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The synergistic effects of *Moringa Peregrina* and *Murraya koenigii* leaf extracts on antimicrobial activity (against *Candida albicans* and *Helicobacter pylori*), antioxidant, and lipase inhibitory properties

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ABSTRACT

This research aimed to evaluate the antimicrobial, antioxidant, and lipase inhibitory properties of leaf extracts from *Moringa peregrina* (*M. peregrina*) and *Murraya koenigii* (*M. koenigii*). Antioxidant capacity was assessed using the ABTS method, while total phenol content was measured via the Folin-Ciocalteu method. The antifungal activity against *Candida albicans* was determined through zone inhibition assays, and the ability to inhibit *Helicobacter pylori* was evaluated by measuring urease enzyme activity. Lipase activity inhibition was assessed using ELISA at 405 nm. Analysis of variance revealed that increasing concentrations of both extracts significantly enhanced antioxidant activity and total phenol content ($p<0.05$). *M. peregrina* and *M. koenigii* exhibited the highest antioxidant activity at 4000 mg/L, while *M. koenigii* showed the lowest activity at 31.25 mg/L. The highest total phenol content occurred at 4000 mg/L for *M. koenigii*. Synergistic effects of both extracts were noted at 1000 mg/L. At 1500 mg/L, *M. peregrina* displayed the largest inhibition zone against *Candida albicans*; conversely, *M. koenigii* showed the strongest inhibition of *Helicobacter pylori* at 2000 mg/L. The greatest lipase inhibitory activity was found at 4000 mg/L for *M. peregrina*, while *M. koenigii* had the least at 2000 mg/L, and there were no synergistic effects. These findings suggest that *M. peregrina* and *M. koenigii* leaf extracts have potential applications in developing anti-diabetic food products.

Keywords: Antibacterial activity; Antioxidant; Lipase inhibitory; *Murraya koenigii*; *Moringa peregrine*.

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1. Introduction

Currently, there is a growing trend towards the use of alternative medications, such as natural or herbal remedies, due to their potential for reduced side effects or toxicity associated with their natural composition. Herbal compounds are increasingly recognized as viable substitutes for synthetic additives in food products due to their antioxidant and antimicrobial properties (Al Harbi et al., 2016). Natural sources of lipase inhibitors are particularly valued for their structural diversity, low toxicity, and wide range of availability. These compounds have been utilized in gastrointestinal, bloodstream, and central nervous system applications without any evident side effects. Pancreatic lipase inhibitors primarily play a role in lipid metabolism by preventing the hydrolysis of macromolecules and thereby reducing the absorption of smaller molecules and fat accumulation (Liu et al., 2020).

To minimize oxidative degradation, antioxidants can be utilized during the production process. The demand for potent antioxidants with lower toxicity and enhanced efficacy in the food industry is increasingly recognized as a critical necessity. As a result, researchers and the food industry are directing their focus toward natural antioxidants (Al Harbi et al., 2016).

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Due to the emergence of antibiotic resistance, there is a rising need to explore new antimicrobial compounds. *Helicobacter pylori* is found in the stomachs of nearly 50% of humans and leads to various gastrointestinal disorders (Mahfuza Shaplaa et al., 2018; Baltas et al., 2016).

Candida albicans is a pathogen; in the general population, the prevalence of oral *Candida albicans* is approximately 75%, and weakened immune system significantly (Gutierrez et al., 2020; Mayer et al., 2013). *Candida albicans* is recognized as the most common fungal pathogen in humans (Zida et al., 2017).

Moringa peregrina (Fiori), is a plant belonging to the Moringaceae family, which consists of 14 species (Chelliah et al., 2017). This species is indigenous to Africa and Asia (Vergara-Jimenez et al., 2017). The *M. peregrina* plant thrives in arid regions, particularly in northeastern Africa, southwestern Asia, and southeastern Iran. In Iran, this tree is observed extensively in Sistan and Baluchestan and Hormozgan provinces. The seeds of *M. peregrina* typically comprise approximately 33% oil, 38% protein, 16% carbohydrates, 5% fiber, and 8% moisture, which render them beneficial for various industries including pharmaceuticals, cosmetics, and food (Abdulkarim et al., 2005). The oil extracted from the seeds has diverse applications, including cooking, soap making, cosmetics, and fuel. The leaves can be utilized as fertilizer, and powdered seeds have been employed to ameliorate bacterial skin infections (Khanna et al., 2015). *M. peregrina* has significant interest among researchers due to its high nutritional content such asvitamins A and E, and amino acids (Lalas, Stavros et al., 2017; Ching & Mohamed, 2001).

The ethanolic extract of *M. peregrina* leaves has been found to contain polyphenols, including quercetin glycosides, kaempferol glycosides, and chlorogenic acid, flavonoids, tannins, anthraquinones, glycosides, alkaloids, saponins, triterpenoids, and reducing sugars (Fatma et al., 2013; Siddhuraju, & Becker 2003; Monica et al., 2015). Various studies have validated the therapeutic effects of *M. peregrina*, including anti-inflammatory and analgesic properties from its root (Koneni et al., 2009). Hepatoprotective and antibiotic properties, chemotherapeutic effects against colorectal cancer cells, anti-inflammatory, antispasmodic, diuretic effects, cholesterol-lowering capabilities, antioxidant properties, and antifungal activity (Farooq et al. 2012; Pari & Ashok Kumar, 2002). The leaves of Moringa are primarily used for medicinal purposes, as well as for human nutrition, due to their richness in antioxidants and other nutrients that are commonly deficient in populations residing in developing countries. Moringa leaves have been applied in the treatment of various ailments, including malaria, typhoid fever, hypertension, and diabetes (Sivasankari et al., 2014). The roots, bark, gum, leaves, pods, flowers, seeds, and oil of *M. oleifera* possess a range of biological activities, including protection against gastric ulcers (Pal et al., 1995), anti-diabetic effects (Oyedepo et al., 2013), blood pressure reduction (Faizi et al., 1998), and anti-inflammatory properties (Rao & Mishra, 1998). *M. koenigii* leaves are a perennial plant from the Rutaceae family, rich in minerals and vitamins A and B, serve as a good source of amino acids, proteins, carbohydrates, and alkaloids (Tee & Lim, 1991). They possess multiple medicinal properties, including anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anti-cancer, and hepatoprotective qualities. The leaves have a mildly pungent, bitter, and acidic flavor that retains these characteristics even after drying (Omankutty et al., 1984). The aqueous part of the plant is non-toxic and is evaluated for its hypoglycemic activity (Kesari et al., 2005). Reportedly, curry species are widely utilized in traditional medicine throughout East Asia, Australia, and Southern Africa. In traditional systems, this plant is recognized for its analgesic, antifungal, anti-inflammatory, antidiarrheal, antipyretic, and soothing properties, and is used to lower body temperature, treat blood disorders, combat dysentery, reduce inflammation, relieve itching, control vomiting, and manage diabetes (Dhongade et al., 2013).

Essential oils of Moringa have high antioxidants (Tachibana et al., 2003; Ningappa et al., 2008), and antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi (Chowdhury et al., 2001).

There is a lack of comprehensive studies examining the antimicrobial and lipase-inhibitory effects of herbal combinations, particularly in the context of *Candida albicans* and *Helicobacter pylori*. Although individual bioactivities (antioxidant, antimicrobial, lipase inhibition) of *Moringa peregrina* and *Murraya koenigii* have been studied, no prior studies have evaluated the synergistic interaction between these two plant species. So far, limited research has addressed the antioxidant, antimicrobial properties against *Candida albicans* and *Helicobacter pylori*, or the inhibition of lipase enzyme aimed at evaluating the reduction of triacylglycerol absorption and weight loss. Despite the medicinal and nutritional value of these plants, no prior research has explored their combined potential as functional food ingredients, particularly in the prevention of infections and obesity-related issues. Link these bioactivities to potential applications in functional foods aimed at improving gastrointestinal health and reducing fat absorption. The study integrates three biofunctional assessments (antioxidant, antimicrobial, enzyme inhibition) within a synergistic herbal context, offering a multifunctional approach for food and pharmaceutical development. Furthermore, no studies have been conducted on the synergistic effects of these two plant extracts. The present study aims to investigate the antimicrobial, antioxidant, and lipase-inhibitory properties of the extracts from curry leaves and *M. peregrina*, as well as the synergistic effects of these two herbal extracts with the goal of utilizing them in the production of functional food products.

2. Material and Methods

2.1. Extraction of extracts from the plants

The leaves of *M. peregrina* are dried in a dark place. The extract from powdered samples (particle size of 40 mesh) of *M. peregrina* and curry leaves was obtained using a solvent mixture of ethanol/water (70:30, v/v) in an ultrasonic extractor at a temperature of 50°C, for 30 minutes, at a frequency of 40 kHz, and with a solid-to-solvent ratio of 1:20 (w/v). To minimize solvent evaporation, glass containers with lids were utilized. The extract was filtered after extraction through Whatman filter paper No. 1, then concentrated and dried using a rotary evaporator at 40°C. The concentrated and dried extract was stored in light-protective containers at 4°C until further testing.

2.2. Antioxidant activity assessment

In the ABTS method, the ABTS cation was prepared using a stock solution of 7 mM ABTS and 45.2 mM potassium persulfate. This mixture was prepared 12 to 16 hours prior to use and kept in a dark environment at room temperature. The prepared ABTS cation solution was diluted with ethanol until achieving an absorbance of

approximately 70% at a wavelength of 734 nm. A volume of 100 µL of the sample solution was added to the diluted ABTS solution, and the absorbance was measured after five minutes. Antioxidant activity was calculated using the following formula (Eq. (1)):

$$ABTS\ (\%) = (1 - \frac{A}{A_0} \times 100) \quad (1)$$

where A and A0 are the absorbance values of the ABTS solution with and without the sample, respectively (Siddhuraju & Becker, 2003).

2.3. Determination of total phenolic content (TPC)

The total phenolic content in the extracts was determined using the Folin-Ciocalteu method. The reaction mixture contained 0.025 mL of extract, 0.125 mL of Folin-Ciocalteu reagent, 1.975 mL of diluted water (1:10), and 0.375 mL of 7.5% sodium carbonate. The compounds were kept in the dark at ambient conditions for 2 hours to complete the reaction. Absorbance was measured at 760 nm using a spectrophotometer. The results were expressed as equivalents of gallic acid (g/100 g extract) (Carloni et al., 2012; Iqbal & Bhanger, 2006).

2.4. Assessment of antifungal activity against Candida albicans

The standard strain of *Candida albicans* ATCC 10231 was obtained in lyophilized form from the Iranian Research Organization for Science and Technology. To activate the strains, procedures for each microorganism were followed. The antifungal activity was evaluated using the paper disc diffusion method. After standardizing the microbial population (approximately 10^8 CFU mL^{-1}), the culture was inoculated onto the medium. Sterile discs with a diameter of 6 mm were soaked in the raw extract solution, dried, and placed on the surface of the SDA medium containing chloramphenicol. The samples were incubated at 4°C for 2 hours and then at 37°C for incubation. Following incubation, the antimicrobial activity was assessed by measuring the diameter of the inhibition zone (Mothana et al., 2009).

In sterilized 96-well plates, serial double dilutions of the selected raw extracts in 5% DMSO broth were prepared to create a concentration range of 1000 to 7.8 µg/mL. A suspension of microbial cells at 1.5 McFarland standards (1.5×10^8 CFU mL^{-1}) was added to each well. Control cultures without extract were also placed in the plates. The plates were incubated for 18 hours at 37°C. The minimum inhibitory concentration (MIC) of the raw extract was subsequently determined, and the endpoint where no growth is observed (Kaewpiboon et al 2012).

2.5. Evaluation of Helicobacter pylori inhibition

The inhibitory capacity against *Helicobacter pylori* in the samples was determined using urease enzyme inhibition. This method is based on the release of ammonium through the Berthelot reaction, and absorbance was measured at 625 nm. The reaction mixture consisted of 850 µL of urea, 50 µL of urease, and 100 µL of an inactivated bacterial suspension in phosphate-buffered saline with sodium phosphate at pH 7.4, to reach a final volume of 985 µL. After an initial incubation for 30 minutes at 30°C, the reaction was completed by the sequential addition of 500 µL of solution A (containing 4.47 g salicylic acid, 2.5 g sodium hydroxide, and 20 mg sodium nitroprusside in 50 mL distilled water) and solution B (containing 1.5 mL chlorinated water and 0.5 g sodium hydroxide in 70 mL distilled water). The resultant mixture was incubated at 37°C for 30 minutes to develop the color, and the percentage of inhibition in the samples was calculated using the following equation (Eq. (2)) (Nabati et al., 2012):

$$I\ (\%) = (1 - \frac{T}{C} \times 100) \quad (2)$$

where I indicates enzyme inhibitory activity, T is the absorbance of the test sample in the presence of the enzyme, and C is the absorbance of the sample without inhibitor in the presence of the enzyme.

2.6. Lipase activity inhibition assessment

0.005 g of lipase enzyme was added to 1 mL of phosphate-buffered saline and mixed using a vortex. A mixture consisting of 40 µL of an inactivated bacterial powder suspension in phosphate-buffered saline, 240 µL of phosphate-buffered saline, 6 µL of substrate, and 4 µL of enzyme was poured into an ELISA plate. All samples were incubated for 30 minutes at 37°C, after which absorbance was read at a wavelength of 405 nm using the ELISA reader. Lipase inhibitory activity was calculated using the following equation (Eq. (3)) (Kaewpiboon et al., 2012):

$$AI\ (\%) = (\frac{A - A_0}{A_0} \times 100) \quad (3)$$

where AI indicates the percentage of lipase activity inhibition, A is the absorbance of the solution without inhibitor, and A_0 is the absorbance of the solution containing an inhibitor.

2.7. Data analysis

The statistical population included hydroalcoholic extracts from two plant samples (*M. peregrina* and *M. koenigii*) at different concentrations (0.25, 0.5, 0.75, and 1 percent). Sampling was carried out randomly, resulting in a total sample volume of 24 (8 treatments) based on three replications. Initially, a normality test was conducted on the data, and due to the parametric nature of the data distribution, one-way analysis of variance (ANOVA) tables were employed for comparison among treatments. Subsequently, Duncan's multiple range test was applied for mean comparison at a significance level of 0.05, using SPSS software. For pairwise comparisons, the Independent Samples t-Test was applied at a significance level of 0.05.

3. Results and Discussion

3.1. Antioxidant properties

Table 1 shows the antioxidant properties of *M. peregrina* and *M. koenigii* leaf extracts, while Table 2 compares the synergistic effects of *M. peregrina* and *M. koenigii*extracts. Increasing the concentration of each treatment significantly affected the antioxidant activity ($p < 0.05$). The highest antioxidant activity was observed at concentrations of 1000, 2000, and 4000 mg/L for *M. peregrina* and

at 4000 mg/L for curry ($p < 0.05$). Furthermore, results showed no significant difference between the concentrations of 1000, 2000, and 4000 mg/L for *M. peregrina* ($p \geq 0.05$). The lowest antioxidant activity for *M. peregrina* and curry was recorded at 31.25 mg/L ($p < 0.05$). The most pronounced synergistic effects between *M. peregrina* and *M. koenigii*were reported at a concentration of 1000 mg/L ($p < 0.05$). In pairwise comparisons, results indicated that *M. peregrina* exhibited higher antioxidant properties than *M. koenigii* ($p < 0.05$). The highest synergistic effects of *M. peregrina*-and *M. koenigii* were reported at a concentration of 1000 mg/L.

Table 1. Comparison of antioxidant properties (percentage inhibition of ABTS free radical) of *Moringa peregrina* and *Murraya koenigii*.

Treatment number	Concentration (mgL^{-1})	Moringa	Murraya
1	4000	88.01 ± 3.07^{a}	86.10 ± 6.50^{a}
2	2000	89.59 ± 1.55^{a}	73.08 ± 7.83^{b}
3	1000	87.45 ± 0.11^{a}	42.44 ± 3.22^{c}
4	500	58.74 ± 0.56^{b}	36.74 ± 0.23^{d}
5	250	29.41 ± 3.01^{c}	13.49 ± 0.46^{e}
6	125	27.55 ± 1.34^{c}	10.64 ± 0.12^{f}
7	62.5	23.23 ± 2.09^{d}	3.68 ± 0.97^{g}
8	31.25	14.48 ± 0.83^{e}	1.40 ± 0.14^{h}

Values with different superscripts on the same column are significantly different ($p < 0.05$).

Table 2. Synergistic antioxidant effects of *Moringa peregrina* and *Murraya koenigii*.

Treatment number	Concentration (ppm)	Moringa	Murraya	Moringa + Murraya
3	1000	87.45 ± 0.11^{a}	42.44 ± 3.22^{a}	40.90 ± 0.64^{a}
4	500	58.74 ± 0.56^{b}	$36.74\pm$ al0.23^{b}	18.87 ± 3.51^{b}

Values with different superscripts on the same column are significantly different ($p < 0.05$).

These findings align with the results obtained from the phenolic compounds present in these plants. The bioactive compounds in Moringa include phenols and flavonoids such as quercetin and kaempferol, which demonstrate potential antioxidant activity (Lin et al., 2018; Mensah et al., 2012; Siddhuraju, & Becker, 2003). Additionally, bioactive compounds such as phenols and flavonoids in *M. koenigii* leaves also possess antioxidant properties (Igara et al., 2016; Ghasemzadeh, et al., 2014). The high levels of phenolic compounds in the extracts are responsible for their strong antioxidant characteristics and play a significant role in preventing the autoxidation of oils. As the concentration of phenolic compounds increases, the presence of hydroxyl groups enhances the likelihood of hydrogen donation to free radicals, consequently increasing the ABTS radical scavenging capacity. It is noteworthy that not all phenolic compounds may possess the ability to scavenge free radicals or exhibit antioxidant activity (Mahdavi et al., 1996). Lin and colleagues (2018) reported that Moringa contains flavonoids with anti-inflammatory and antioxidant effects (Lin et al., 2018). Tripathi et al. (2018) confirmed the antioxidant activity and free radical scavenging capability of *Murraya koenigii* leaf essential oil. The radical scavenging ability greatly depends on the number and position of hydroxyl groups, and the molecular weight of the phenolic compounds (Mahdavi, et al., 1996). Lamou et al. (2016) measured the effects of *M. oleifera* aqueous extract on parameters of oxidative stress (superoxide dismutase, catalase, glutathione, and malondialdehyde) in mice, observing increased antioxidant enzyme activity and reduced blood malondialdehyde concentration. Iqbal and Bhanger (2006) demonstrated that season and growing location have a significant impact on the antioxidant activity of *M. oleifera leaves*. Rajendran et al. (2014) validated the antioxidant activity of essential oils from *M. koenigii* leaves sourced from the southern Tamil Nadu region of India. In phenolic compounds with lower molecular weights, hydroxyl groups are more readily accessible (Mahdavi et al., 1996).

Phenolic compounds exert their antioxidant activity through various mechanisms, including the termination of chain oxidation reactions, degradation of hydroperoxides, and binding with metal ions. Polyphenols have the ability to trap free radicals, thereby terminating the cycle of oxidation reactions. Phenolic compounds can readily transfer a hydrogen atom to free radicals, producing more stable and less reactive radicals. Additionally, it has been established that phenolic compounds can neutralize radicals by electron transfer (Mahdavi, et al., 1996).

Similar results to those observed in the current study have been reported by other researchers. Siddhuraju and Becker (2003) showed that *M. oleifera* leaf extracts could scavenge proxyl and superoxide radicals, as well as DPPH radicals. Methanolic and ethanolic extracts exhibited the highest antioxidant activity in the β-carotene-linoleic acid system. Furthermore, Ningappa et al. (2008) indicated that *M. koenigii* could serve as a natural antioxidant source and inhibit DPPH and hydroxyl radicals, lipid peroxidation, and superoxides. Das et al. (2011) reported that *M. koenigii* leaf powder at a concentration of 0.2% was a highly effective inhibitor of primary and secondary oxidation products in raw and cooked goat meat, serving as a natural antioxidant in both raw and cooked meat systems. Erkan et al. (2012) noted that DPPH radical scavenging activities of *M. koenigii* leaf essential oil were relatively low, whereas Kaewpiboon et al. (2012) affirmed the antioxidant activity of Moringa extract through DPPH radical inhibition. Patil et al. (2012) concluded that the methanolic extract of *M. koenigii* leaf (due to its high antioxidant properties) has significant potential for therapeutic and preventive applications (Patil, et al., 2012; Ghasemzadeh et al., 2014).

3.2. Total phenolic content (TPC)

Table 3 illustrates the total phenolic content of the two plant species, *Moringa* and *M. koenigii*, while Table 4 compares the synergistic effects of the extracts of *M. peregrina* and *M. koenigii*. The increase in concentration significantly affected the total phenolic content ($p < 0.05$). The highest total phenolic content was recorded at a concentration of 4000 mg/L for the *M. koenigii* plant, while the lowest total phenolic content was observed in the *M. koenigii* sample at a concentration of 75 mg/L ($p < 0.05$). The highest synergistic effects of *M. peregrina-M. koenigii* were reported at a concentration of 1000 mg/L ($p < 0.05$).

In the present study, the highest and lowest total phenolic contents were observed at concentrations of 4000 and 75 mg/L, respectively, for the *M. koenigii*. The highest synergistic effects of *M. peregrina-M. koenigii* were noted at a concentration of 1000 mg/L. Any compound that contains at least one aromatic ring connected to a hydroxyl group can be classified as a phenolic compound. Given their wide range of beneficial biological effects, including antioxidant properties, phenols hold significant applications in food, chemistry, pharmacy, and medicine. Phenolic compounds are secondary metabolites of many plants, especially medicinal plants (Mahdavi et al.,1996). The phenolic compounds in

M. peregrina and *M. koenigii* have been reported in various studies. Siddhuraju and Becker (2003) demonstrated that *M. oleifera* leaf extracts contain bioactive compounds such as phenols and flavonoids. Iqbal and Bhanger (2006) confirmed the total phenolic and flavonoid content of methanolic extracts of *M. oleifera* leaves in Pakistan. These compounds exhibit high antioxidant potential and are effective in removing and preventing the formation of free radicals. Specifically, these compounds can scavenge free radicals and facilitate the precipitation of oxidizing elements such as iron (Mahdavi, et al., 1996). Bamishaiye et al. (2011) identified nutritious elements such as phenolics and flavonoids in dried *M. oleifera* leaves. Mensah et al. (2012) demonstrated that dried powder of *M. oleifera* leaves is a good source of chemicals, secondary metabolites, and nutrients such as tannins, saponins, alkaloids, phenolics, flavonoids, and glycosides. The ability to donate electrons or hydrogen, form complexes with metals, and counteract radical activity is closely related to the number of hydroxyl groups present in the aromatic ring and their positioning (Mahdavi et al., 1996). Ghasemzadeh et al. (2014) confirmed the presence of bioactive compounds such as quercetin, catechin, epicatechin, naringenin, and myristin, as well as total phenol levels in the *M. koenigii* leaf extract from three different locations in Malaysia. Igara et al. (2016) verified the presence of active bioactive constituents, including flavonoids and phenolics in *M. koenigii* leaves, which exhibit antioxidant properties.

Table 3. Comparison of total phenolic content (mg Gallic acid/L) of *Moringa peregrina* and *Murraya koenigii*.

Treatment number	Concentration (ppm)	Moringa	Murraya
1	4000	33.33 ± 0.00^{a}	35.37 ± 0.00^{a}
2	2000	15.57 ± 0.00^{b}	16.80 ± 0.00^{b}
3	1000	10.57 ± 0.14^{c}	10.16 ± 0.14^{c}
4	500	6.39 ± 0.00^{d}	6.80 ± 0.00^{d}
5	250	2.51 ± 0.00^{e}	2.31 ± 0.00^{e}
6	125	2.31 ± 0.00^{f}	1.49 ± 0.00^{f}
7	62.5	2.00 ± 0.14^{g}	0.98 ± 0.14^{g}

Values with different superscripts on the same column are significantly different ($p < 0.05$).

Table 4. Synergistic effects of phenolic content (mg Gallic acid/L) of *Moringa peregrina* and *Murraya koenigii*.

Concentration (ppm)	Moringa	Murraya	Moringa + Murraya
1000	10.57 ± 0.14^{a}	10.16 ± 0.14^{a}	0.47 ± 0.00^{a}
500	6.39 ± 0.00^{b}	6.80 ± 0.00^{b}	0.00 ± 0.00^{b}

Values with different superscripts on the same column are significantly different ($p< 0.05$).

3.3. Antifungal activity against Candida albicans

Table 5 displays the antifungal properties of *M. peregrina* and *M. koenigii* against *Candida albicans*. The results of this study revealed that the highest zone of inhibition occurred at a concentration of 1500 mg/L for the *M. peregrina* plant. The antifungal activity of *M. peregrina* has been previously reported (Patel et al., 2014; El–Mohamedy & Abdalla, 2014). Further, the results showed that the concentration of 1500 mg/L significantly inhibited the growth of *Candida albicans* compared to *M. koenigii*.

In fact, the *M. koenigii* plant did not exhibit antifungal properties at this specific concentration. Previous studies have also reported the antifungal activity of Moringa (Patel et al., 2014; El–Mohamedy & Abdalla, 2014). Patel et al. (2014) confirmed that *M. oleifera* possesses antifungal activity against several fungi, including *Candida albicans* and *Candida tropicalis*. Both water and ethanol extracts of the leaves showed maximum activity and the largest inhibition zone against *Saccharomyces cerevisiae*. Tripathi et al. (2018) also confirmed the antifungal activity of the essential oil of *M. koenigii* leaves.

El-Mohamedy and Abdalla (2014) demonstrated that extracts from the roots, leaves, and pods of *M. oleifera* significantly inhibited the growth, germination of spores, and mycelial performance of *Fusarium oxysporum*, *Fusarium solani*, *Alternaria solani*, *Alternaria alternata*, and *Rhizoctonia solani*. Extracts of *M. oleifera* exhibited varying degrees of antifungal activity against the tested pathogens, with an increasing reduction effect on tested pathogens corresponding to the concentration of *M. oleifera* extract. *F. oxysporum*, *F. solani*, and *A. solani* were significantly affected by *M. oleifera* extract compared to *R. solani*, *S. rolfsii*, and *M. phaseolina*.

Table 5. Comparison of the diameter of the inhibition zone (mm) against *Candida albicans* in *Moringa peregrina* and *Murraya koenigii*.

Treatment number	Concentration (ppm)	Moringa	Murraya
1	1500	13.00 ± 0.01	-

3.4. Inhibitory activity against Helicobacter pylori

Fig. 1 present the inhibitory properties of *M. peregrina* and *Murraya koenigii* against *Helicobacter pylori*, and the synergistic effects of *the* extracts. Based on a previous study (data not shown), the highest inhibitory effects against *Helicobacter pylori* at a concentration of 4000 mg/L were attributed to both *M. peregrina*- and *Murraya koenigii*- ($p < 0.05$). The results of this research indicated that the highest inhibitory effect against *Helicobacter pylori* at a concentration of 2000 mg/L was attributed to the *Murraya koenigii* plant. The most significant synergistic effects of *Moringa - Murraya* were reported at a concentration of 500 mg/L. Similar results regarding the antibacterial properties of these two plants against other pathogenic bacteria have been previously reported. Walter et al. (2011) demonstrated that *M. oleifera* extract has inhibitory effects against *Salmonella typhimurium*, *Vibrio cholera*, and *Escherichia coli*. Mensah et al. (2012) reported that the zone of inhibition of dried *M. oleifera* leaf powder against *Staphylococcus aureus*, *Pseudomonas*, *Klebsiella*, and *Escherichia coli* was very minimal or completely absent.

Erkan et al. (2012) observed a complete inhibition of *Listeria innocua* growth at 400 and 600 µg/mL of *Murraya koenigii* leaf essential oil, demonstrating this plant's potential as a natural antimicrobial agent. Rajendran et al. (2014) confirmed the antimicrobial activity of essential oils from *Murraya koenigii* leaves from the southern Tamil Nadu region of India, with a maximum zone of inhibition against *Corynebacterium tuberculosis*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*. Al Harbi et al. (2016) showed the antibacterial effects of ethanol and methanol extracts of *Murraya koenigii* leaves against *Staphylococcus*, *E. coli*, *Streptococcus*, and *Proteus*. *Murraya koenigii* extracts exhibited antibacterial effects, particularly against *E. coli* and *Staphylococcus*, when compared to

Gentamicin and Amikacin. Kaewpiboon et al. (2012) assessed the antimicrobial activity of *M. oleifera* extracts against both Gram-positive and Gram-negative bacteria as well as yeasts. The strongest antimicrobial activity was observed against *Pseudomonas aeruginosa* and *Candida albicans*. Bhushan et al. (2020) studied the antibacterial activity of *Murraya koenigii* against *Escherichia coli*, *Staphylococcus aureus*, and subsequently *Bacillus subtilis* and *Pseudomonas aeruginosa*, indicating a moderate sensitivity of *Murraya koenigii* aqueous extract to all pathogens.

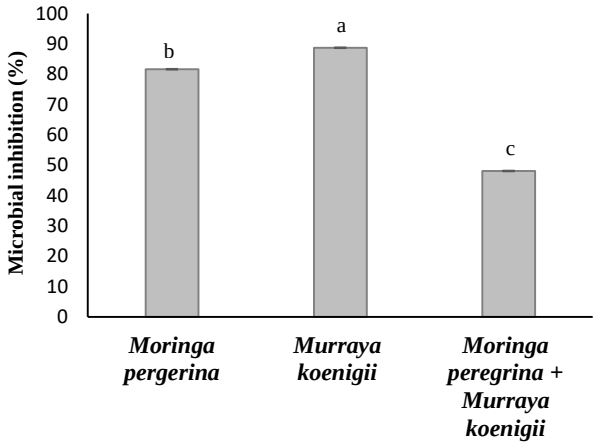

Fig 1. Inhibition of *Helicobacter pylori* of *Moringa peregrina*, and *Murraya koenigii* and synergistic effects of them. Different lowercase letters show significant differences ($p < 0.05$).

Overall, various essential oils and extracts demonstrate antibacterial effects against different microorganisms in food and laboratory models. The antibacterial activity of plant extracts is attributed to the presence of phenolic compounds, which are the primary constituents of the extracts. This property relates to the destructive effect of the essential oils on the lipid membranes of microorganisms, destabilizing the cytoplasmic membrane and leading to cell death. Furthermore, research indicates that different essential oils and extracts increase membrane permeability, allowing their constituents to dissolve in the membrane, causing swelling and reducing membrane functionality. Other factors that lead to membrane disruption and rupture include variations in pH, electrical potential, and alterations in ion transfer or depolarization, resulting in structural changes in the membrane, interference with the cell's energy production system (ATP), and inhibition of enzyme activity necessary for energy production. Although the occurrence of antimicrobial activity is often quite evident, its mechanism of action is not fully understood. Generally, plant-derived products lead to cytoplasmic granulation, rupture of the cytoplasmic membrane, inactivation or inhibition of the activity of intracellular and extracellular enzymes essential for microbial growth, and disruption of the cell wall. Different essential oils and extracts exert their antimicrobial effects by altering the structure and function of the cell membrane. Studies conducted regarding the mechanisms of action of various essential oils and extracts have shown that these compounds enhance membrane permeability. The components of the essential oils and extracts penetrate the membrane, leading to membrane swelling, which ultimately affects its function and results in cell death. The chemical compounds and the synergistic roles that the individual compounds play with other constituents have a significant impact on the antimicrobial and antioxidant activities of the plants (Burt, 2004; Noui Mehidi et al., 2024).

3.5. Inhibitory activity against lipase

Table 6 presents the lipase inhibitory properties of the two plants, *M. peregrina* and *M. koenigii*, *and* the synergistic effects of the extracts. The results indicated that the highest lipase inhibitory activity was found for *M. peregrina*, whereas the lowest inhibition was observed for *Murraya koenigii*. There is no significant synergistic effect for two extracts.

Lipase inhibitors can impair the enzyme's ability to break down fats, which may help control the intake of fats from the bloodstream for lipid-reducing effects (Liu et al., 2020). The results of this study showed that the highest lipase inhibitory activity was observed at a concentration of 4000 mg/L for *M. oleifera*, while the lowest inhibitory activity was noted at a concentration of 2000 mg/L for *Murraya koenigii* (data not shown). Kaewpiboon et al. (2012) confirmed the lipase inhibitory activity of *M. oleifera* extract. Inhibitory activity of lipase from other plant extracts has also been previously reported (McDougall et al., 2009; Kim et al., 2010).

Table 6. Comparison of lipase activity inhibition (%) by *Moringa peregrina* and *Murraya koenigii*.

Concentration (ppm)	Moringa	Murraya	Moringa + Murraya
2000	58.23 ± 0.05^{a}	49.05 ± 0.07^{c}	53.75 ± 1.06^{b}

Values with the different superscripts are significantly different ($p < 0.05$).

4. Conclusion

The findings of this study support the potential of *M. peregrina* and *M. koenigii* leaf extracts as effective agents for antimicrobial activity, antioxidant properties, and lipase inhibition. The synergistic effects observed between the two extracts highlight their potential to work collaboratively in enhancing health benefits, particularly against *Candida albicans* and *Helicobacter pylori*, as well as their capacity to reduce lipase activity, which is relevant for lipid metabolism and obesity management. The integration of these extracts into functional food formulations could be a significant advancement in dietary strategies for improving health outcomes and managing metabolic disorders. Their antimicrobial properties may serve as natural preservatives in food, extending shelf life and ensuring food safety without the adverse effects associated with synthetic additives. Moreover, their antioxidant capacity can contribute to the prevention of oxidative stress-related diseases. Such applications could be especially relevant in the food industry, where there is an increasing demand for natural and health-promoting ingredients.

The antimicrobial properties of the extracts make them suitable candidates for natural preservatives in various food products, potentially reducing the reliance on artificial additives. The lipase inhibitory effects suggest potential applications in weight management or obesity treatment products, and further research could lead to the development of herbal medicines or nutraceuticals.

Despite the promising results, there are notable limitations that must be addressed. The current study was conducted in vitro, and in vivo validation is crucial to confirm the effectiveness and safety of

these extracts. Further research is necessary to investigate the pharmacokinetics, optimal dosages, and potential side effects when consumed as part of a diet or supplement. Additionally, large-scale production and standardization of the extracts for consistent quality and efficacy will be essential for actual application in food or pharmaceutical products. Finally, research exploring the interactions of these extracts with other food components in complex matrices is needed to fully understand their utility in real-world applications. Essential oils and terpenoids in *M. koenigii* leaves may enhance membrane permeability of Gram-negative bacteria like H. pylori, making them more susceptible. The effectiveness against fungal pathogens like *Candida albicans* may be attributed to the high content of flavonoids and saponins, which impair fungal cell wall synthesis and promote membrane rupture.

M. peregrina also contains anthraquinones and tannins, known for their antifungal properties. While previous studies confirm the antimicrobial potential of both plants, the differential spectrum observed here may stem from variability in extract composition, extraction method, or synergistic interactions unique to the combined formulation. This highlights the need to standardize extract profiles and explore target-specific phytochemicals in future investigations.

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Conflict of interest

The authors declare that there is no conflict of interest.

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