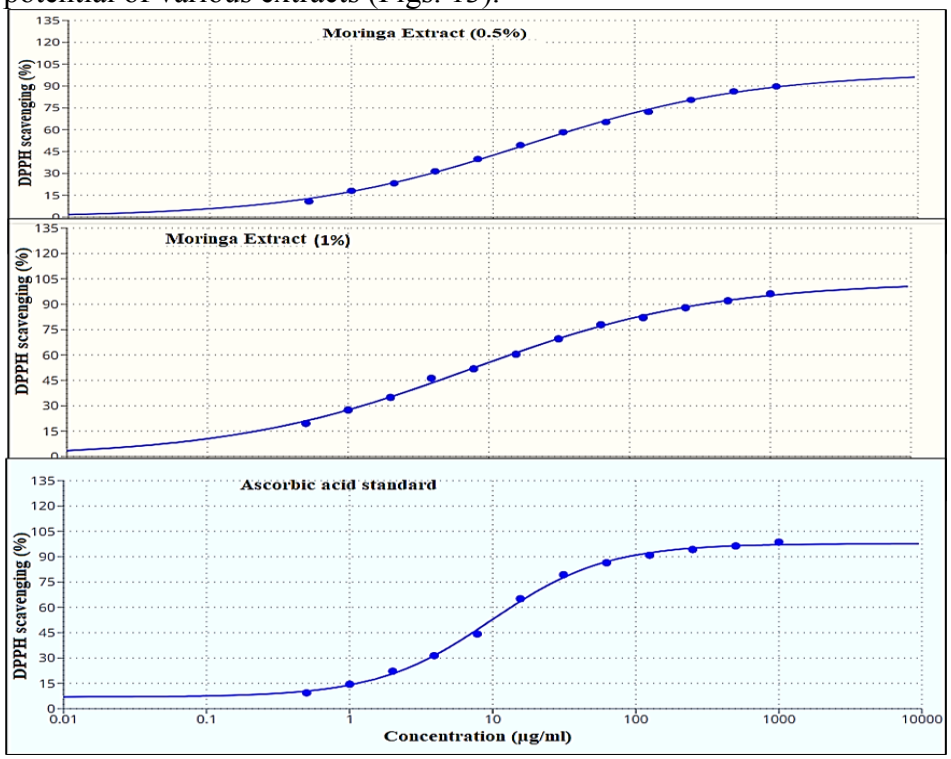


**Fig. 13:** Radical scavenging activity of different treatments by DPPH method at different levels

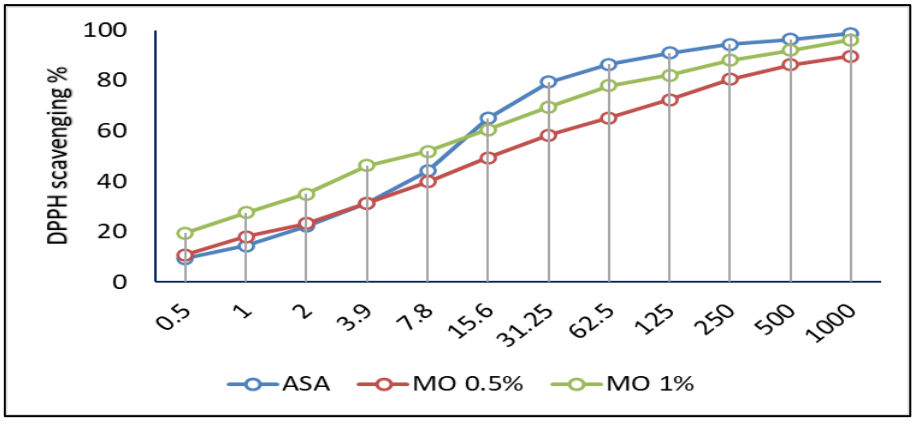


**Fig. 14:** Radical scavenging activity of different extracts by DPPH method at different concentrations.

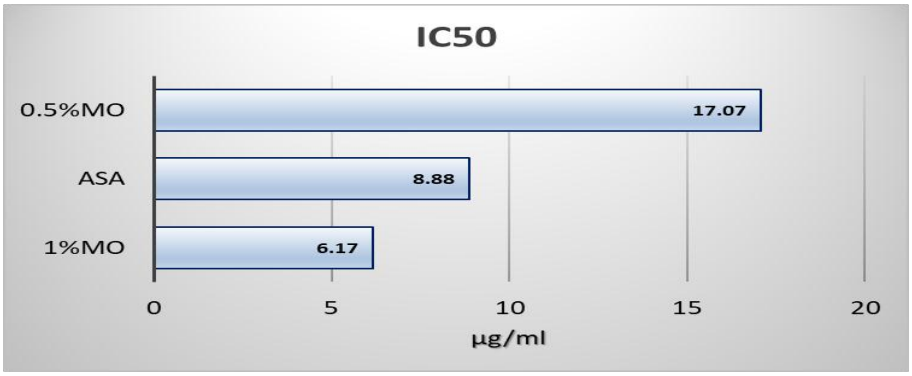


**Fig. 15:** DPPH (IC50 (µg/ml) at different treatments
ASA: ascorbic acid, MO: moringa leaf extract

Antimicrobial activity: Table 4 and Figure 16 show the antimicrobial effects of *Moringa oleifera* leaf (both 0.5 and 1% ethanol extracts) against two gram-positive bacterial strains (*Staphylococcus aureus* RCMB 25923 and *Bacillus subtilis* RCMB 015 (1) NRRL B-543), two gram-negative bacterial strains (*Escherichia coli* ATCC 25922 and *Proteus vulgaris* RCMB 004 (1) ATCC 13315), as well as two fungal strains, namely a mould (*Aspergillus fumigatus*, RCMB 002008) and a yeast (*Candida albicans*, RCMB 005003 (1) ATCC 10231). With (MO-1%+CHNPs), the highest zone of inhibition was observed for *Escherichia coli* at 32.8 ± 2.17 mm, *Aspergillus fumigatus* at 29.4± 3.98, *Bacillus subtilis* at 28.62 ± 5.54 mm, and *Staphylococcus aureus* at 26.88 ± 6.34 mm.

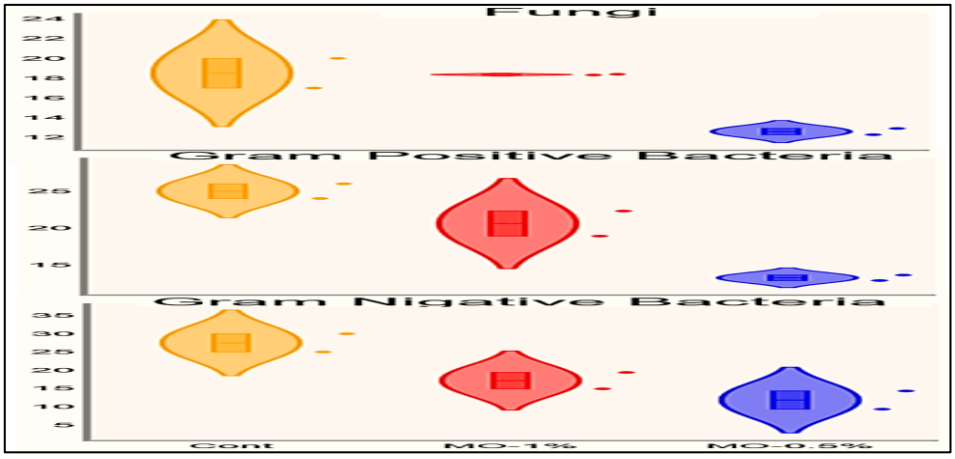


**Fig.16:** Violin pox of antimicrobial activity of moringa extracts (mean±SD) on overall gram negative, gram positive and fungi

Finally, moringa 1% has significantly inhibition effects on most studies pathogenic microorganism comparing to the corresponding control for each group followed by moringa at 1% alone on fungi.
While, both MO at 0.5% the results were not promising because they gave (12.9 mm), (12.28 mm), (13.6 mm), (12.86 mm), (14.3 mm) and (9.3 mm) for *Aspergillus fumigatus, Candida albicans, Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Proteus vulgaris*, respectively.

**Table 4. Antimicrobial activity of moringa extracts (mean±SD)**

| Microbial Strain | Diameter of inhibition zones (mm) | | | |
|---|---|---|---|---|
| | MO-0.5% | MO-1% | Control | LSD |
| **Fungi** | | | ***Ketoconazole*** | |
| ***Aspergillus fumigatus (RCMB 002008)*** | 12.9 ± 3.11 | 18.4 ± 3.63 | 17 | 1.47 |
| ***Candida albicans RCMB 005003 (1) ATCC 10231*** | 12.28 ± 2.95 | 18.32 ± 3.36 | 20 | 2 |
| **Gram Positive Bacteria:** | | | ***Gentamycin*** | |
| ***Staphylococcus aureus ATCC 25923*** | 13.6 ± 2.38 | 22.3 ± 2.86 | 24 | 1.9 |
| ***Bacillus subtilis RCMB 015 (1) NRRL B-543*** | 12.86 ± 1.64 | 18.9 ± 2.45 | 26 | 1.68 |
| **Gram Negative Bacteria:** | | | ***Gentamycin*** | |
| ***Escherichia coli ATCC 25922*** | 14.3 ± 3.8 | 19.4 ± 2.1 | 30 | 2.41 |
| ***Proteus vulgaris RCMB 004 (1) ATCC 13315*** | 9.3±1 | 14.9 ± 1.11 | 25 | 2.1 |

The present results are agree with the findings of **Kapoor (1999)** who reported increase in the diameter zone of inhibition with increase in concentration of Moringa oleifera. According to **Napoleon *et al.*, (2009)**,

*Escherichia coli*, *Staphylococcus typhi*, and *S. aureus* were all susceptible to 200 mg ml-1 of *M. oleifera* seed ethanol extract. According to **Bako *et al.*, (2010)**, *Bacillus subtilis, Bacillus cereus, Proteus mirabilis, Staphylococcus pneumonia, and Esherichia coli* were all inhibited by the crude extract of *M. oleifera*. According to **Bukar *et al.*, (2010)**, this might be because of the natural phytochemicals (alkaloids, saponins, tannins, flavonoids, steroids, phenols, phylobatannin, etc.) and the ease with which they dissolve in ethanol as opposed to water. This was in line with research by **Gundogan *et al.*, (2006) and Malu *et al.*, (2009),** who found that ethanol extracts of herbs and spices had the strongest inhibitory impact because the bioactive ingredients were more soluble in organic solvents than in water. The meat industry may thus take advantage of *Moringa oleifera* leaves' potential as a naturally occurring plant-based preservative to reduce bacterial contamination (**Liga *et al.*, 2025**), even though the antibacterial efficacy of various extraction methods varies.

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## تقييم المحتوى الفينولي والنشاط الحيوي لمستخلص أوراق المورينجا

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إن استخدام مضادات الأكسدة الطبيعية الجديدة ومضادات الميكروبات في الأطعمة أمرٌ مُلِحّ، لأن بعض مضادات الأكسدة ومضادات الميكروبات الإصطناعية لها آثار ضارة ومُطَفِّرة ومسرطنة.

**يمكن تلخيص أهم نتائج هذا البحث على النحو التالي:**

1. **التركيب الكيميائي لأوراق المورينجا:-**

• وفقًا لوزنها الجاف، تحتوي أوراق المورينجا على التركيب الكيميائي التالي: 6.9% رطوبة، و24.1% بروتين خام، و7.19% دهن خام، و15.23% رماد كلي، و6.29% ألياف، و40.32% كربوهيدرات.

2. **تأثير اختلاف نسب المذيبات الإيثانولية على النشاط المضاد للأكسدة في أوراق المورينجا:-**

• وفقًا للنتائج، سجّل المستخلص الإيثانولي 70% أعلى نسبة DPPH، وهي أعلى بقليل من نسبة المستخلص الإيثانولي 100% (بلغت نسبة DPPH للمستخلص 70% 91.59%، وللمستخلص 100% 91.47%). كان كلا النشاطين المضادين للأكسدة مماثلين لنشاط حمض الأسكوربيك. وفقًا لهذه النتائج، فإن المذيب الأمثل لإستخلاص مكونات مضادات الأكسدة من أوراق المورينجا هو 70٪ من الإيثانول.

**٣- المركبات الفينولية والفلافونويدية الكلية ونشاط مضادات الأكسدة في أوراق المورينجا:-**

• تم الحصول على أعلى إنتاجية (٦٤,٤٩٧ ± ٠,٤٦٥ ملجم من GAE/جم من المستخلص أو ٦,٤٥٠ ± ٠,٤٦٥ جم من GAE/١٠٠ جم من المستخلص) من حساب الفينول الكلى لمستخلص أوراق المورينجا الإيثانولي (MO ١٪)، بالإضافة إلى مستخلص المورينجا (٠,٥٪) والذى انتج ٤٢,٠٢٧ ± ٠,٨٢٦ ملجم من GAE/جم)

• أظهر مستخلص أوراق المورينجا الإيثانولي (1%) أعلى كمية من الفلافونويدات (30.86 ± 0.248 ملجم QE/جم من المستخلص)، يليه MO 0.5%، (24.067 ± 0.179 ملجم QE/جم)

• تم تقييم قدرة المعاملات المختلفة على التخلص من الجذور الحرة بناءً على قدرتها على التخلص من DPPH الاصطناعي. يُستخدم حمض الأسكوربيك للمقارنة مع المعاملات (المورينجا).

4. **استخدام جهاز HPLC لتقدير المركبات الفينولية في مستخلص أوراق المورينجا كميًا:-**

- أظهر تحليل HPLC لمستخلص أوراق المورينجا وجود 20 مركبًا فينوليًا تتراوح نسبتها بين <LOQ و 28.12%.
- بمقارنة مدة بقاء المواد الكيميائية وفقًا للمعايير الأصلية، تبيّن أن المكونات المعترف بها حمض الكلوروجينيك ، وحمض الجالك، والنارينجين، والفانيلين هي أكثر المواد الكيميائية وفرةً في مستخلصات المورينجا (مع اختلافات في كميتها). بينما تراوحت النسب المئوية المنخفضة للمواد الكيميائية الأخرى بين 2.81% وأقل من 0.17%.
- أظهرت خمسة من أصل عشرين مركبًا، وهي حمض الكوماريك، وحمض السيناميك، والديزين، والكاتشين، والهسبريتين أن أحماض الجالك والكلوروجينيك، ذات الخصائص المضادة للفطريات والفيروسات ومضادات الأكسدة، تُشكل غالبية المكونات الفينولية النشطة الموجودة في أوراق المورينجا أوليفيرا. وهذا قد يُساعد في منع الضرر التأكسدي.
- بالإضافة إلى ذلك، تُعدّ الأوراق غنيةً بالفلافونويدات، والتي تشمل البروتين، والكامبفيرول، والكيرسيتين. تشتهر هذه المركبات بتأثيراتها المضادة للالتهابات، والحساسية، والسرطان، والتشنجات.