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Usage of *Moringa Oleifera Leaves Extract* in Production of Hard Candy to Enhance the Efficiency of the Respiratory System

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MORINGA leaf extract products represent a promising alternative for treating respiratory infections. *Moringa oleifera* is a tree rich in various active phytochemicals with multiple health benefits. Antimicrobial property, particularly against pneumonia, is among the reported health benefits. The goal of this study is to produce hard candies containing *Moringa* leaf extract as a functional product after investigating in vitro how *Moringa* leaf extract affects lung cells and treats respiratory infections. According to the obtained data, no cytotoxic effect was reported during usage of *Moringa* leaf extract (50, 100, 150, and 200 µg/mL) on W138 cells, and cell viability increased gradually through the use of these concentrations. Also, concentrations of 150 and 200 µg/mL *Moringa* leaf extract showed antimicrobial activity against *Klebsiella pneumoniae, Streptococcus pneumoniae*, and *Candida pneumoniae.* According to the results of sensory evaluation on samples of *Moringa* hard candy, there is no significant difference($p < 0.05$) between the treated samples (A:1.8 mg *Moringa* leaf extract /100 g hard candy), (B: 2.7 mg *Moringa* leaf extract /100 g hard candy)and (C: 3.6 mg *Moringa* leaf extract / 100 g hard candy) and the control sample,with the exception of color and odor characteristics in samples C and A, respectively. Both the color and the order ranking have good acceptance.Finally, the development of *Moringa* hard candy appears to be a promising preventative product.

Keywords: *Moringa oleifera*, Antimicrobial activities, Hard candy as food functions.

Introduction

Some edible wild native plant varieties are considered an alternative source of functional food due to their high content of active substances required for public health.One of those trees is the *Moringa oleifera* Lam, which is indigenous to the Himalayas, India, Pakistan, Asia Minor, Africa, and Arabia. Since 1598, *Moringa oleifera* has been utilized in food processing. The nutritional, cosmetic, and therapeutic uses of tree parts include leaves, flowers, green pods, seeds, and roots(Ambawat et al., 2022).

Moringa oleifera (family: *Moringa ceae*) is considered a highly valued plant, having an impressive array of active substances with high medicinal uses and nutritional value. Different parts of this plant contain many important minerals. It is also a good source of protein, vitamins, B-carotene, various amino acids, and phenols. *Moringa* contains a distinct and abundant blend of zeatin and quercetin, as well as Kaempferol and a variety of other phytochemicalsthat have antimicrobial effects (Ambawat et al., 2022; Kinase, 2014). *Moringa* plant extracts are used as anti-tumor, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer, anti-spasmodic, hypotensive, cholesterol-lowering, antioxidant, anti-diabetic, anti-bacterial, and anti-fungal agents. *Moringa* is one of the most important plants that contain a high percentage of antioxidants, particularly in the leaves, and the antioxidants present in *Moringa* leaves include tannins, steroids, triterpenoids, flavonoids, saponins, quinones, and alkaloids. These antioxidants have the ability to reduce inflammation (Windari et al., 2021).

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Respiratory infection is one of the most important diseases for which researchers seek to provide effective treatment. A respiratory infection affects the respiratory tract, which is the part of the body in charge of breathing. These infections can cause problems in the sinuses, throat, lungs, and airways. Respiratory infections are classified into two types; upper and lower respiratory infections. The causative of these infections may be due to viral, bacterial or fungus agents. Viral infections are frequently associated with secondary bacterial infection and, depending on the severity of the infection, may progress to a fungal infection (Ivanova et al., 2022). From the most causative of respiratory infections was *Streptococcus pneumoniae*, also known as *pneumococcus*, which is an aero-tolerant anaerobic gram-positive spherical bacterium that is alpha-hemolytic (under aerobic conditions) or β-hemolytic (under anaerobic conditions). They are noticed in pairs (diplococci), do not produce spores, and are non-motile. *S. pneumoniae* was identified as a major cause of pneumonia in the late centuries and is the subject of numerous humoral immunity studies (Siemieniuk et al., 2011). A gram-negative, encapsulated, non-motile bacterium known as *Klebsiella pneumoniae* is present in the environment and has been linked to pneumonia in patients with diabetes mellitus or alcohol use disorder. The bacterium characteristicallycolonizes the oropharynx and gastrointestinal (GI) tract mucosa of humans. The bacterium can exhibit high levels of virulence and antibiotic resistance once it has entered the body. *K. pneumoniae* is the most common cause of hospital-acquired pneumonia, which is responsible for 3% to 8% of all nosocomial bacterial infections (Yahya, 2022). The oropharyngeal flora and upper respiratory tract normally contain *candida*. However, *Candida pneumonia* is a rare infection of the lungs that is frequently diagnosed as a result of a propagated *Candida* infection linked to risk factors in the clinical setting, such as prolonged antibiotic use, severe immunosuppressive conditions, or hematologic malignancies. *Candida pneumonia* cannot be distinguished from other types of *candidiasis* using a clinical predictive model or clear definition. Additionally, *Candida pneumonia* has a high mortality rate (Güntsch et al., 2006).The goal of this study was to assess the cytotoxicity of *Moringa* leaf extract on lung cells, as well as its effect on microorganisms that cause pneumonia, and to use it as a functional food suchas *Moringa Oleifera*hard candy.

Materials and Methods

Materials

The dried leaves of *Moringa Oleifera* plants were obtained from National Research Center, Giza, Egypt. Anti-microbial sensitivity test, IC_{50} assay, W138 fibroblast cells,HPLC analysis for quantification of phenolic acids were done in the Laboratory of the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. All media, chemicals, and reagents needed for the previous tests were provided by the Laboratory of the Regional Centre for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Sucralose, glucose syrup, mannitol, and citric acid were provided from local markets.

Methods

Preparation of dried Moringa leaves extract

Dried *M. Oleifera* leaves (200 g) were socked in 1000 mL of 80% ethanol for 24 h at 60 °C with stirring. The extract was centrifuged (model: DM0412, brand: DLAB, Russia) at 6500 x g for 10 min. at 4 °C and the supernatant was filtered through filter paper (No. 3 Whatman). The extract faltered solution was concentrated using a rotary evaporator (model: RE-501, brand: HNZXIB, China) under vacuum at 60 °C to remove ethanol (yield 18% weight/weight (w/w)). The sample was freeze dried (model: LY-1 ON, brand: MCGS, USA) and stored at 4 ºC until required.

Proximate Analysis and phytochemical screening of Moringa oleifera leave extract

Moisture (oven method at 105°C; AOAC, 934.01), crude protein (micro-Kjeldhal, N x 6.25; AOAC, 981.10), ash (550°C overnight; AOAC, 930.05), fat (Soxhlet extraction with diethyl ether; AOAC, 991.36), crude fiber (successive hydrolysis with 100°C 0.05 N H_2SO_4 and 0.05 N NaOH for 30 min each) were determined (AOAC 2000). TotalCarbohydrate content was calculated by difference (James 1996).Qualitative Determination of phytochemicals, alkaloids,tannins sterol, saponins and glycoside were determined by the method described by Vasini et al, (2023). Total phenol was determined by the formation of blue-green or black coloration due to the addition of 2 mL of the extract to 2 mL of ferric chloride (FeCl3 2%) solution., and the total flavonoid was determined using a mixture of 2 mL of extract with few fragments of magnesium ribbon and concentrated HClas described by Harborne, (1998).

HPLC analysis for phenolic acids and flavonoids compounds of Maringa Oleifera leaves extract

HPLC analysis for quantification of phenolic acidsand flavonoids extract were carried out Flavonoids were determined by HPLC according to the method of Abo El-Fadl et al., (2020). The Eclipse plus C18 column (4.6250 mm, i.d. 5 m) was used for the separation. At a flow rate of 1 mL/min, the mobile phase was composed of water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B). The mobile phase was programmed in

the following order: 0 min (80% A), 0-5 min (80% A), 5-8 min (40% A), 8-12 min (50% A), 12-14 min (80% A), and 14-16 min (80% A). At 280 nm, the multi-wavelength detector was monitored. The injection volume was ten microliters (series 1200). The column temperature was kept constant at 35 °C.

Cytotoxicity effect ofMaringa Oleifera leaves extract

The cytotoxicity study of *M. oliefera* leaf extract was conducted using the MTT assay. W138 fibroblast cells were planted in 96-well plates in DMEM supplemented with 10% FBS. The cells were grown in 96-well plates for the formation of a monolayer until 70–80% confluence was reached after an incubation period of 24 hours at 5% humidity and 37 °C. After having the desired cell density, *M. oliefera* leaf extract was given with the different dilutions at 50, 100, 150, and 200 µg/mL and was extra incubated for 24 h. The next day, 20 µL of MTT solution was added to each well, the plate was kept, and it was re-incubated for 3 hrs. 100 µL of DMSO was added to dissolve the formazon crystals. Later, the absorbance of the plate was measured at 570 nm by using 96-well microplate readers (Hegazi et al., 2019).

Antimicrobial sensitivity test Maringa Oleifera leaves extract

Different concentrations (50, 100, 150, and 200 µg/mL) of the extract were prepared by mixing with known concentrations of ethanol. Then they were loaded on discs prepared with Whatmann No. 1 filter paper. The discs were added to investigate the antimicrobial activity of the extract against clinical isolates grown in specific media, such as *Klebsiella pneumoniae, Streptococcus pneumoniae*, and *Candida pneumoniae* (Bauer, 1966; Güntsch et al., 2006).

Preparation of Moringa Oleifera hard candy

The main ingredients in the *Moringa* hard candy were precisely weighed amounts of sucralose, glucose syrup, mannitol, citric acid, and dried *Moringa* leaves extract, dosage was added according to the results obtained from cytotoxicity and antimicrobial sensitivity tests. Sucralose and glucose syrup were dissolved in water to create a candy base, which was then used to make hard candy. The temperature of the candy was raised between 145 and 156 °C, it was allowed to cool, and then acidulate and flavoring were added during mixing, followed by *Moringa* extract. The candy mass was put into the molds. The weight of the candy pieces was verified, and then they were separately packaged (Luetragoon et al., 2021).

The formula of the control hard candy: sucralose 30%, glucose 30%, mannitol 9%, citric acid 1%, and water 30%.

The formula of Moringa Oleifera hard candy (three treatments A, B and C): sucralose 30%, glucose 30%, mannitol 9%, citric acid 1%, water 30%, and dried *Maringa*extract was dissolved in the water in 3 treatments according to the results obtained from cytotoxicity and antimicrobial sensitivity tests. (A: 1.8 mg*Moringa* leaf extract /100 g hard candy), (B: 2.7 mg*Moringa* leaf extract /100 g hard candy) and (C: 3.6 mg*Moringa* leaf extract / 100 g hard candy)

Sensory evaluations of Moringa Oleifera hard candy

Hard candy samples were evaluated organoleptically by 10 panelists according to Bajaj et al., 2006. The tested characteristics including: color, odor, taste, flavor, and overall accessibility. All sensory items were evaluated through 10 marks.

Statistical analysis

Results were analyzed by (ANOVA) using SAS (1999) statistical package of the general linear model (GLM). The results average was based on three-replicates ($p \leq 0.05$) (SAS Statistical Analysis System, 1999).

Results and Discussion

Proximate Analysis and phytochemical screening of Moringa oleifera leaves

Data represented in Table 1 illustrate the approximate analysis of *Moringa* leaves, which contains 5.84±0.34% moisture, 7.39±0.50% ash, 26.97±0.82% crude protein, 4.86±0.43% fat, 18.75±1.10% crude fibers, and 36.19±2.25% total carbohydrates. Also, Table 2 assesses the phytochemical compounds screening of *Moringa* leaves extract, which represents the presence or absence of these compounds. Because of the fat content of *M. oleifera*, its extract is in high demand for its medicinal value. The crude fiber content of *M. oleifera* leaf extract was found to be beneficial to digestive system health. The high protein value of *M. oleifera* leaf extract makes it a good potential source of supplementary protein in functional foods. *Moringa oleifera* leaves are a respectable source of proteins, which have to be exploited to define their commercial sustainability (Asghar et al., 2022).

As shown in Table 2, secondary metabolites (such as polyphenols, tannins, saponins, etc), which are chemical compounds that are biologically active, are typically present in *M. oleifera* leaf extract. These secondary metabolites can be used as pharmacologically active substances or in nutrition (Ampitan & Adelakun, 2023). It was decided to look into *M. oleifera* leaf extract as a phyto-therapeutic agent to fight infectious agents after it was discovered to have antibacterial and anti-inflammatory properties (Singh & Kumar 2020).

TABLE 1. Proximate analysis of *Moringa oleifera*leaves extract.

Components	(%)
Moisture	5.84±0.34
Ash	7.39±0.50
Crude protein	26.97±0.82
Fat	4.86±0.43
Crude fibers	18.75±1.10
Total carbohydrates	36.19±2.25

TABLE 2. Preliminary phytochemical screening of *Moringa oleifera* leaves extract.

Compound	Representation
Phenols	+
Flavonoids	+
Tannins	+
Saponin	+
Alkalois	+
Steroids	-
Glycoside	+

Polyphenols in Moringa oleifera leaves extract

HPLC data in Table 3 show the polyphenolic contents of the ethanolic extract of dried *M. oleifera* leaves. Dried *M. oleifera* leaves extract exhibited totally twelve separated polyphenolic compounds. Only, five compounds were identified as flavonoids: Naringenin 12.30 mg/g, Rutin 5.34 mg/g, Quercetin 4. 66 mg/g, Kampherol 11.79 mg/g, and Apegenin 5.67 mg/g. On the other hand, there were seven compounds identified as phenolic compounds: Chlorogenic acid 5.11 mg/g, Caffeic acid 1.02 mg/g, Syringic acid10.36 mg/g, Gallic acid2.10 mg/g, Ellagic acid8.41 mg/g, Catechol 4.87 mg/gand Benzoicacid12.29 mg/g. this result is confirmed with Wei et al., (2023). Polyphenolic compounds are abundant, varied, all-pervasive, and widely distributed in nature. The importance of bioactive natural polyphenolic secondary metabolites is enormous and highly significant. The biological activities of these compounds are known to include antimicrobial, antioxidant, and anti-inflammatory effects (Al Mamari, 2021).

In vitro effect of Moringa Oleifera leafextract on W 138 cells

The MTT assay was used to evaluate the cytotoxicity of *M. oleifera* leaf ethanolic extracts on lung fibroblast W138 cell lines at various concentrations of 50, 100, 150, and 200 µg/mL. The percentage of cell viability of W138 cells after 24 hours of treatment incubation as compared to controls is depicted in the data in Fig. 1. According to the findings, a dose of 50 µg/mLof the ethanolic extract from *M. oleifera* leaf differed significantly from the normal control and the other three doses ($P < 0.05$). Interestingly, at other concentrations (100, 150, and 200 µg/mL) compared to control, no discernible cytotoxic effect was seen; this finding was supported by Bhadresha et al (2022). This result indicates that *M. oleifera* is safe to use in food products.

Evaluation of Moringa Oleifera Antimicrobial activity

Data existing in Table 4 show the antimicrobial effect of *M. oleifera* leaf extract on the growth of two strains of bacteria (*Streptococcus pneumoniae* (gram-positive) and *Klebsiella pneumoniae (gram-negative)* and one strain of yeast (*Candida pneumonia*). The results show that there were clear inhibition zones around the studied extract concentrations. The obtained data displayed that *M. oleifera* leaf extract concentrations of 100 mg/mL, 150 mg/mL, and 200 mg/mL had a noticeable antibacterial effect on the growth of both positive and negative *pneumoniae* investigated strains. Moreover, *M.oleifera* leaf extract concentrations (150 mg/mL and 200 mg/mL) were found to be effective as an antifungal on the *Candida pneumoniae* strain. According to this finding, increasing the concentration of *M. oleifera* extract increased its antimicrobial activities. According to Peixoto et al. (2011), aqueous and methanolic *M. oleifera* leaf extracts contain compounds with broad-spectrum antimicrobial activity capable of inhibiting the growth of *pneumonic* microorganisms, which supports the findings.

TABLE 3. HPLC analysis for quantification of polyphenols (phenolic acids and flavonoids)of dried *Moringa oleifera* leaves extract.

Polyphenolic compounds of dried *M.Oleifera* leave extract	quantity (mg/g)
Naringenin	**12.30**
Rutin	**5.34**
Quercetin	**4. 66**
Kampherol	**11.79**
Apigenin	**5.67**
Chlorogenic acid	**5.11**
Caffeic acid	**1.02**
Syringic acid	10.36
Gallic acid	2.10
Ellagic acid	8.41
Catechol	4.87
Benzoic acid	12.29

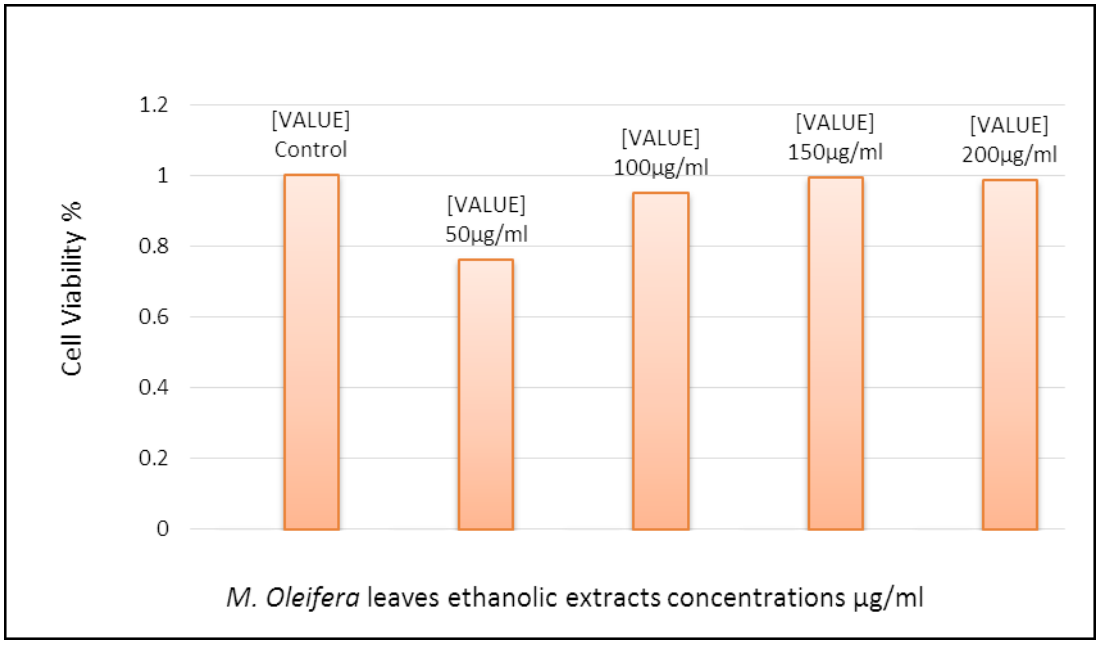

Fig. 1. Effect of *Moringa Oleifera* leafextract on W 138 cell viabilities.

TABLE 5. Antimicrobial activities screening of *M. oleifera*leaves extract.

Microorganism	Type	Antimicrobial activity of *M. oleifera* leave extract			
		50 µg /mL	100 µg /mL	150 µg /mL	200 µg/mL
K. pneumoniae	**Gram negative bacteria**	-	+	++	+++
S. pneumoniae	**Gram positive bacteria**	-	+	++	+++
C. pneumonia	**Fungus**	-	-	+	++

Sensory evaluations of Moringa Oleifera hard candy

According to the results of sensory evaluation in Table (6) conducted on samples of *Moringa* hard candy, the ANOVA data shows that there is no significant difference between the treated samples and control sample (without adding *M. Oleifera* extract) except the characteristic of color and oder, there was a significant difference($p < 0.05$) between the control sample (8.1 ± 0.11^{d}) and all treatments ($p < 0.05$), where is C sample ranked the highest score (8.9 ± 0.15^{a}), followed by B sample (8.7 ± 0.15^{b}), then A sample (8.2 ± 0.11^{c}). C sample referring to the excess of the greenish color of *M. Oleifera* extract. On the other hand, there was a significant difference ($p < 0.05$) between the control sample (8.1 ± 0.10^{c}) and treatment A (8.8 ± 0.10^{a}). At the same time, the latter will discover a statistically significant difference between it and samples B and C (8.5 ± 0.13^{b} and 8.4 ± 0.13^{b} respectively), which also recorded significant difference ($p < 0.05$) between them and the control sample.

Based on the results, the color of *Moringa* candy showed good acceptance, especially for sample C (ranking 8.9 ± 0.15^{a}) than B sample (8.7 ± 0.15^{b}), this may be due to the difference in *Moringa* extract concentration. The highest odorcharacteristic was A sample∧.8 ± 0.10^{a} (which detects desired foods, risks, pheromones, and plays a role in taste), The rest of the sensory evaluation, such as taste (which expresses the product's sense of taste), flavor (which expresses the taste and smell of food), and overall acceptability did not show statistically significant differences ($p < 0.05$) between *Moringa* hard candy samples and the control.

Finally, despite the increased concentration of *Moringa* extract in samples A, B and C, the product received positive feedback from panelists in general. That means the product's organoleptic qualities were unaffected by the increasing *Moringa* extract concentrations.

Conclusion

The goal of this study is to use the functional role of *Moringa* leave extract to produce a functional food that treats respiratory tract infections in the form of hard candy.*Moringa* extract increased the vitality of linear lung fibroblast cells while having no negative effects, indicating that the plant can be used safely in food processes. *Moringa* also has an antimicrobial effect on respiratory tract infections, making it a good candidate for use as an additive in functional foods in these cases. Finally, the development of *Moringa* hard candy appears to be a promising preventative product. Traditional medicine encourages and promotes the use of alternative therapeutic methods. However, more research is needed to determine the clinical benefit of this new product against respiratory infections.

TABLE 6. Organoleptic qualities of *Moringa* hard candy.

Sample	Color	Oder	Taste	Flavor	Overall accessibility
Control	8.1 ± 0.11^{d}	8.1 ± 0.10^{c}	8.4 ± 0.12^{ab}	8.0 ± 0.10^{a}	7.6 ± 0.10^{ab}
A (1.8 mg/100 g)	8.2 ± 0.11^{c}	8.8 ± 0.10^{a}	8.7 ± 0.13^{a}	8.0 ± 0.10^{a}	7.7 ± 0.10^{a}
B (2.7 mg/100 g)	8.7 ± 0.15^{b}	8.5 ± 0.13^{b}	8.4 ± 0.12^{ab}	8.1 ± 0.08^{a}	7.7 ± 0.10^{a}
C (3.6 mg/ 100 g)	8.9 ± 0.15^{a}	8.4 ± 0.13^{b}	8.5 ± 0.13^{ab}	8.1 ± 0.08^{a}	7.5 ± 0.10^{ab}

Values represent the mean ± S.E.M. (n = 3), with significant different at ($p < 0.05$).

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