EFFECT OF *MORINGA OLEIFERA* LAM. LEAF EXTRACT IN TREATING PNEUMONIA

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Moringa plays a critical role in acute inflammation related to pneumonia. *Moringa oleifera* is a tree rich in various phytochemicals with health benefits. Among the reported health benefits are antioxidant and anti-inflammatory properties. The purpose of this study was to investigate whether dried *M. oleifera* leaves ethanolic extract would resist acute lung-induced inflammation in W138 cells by lipopolysaccharide (LPS) *in vitro* models. *M. oleifera* leaves were analyzed for phytochemicals using standard methods. The screening showed the presence of tannins, saponins, flavonoids, phenolic acids, alkaloids, terpenoids, and steroids. HPLC analysis confirmed high levels of bioactive polyphenols in the ethanolic extract (7 phenolic acids and 5 flavonoid compounds) according to the standards used. The ethanolic extract of leaves showed the highest cell viability and more than 85% of the cells were viable at 1, 10, and 100 µmol/ml values in comparison with dexamethasone as a standard anti-inflammatory drug ($P < 0.05$). Leaves ethanolic extract EC_{50} value was 0.79 µmol/ml and significantly suppressed the production level of IL-1β (14.46 ±1.92 mg/ml) and IL-6 (51.67± 2.0 mg/ml) in comparison to controls ($P < 0.05$). These outcomes support the traditional use of the *M. oleifera* plant as a promising source of bioactive compounds with potential antioxidants for inflammation-related diseases/disorders, especially inflammation-related Pneumonia.

Keywords: Moringa, leaves extract, pneumonia, pro-inflammation

INTRODUCTION

The immune response that underlies many physiological and pathological processes in the body is known as inflammation, which serves as a defense mechanism against the causative. However, some infections can also trigger a severe localized or systemic inflammatory response, which can result in fatal conditions like pneumonia. According to present knowledge, preventing the cytokine storm may be a crucial factor in helping patients with severe pneumonia, who are infected with highly pathogenic agents survive. In

clinical applications, some natural anti-inflammatory therapeutics have been shown to be effective (Mehta et al., 2020).

Both the 2019 coronavirus disease (COVID-19) and the multidrug-resistant bacterial infection that causes acute respiratory syndrome have posed serious risks to global health. Most patients suffering from these infections of the respiratory system develop pneumonia as well as acute respiratory distress syndrome (ARDS) (Ye et al., 2020). A cytokine storm, or elevated levels of pro-inflammatory cytokines, is what causes ARDS, a leading cause of death. Through dysfunction of the respiratory epithelium, interleukins, such as interleukin-6 (1L-6) and interleukin-1β (IL-1β), contribute significantly to lung damage in ARDS patients. Acute overproduction and unregulated release of pro-inflammatory markers, both locally and systemically, are referred to as a cytokine storm or cytokine release syndrome (CRS) (Grasselli et al., 2021).

Medicinal plants are still a potential source of innovative unique medicines essential to maintaining human health. They support the development of novel drugs, which are necessary for the management of numerous disorders. *Moringa oleifera* Lam., also known as the miracle tree, is a member of the Moringaceae family and is native to tropical Northern India. It has since spread throughout the world, with popularity in the Mediterranean and Red Sea regions. It is used as a food additive and supplements (Dhakad et al., 2019). *M. oleifera* has an abundance of nutritional components, including essential amino acids, oleic acids, vitamins, and minerals, making it suitable for applications in food and medicine. Almost every part of the plant has been found to contain bioactive *M. oleifera* components. Flavonoids, phenolic acid, alkaloids and sterols, which are all distributed in the leaves, roots, and seeds, as well as terpene, which is distributed throughout the pods, are the main constituents isolated from *M. oleifera* (Liang et al., 2019).

M. oleifera is also known for its therapeutic applications, including the treatment of various infections, immune system modulation, and manifestation of antioxidant, anti-diabetic, or anti-tumor effects. The constituents isolated from the leaves of *M. oleifera* are reported to function in treating about 80 diseases, most of which fall under the categories of oxidative stress, disorders of glucose metabolism, tumors, organ damage, and immune-related diseases. More than 40 naturally occurring antioxidant compounds are found in *M. oleifera*, which is well known for its ability to neutralize free radicals. *M. oleifera* has a variety of initiatives to fight infectious diseases. All plant parts can be used to create a variety of antibacterial, antifungal, antiviral, and anti-parasitic formulations (Pakade et al., 2013).

In this light, treating cytokine release syndrome (CRS) or cytokine storm, and preventing secondary infection may be serious approaches to infection therapy. As a result, developing anti-inflammatory drugs based on natural products such as bioactive *M. oleifera* compounds is crucial for the treatment of CRS patients.

MATERIALS AND METHODS

The dried leaves of *M. oleifera* plant were obtained from the National Research Center, Giza, Egypt. The leaves preparation and extraction were done at the Desert Research Center, Cairo, Egypt. EC_{50} assay, W138 fibroblast cells, interleukins and other chemicals needed for cytotoxicity test were provided by Global Research Labs, 3 Dr. Mahmoud Fathi Street; Medical Center 2; Nasr City, Cairo 11528, Egypt. HPLC analysis for quantification of phenolic acids was done in the Laboratory of the Regional Center for Mycology and Biotechnology, Al- Azhar University, Cairo, Egypt.

1. Preparation of Dried Leaves Extract

Dried *M. oleifera* leaves (100 g) were socked in 500 ml of 80% ethanol for 24 h at 60°C with stirring. The extract was centrifuged at 6500 x g for 10 min at 4°C and the supernatant was filtered through filter paper (No. 3 Whatman) and dried by using a rotary evaporator under vacuum at 60°C to remove ethanol (yield 10% w/w). The sample was stored at 4ºC until required.

2. Qualitative Phytochemical Screening of Leaves Extract

The method described by Savithramma et al. (2011) was used to determine the qualitative levels of phytochemicals, alkaloids, tannins, sterols, saponins, and terpenoids. Total phenol was determined by forming a blue-green or black coloration after adding 2 ml of extract to 2 ml of ferric chloride ($FeCl_3$ 2%) solution, and total flavonoid was determined through the addition of 2 ml of extract with a few fragments of magnesium ribbon and concentrated HCl, as described by Harborne (1998).

3. Quantitative Analysis of Leaves Extract Polyphenols Contents

M. oleifera leaves extract produced by the previous method was analyzed using the HPLC technique to identify the existence of bioactive polyphenolic compounds. The method of Goupy et al. (1999) was used for the quantification of phenolic acid extraction. Flavonoids were determined using the method described by Pirjo et al. (2000). Drying was used to evaporate the pure compound, and the precipitated residue was dissolved in HPLC-grade methanol. The extract was injected into the Thermo-hypersil reversed phase C18 column at 25ºC for 30 min. The mobile phase is made up of 0.05% tri-fluoroacetic acid/acetate (solvent A) and distilled water (solvent B). The UV absorption spectra of the standards and samples were measured between 230 and 400 nm. The mobile phase, as well as the sample and standard solutions, were degassed and filtered through a 0.45 µm membrane filter (Millipore). The compounds were identified by comparing their retention times and UV absorption spectra to those of the standards.

4. *In Vitro* Investigation of Leaves Extract as Anti-inflammatory Agent

4.1. Cell viability and calculation of EC_{50} of leaf extract in lung fibroblasts

As a tested compound, *M. oleifera* leaf extract was provided in a liquid form "oily" at a concentration of 100 µmol/ml suspended in 200 µl solvent. A 100 µmol stock solution was kept at 4°C until needed. The final concentrations of the test compound were prepared for all experiments by diluting the stock with medium. The control cells were treated with the carrier solvent (0.1% DMSO). Dexamethasone was used as a positive control for drugs.

According to Fard et al. (2015), W138 cells were seeded in a 96-well culture plate the day before the experiment. In 200 µl of Mammalian Cell Culture Medium (RPMi-1640) (Gibco, Thermosientific, Germany) containing 10% fetal bovine serum (FBS) (Gibco, Thermosientific, Germany) and 1% penicillin G sodium (10.000 UI), streptomycin (10 mg), and 25 g (PSA) amphotericin B (Gibco, Thermosientific, Germany). Culture plates were incubated for 24 h at 37°C in a 5% CO_2 atmosphere to achieve 70% confluence. The next day, *M. oleifera* leaf extract was serially concentrated in the following concentrations: "100, 10, 1, 0.1 and 0.01 µmol". Furthermore, for control cells, the carrier solvent (0.1% DMSO) was used, and dexamethasone "potent anti-inflammatory" was used as drug-positive control, the dose of dexamethasone was adjusted to 2.5 µM. The treated lung fibroblast cells were incubated at 37°C in an atmosphere of 5% CO_2 for 48 h, then the cell proliferation assay was conducted.

The Vybrant® MTT Cell Proliferation Assay Kit, cat no: M6494 (Thermo Fisher, Germany) was used for the cell cytotoxicity assay. W138 cells (8103 cells per well) were seeded in 96-well culture plates in RPMI media and incubated for 48 h at 37°C with 5% CO_2 before being removed and replaced with new media. Each well received 20 µl of 2,4-diphenyltetrazolium bromide (MTT) solution (1 mg/ml) (Invitrogen, ThermoScientific, Germany) and the plates were incubated for 4 h at 37°C and 5% CO_2. Finally, MTT solution was removed from the wells and 100 µl of sodium dodecyl sulfate with hydrochloric acid (SDS-HCl) was added. The optical density at 570 nm on a microscope was used to determine cell viability on a spectrophotometer (ELx 800; Bio-Tek Instruments Inc., Winooski, VT, USA). After conducting the cell proliferation assay, the percentage of viability was determined which represents the W138 cell response to serial doses (0.01, 0.1, 1.0, 10, and 100 µM) of the provided extract. The XY curve was plotted to illustrate the relation between the log doses of the stimulator (*M. oleifera* leaves extract) versus the normalized response. The best-fit point was determined by linear regression analysis. Calculation of half-maximal stimulatory concentration (EC_{50}): The EC_{50} was calculated using the Graph pad prism software 9.

4.2. *In vitro* assessment of the anti-inflammatory effect of leaves extract using the calculated EC_{50} on an acute inflammatory model of lung fibroblast cells

Lung fibroblast cells were treated for 48 h with 1 g/ml of lipopolysaccharide (LPS) (Sigma Aldrich, Germany) to induce the *in vitro* acute inflammatory model. In RPMI-16 medium, the LPS stock (1 mg/ml) was diluted. The cells were treated with the EC_{50} for *M. oleifera* leaves extract as determined after 48 h of incubation at 37°C with 5% CO_2. The negative control is represented by the untreated "inflammatory reaction model" W138 cells. Using an enzyme-linked immunosorbent assay (ELISA), cells are incubated for 48 h at 37°C with 5% CO_2. After this time, cultured media is collected and used to test for the release of inflammatory mediators like IL6 and IL1. IL-6 was measured using Human Interleukin 6 (IL-6) ELISA Kit Cat No. MBS261259, My BioSource, San Diego, USA. IL-1β was measured using Human IL-1β (Interleukin 1 Beta) ELISA Kit Cat No.EH0185, Fine Biotech, Wuhan, Hubei, China.

5. Statistical Analysis

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

RESULTS AND DISCUSSION

1. Qualitative Phytochemical Screening of Leaves Extract

The data in the current study for the ethanolic *M. oleifera* leaves extract revealed the presence of the following phytochemical constituents; phenols, flavonoids, tannins, saponins, steroids, terpenoids and alkaloids as shown in Table (1) as secondary metabolites.

Table (1). Qualitative analysis of ethanolic extracts of *Moringa oleifera* leaves.

Compound	Representation
Phenols	+++
Flavonoids	+++
Tannins	++
Saponin	++
Alkaloids	++
Steroids	+
Terpenoids	+

+++ = very much ++ = much + = little

The presence of polyphenols in *M. oleifera* leaves gives it numerous purposes as an anti-inflammatory by blocking specific enzyme receptors that

cause inflammatory disorders. Also, they act as anti-allergies, anti-diuretics, anti-microbial, and anti-clotting agents by modifying the prostaglandin pathways. Moreover, *M. oleifera* leaves polyphenolic compounds act as anti-ulcers and immune enhancers. *M. oleifera* leaves phenolic compounds that act as antioxidants and play significant roles in liver detoxification, virus invasion, and tumors (Kumar et al., 2010). Phenols and flavonoids have many properties, including the ability to lower fever, be powerful antioxidants, relieve pain, and inhibit spasms (Krishnaiah et al., 2009). *M. oleifera* leaves contain a lot of phenolic compounds, which act as stimulating agents (as an aphrodisiac) by modulating hormones (Okwu et al., 2009).

Terpenoids have a wide range of biological properties that are linked to antibacterial, antifungal, antiviral, cytotoxic, analgesic, and anticancer effects, which underscores the significance of *M. oleifera* leaves extract. HIV can be inhibited by terpenoids (Cowan, 1999). Terpenoids have been credited in clinical studies with strengthening skin, increasing blood supply to inflamed tissues, increasing antioxidant concentrations in wounds, and other processes. Because of the presence of terpenoids, *M. oleifera* leaves are used as rejuvenating agents and have been discovered to be extremely beneficial for anti-aging and overall beauty enhancement. Terpenoids have numerous essential roles in herbal medicine, including treating burns and psoriasis, preventing scarring after surgery, and aiding in the treatment of various ailments. Terpenoids have also been shown in studies to lower blood sugar levels, reduce complications related to diabetes, and reduce insulin resistance in diabetics by 30 to 50% (Krishnaiah et al., 2009).

Because *M. oleifera* leaves are rich in alkaloids and saponins, which have anti-inflammatory properties, they are used to treat inflammations (Kenner and Requena, 1996). Alkaloids and saponins in *M. oleifera* leaves are what give them their antimicrobial properties (Osborn, 2003). According to Kenner and Requena (1996), saponins are used in the medical field as a gentle blood cleanser. To reduce serum cholesterol and prevent cholesterol reabsorption, saponins are crucial in the treatment of hypercholesterolemia (Olaleye, 2007). They bind to cholesterol to form insoluble complexes that are then excreted via the bile. Saponins reduce the cholesterol in blood and blood pressure. As a result, saponins lower the risk of cardiovascular conditions like hypertension.

Due to the content of steroids in *M. oleifera* leaves, it can act as hormone modulators. Some kinds of steroids are sex hormones, while others have cardio-tonic effects. Female hormones, such as progesterone and estradiol, contribute to the development of female sexual characteristics, regulate the menstrual cycle, and maintain pregnancy. Progestogens and estrogens used in oral contraceptives are modified versions of these hormones. It is known that some types of breast cancer can be affected by estrogen. Androgens are male sexual hormones, such as testosterone. Among their other

attributes is that they have an anabolic effect, which stimulates the growth of muscle (Xiao et al., 2020). The adrenal cortex produces cortical steroids, such as cortisone, and these hormones have two main purposes: controlling the balance of minerals in the body, converting protein into carbohydrate, and storing it as glycogen. Cortical steroids have anti-inflammatory and immunosuppressive properties. They are used to treat rheumatoid arthritis, asthma, and to reduce inflammation in creams. To maintain hormonal balance during pregnancy and lactation, plants high in steroids like M. oleifera are consumed as vegetables since the steroidal structure serves as a building block for steroid hormone synthesis (Edeoga et al., 2005).

2. Quantitative Analysis of Leaves Extract Polyphenols

In the plant kingdom, polyphenolic compounds are widely distributed and are frequently found in the leaves, flowering tissues, and pollen. Due to their antioxidant activity and anti-inflammatory action, these substances are used in human nutrition or medications to modulate many other disorders.

Seven phenolic acid compounds were detected, identified, and quantified according to their retention time and the spectral characteristics of their peaks compared with the standards used. Quantitative HPLC analysis showed a higher content of bioactive compounds in leaves ethanolic (80%) extract. Phenolic compounds namely chlorogenic acid at 5.11 mg/g, caffeic acid at 1.02 mg/g, syringic acid at 10.36 mg/g, gallic acid at 2.10 mg/g, ellagic acid at 8.41 mg/g, catechol at 4.87 mg/g and benzoic acid at 12.29 mg/g were detected as represented in Fig. (1). HPLC was used to identify and quantify five flavonoids in the ethanolic *M. oleifera* leaves extract according to the standards used. The results displayed in Fig. (2) show that naringenin at 12.30 mg/g, rutin at 5.34 mg/g, quercetin at 4.66 mg/g, kaempferol at 11.79 mg/g, and apigenin at 5.67 mg/g were detected.

The dried leaves of *M. oleifera* are an amazing source of polyphenols. (Zhang et al., 2011). *M. oleifera* leaf polyphenols concentration exceed those in fruits and vegetables (Fu et al., 2011). The wide range of reported values for M. oleifera leaves' polyphenol content may be attributed to various factors such as environmental conditions in different origin countries, harvesting season, plant genetics, drying method, leaves development stage, and extraction method. It is worth noting that flavonoids and phenolic acids constitute the major polyphenols present in M. oleifera leaves (Sultana et al., 2009).

A subset of phenolic compounds called phenolic acids is derived from the naturally occurring hydroxyl-benzoic acid and hydroxyl-cinnamic acid found in plants. The importance of phenolic acids found in food is a topic that is gaining more attention due to their well-documented effects on human health. These compounds are specifically being investigated for their well-documented anti-inflammatory, anti-mutagenic, antioxidant, and anticancer

properties. (Verma et al., 2013). In the current study, according to the standards used, the concentration of benzoic acid was 12.29 mg/g, syringic acid was 10.36 mg/g and ellagic acid was 8.41 mg/g, which recorded the highest values.

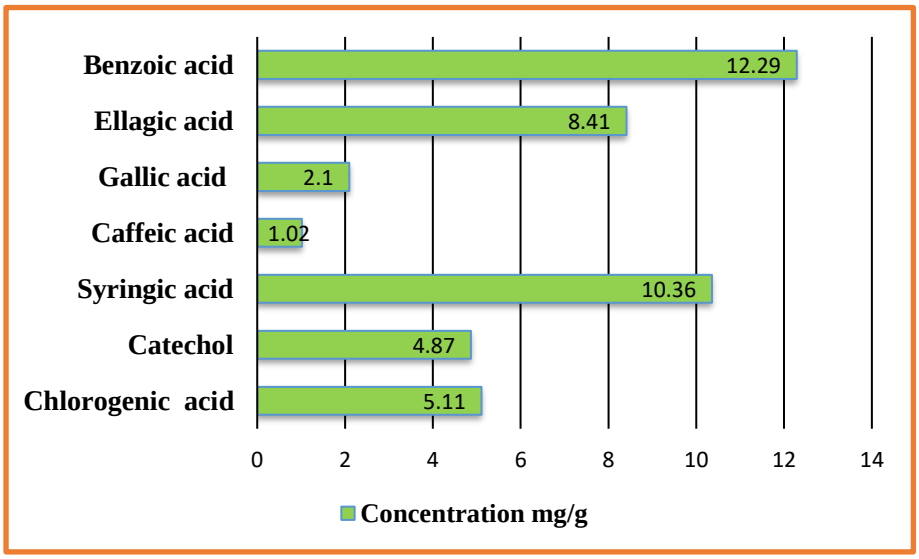

Fig. (1). HPLC analysis for quantification of phenolic acids in *Moringa oleifera* leaf extract.

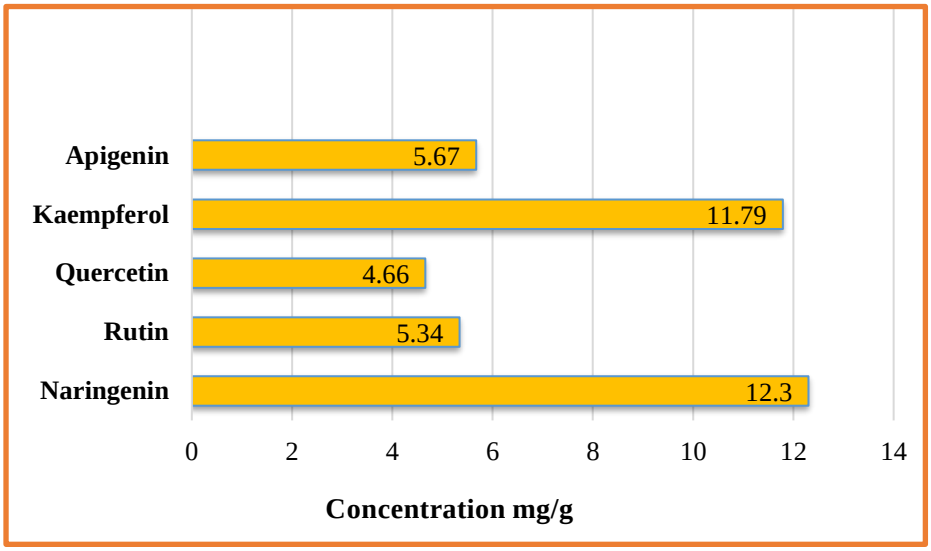

Fig. (2). HPLC analysis for quantification of flavonoids in *Moringa oleifera* leaf extract.

The chemical formula for benzoic acid, an organic substance, is C_6H_5COOH. It is made up of a benzene ring and a carboxyl group. Benzoic acid is therefore considered to be an aromatic carboxylic acid. Under regular conditions, this compound exists as a crystalline, colorless solid. The esters and salts of C_6H_5COOH are referred to as "benzoates". Benzoic acid has many important applications, including the production of phenols, the use of ointments to treat or prevent fungal infections of the skin, and its use as a preservative in many cosmetic products and the food industry. Additionally, it serves as a precursor to benzoyl chloride, which is used to create a variety of other substances, including medicines, dyes, perfumes, and herbicides (Burenjargal et al., 2023).

A common metabolite of plants, syringic acid is a naturally occurring phenolic compound with di-methoxybenzene. Additionally, the potent antioxidant properties of syringic acid may contribute to its favorable effects on human health. It has antioxidant, antimicrobial, anti-inflammatory, anti-endotoxic, neuro, and hepato-protective properties. It exhibits a broad range of therapeutic applications in the prevention of diabetes, cancer, and cerebral ischemia. It reduces the oxidative stress markers and is a powerful free radical scavenger (Srinivasulu et al., 2018).

Ellagic acid, a bioactive polyphenolic substance, is a secondary metabolite that naturally occurs in many plants. Ellagic acid is structurally a di-lactone of hexa-hydroxy di-phenic acid, a dimeric derivative of gallic acid, primarily produced by the hydrolysis of ellagitannins, a diverse class of secondary metabolites. Ellagic acid's anti-inflammatory, anti-proliferative, and antioxidant properties have gained popularity. It has demonstrated pharmacological effects *in vitro* and *in vivo* model systems and has protective properties for the heart, liver, kidneys, and brain (Rani et al., 2023).

Moreover, the quantitative HPLC results showed that *M. oleifera* leaves ethanolic extract contains 5.11 mg/g chlorogenic acid and 4.87 mg/g catechol in moderate amounts. A polyphenol called chlorogenic acid is created when quinic acid and caffeine react to form an ester. Some medicinal herbs' main active component is chlorogenic acid. It is well known that chlorogenic acid may offer protection against oxidative stress-induced cell damage by scavenging hydroxyl and superoxide radicals. Additionally, it was noted that chlorogenic acid has anti-inflammatory effects by reducing the production of pro-inflammatory cytokines. Additionally, it has been demonstrated that chlorogenic acid has anti-bacterial and anti-carcinogenic properties (Ahmad et al., 2023).

Catechol, also known as 1, 2-dihydroxybenzene, has the chemical formula $C_6H_4(OH)_2$. In trace amounts, this colorless substance is present naturally in fruits and vegetables. Destructive distillation of the plant extract catechin led to its initial discovery. Currently, catechol is produced synthetically on an annual basis as a common organic chemical, primarily as

a building block for pesticides, flavors, and fragrances. Catechol can function as pro-oxidants that harm macromolecules like DNA and proteins and as antioxidants that stop lipid peroxidation. Due to their redox cycling activity when used as an antimicrobial agent, catechol can also damage membrane functionality (Maślanka et al., 2023).

At least, 2.10 mg/g gallic acid and 1.02 mg/g caffeic acid are present in the HPLC quantification results in the lowest amounts. Gallic acid (3, 4, 5-trihydroxybenzoic acid), a naturally occurring low molecular weight tri-phenolic compound, has shown promise as a potent antioxidant and an effective apoptosis inducer. Starting with the bioavailability and biosynthetic process of gallic acid, numerous *in vitro*, *in vivo*, and *in silico* studies have been conducted to provide the mechanism of action, radical scavenging activity, ability to inhibit lipid peroxidation, maintenance of endogenous defense systems, and metal ion chelation by this tri-phenolic molecule, along with a thorough overview of the factors causing its high antioxidant activity. Gallic acid derivatives have also been discovered in several phyto-medicines, which have a variety of biological and pharmacological effects, such as inhibiting cell signaling pathways, attacking cancer cells through apoptosis, and radical scavenging (Badhani et al., 2015).

Caffeic acid is a hydroxyl-cinnamic acid, which is naturally present in many plants, including *M. oleifera*. Caffeic acid may have anti-inflammatory, antioxidant, and anti-cancer properties. Caffeic acid, when administered, works as an antioxidant, prevents oxidative stress, thereby preventing DNA damage brought on by free radicals, and inhibits the growth of cancer cells (Espíndola et al., 2019).

Flavonoids are a subgroup of polyphenolic compounds with a benzo--pyrone structure that are widely present in plants. The leaves of the *M. oleifera* are an interesting source of flavonoids. A high intake of flavonoids has been shown to have protective effects against many infectious (bacterial and viral) and degenerative diseases, including cardiovascular diseases, cancers, and other age-related diseases (Kumar and Pandey, 2013).

According to the standards used in the present study, the measured levels of naringenin (12.30 mg/g) and kaempferol (11.79 mg/g) were the highest. Naringenin is a flavonoid widely distributed in several *Citrus* fruits. This phytochemical has been linked to several biological activities, including antioxidant, antitumor, antiviral, antibacterial, anti-inflammatory, and cardio-protective effects (Salehi et al., 2019). Kaempferol (3, 4′, 5, 7-tetra-hydroxy-flavone) is a secondary metabolite that is present in a wide variety of plants, foods derived from plants, and traditional medicinal products. It is thought to have a bitter flavor. Kaempferol has anti-inflammatory, antimicrobial, antioxidant, neuroprotective, and cardiovascular benefits. The beneficial effects of dietary kaempferol in lowering the risk of chronic diseases, particularly cancer, have been described in numerous studies (Silva dos Santos et al., 2021).

Additionally, the quantitative HPLC results revealed that the ethanolic extract of *M. oleifera* leaves contains low concentrations of apigenin (5.67 mg/g), rutin (5.34 mg/g), and quercetin (4.66 mg/g). Apigenin (4′, 5, 7-trihydroxyflavone) is a bioflavonoid that appears to reduce anxiety, affect immune health, modulate hormones, brain function, oxidative stress and inflammation. Rutin is a rutino-side that is quercetin with the hydroxy group at position C-3 substituted with glucose and rhamnose sugar groups. It is a plant pigment that is found in certain fruits and vegetables. Rutin might have antioxidant and anti-inflammatory effects. It might also offer some protection against cancer and other diseases (Semwal et al., 2021). Quercetin is a penta-hydroxy-flavone having the five hydroxy groups placed at the 3-, 3'-, 4'-, 5- and 7-positions. It is one of the flavonoids that is found most frequently in edible fruit and vegetables. It functions as an antibacterial, antiviral, antioxidant, protein kinase inhibitor, an antineoplastic, a plant metabolite, a phytoestrogen, a radical scavenger, a chelator and an Aurora kinase inhibitor (David et al., 2016).

This study concluded that *M. oleifera* leaves ethanolic extract, which contains a variety of bioactive compounds, is essential in modulating diseases based on the prior data. In addition to attacking pathogens, *M. oleifera* can also reduce inflammation, which is a principal factor in conditions like ulcerative colitis, asthma, and many metabolic disorders.

3. In Vitro Investigation of Leaves Extract as Anti-inflammatory Agent

Inflammation is the body's well-known physiological response to infection and tissue damage, which helps the body heal and protect itself from further damage. However, various inflammatory-related diseases and disorders can be brought on by acute inflammation. Inflammation process of cellular dysfunction can be induced through microbial stimulus; LPS is a common prototypical endotoxin.

M. oleifera bioactive ethanolic extract was examined for cytotoxicity study with MTT assay. The ethanolic extract showed that the highest cell viability and more than 85% of the cells were viable at 1 µmol/ml, 10 µmol/ml and 100 µmol/ml values in comparison with dexamethasone as standard anti-inflammatory drug ($P < 0.05$) (Fig. 3), and the EC_{50} value was 0.79 µmol/ml (Fig. 4). Consequently, EC_{50} value of *M. oleifera* ethanolic bioactive leaves extract used for anti-inflammatory experiments.

The anti-inflammatory activity of ethanolic extract of *M. oleifera* leaves was investigated against pro-inflammatory mediators secreted by LPS-induced inflammation. The cytokine production levels of IL-1β and IL-6 in the cell-free culture supernatants were significantly enhanced in response to LPS induction (IL-1β 23.25±1.17 mg/ml, IL-6 134.52±2.21 mg/ml) as a positive control, which has proven the successful establishment of *in vitro* inflammatory model, in comparison to normal control (IL-1β 5.67± 0.91

mg/ml, IL-6 63.10±2.028 mg/ml) ($P < 0.05$). *M. oleifera* ethanolic bioactive leaves extract significantly reduced the production level of IL-1β (14.46 ±1.92 mg/ml) and IL-6 (51.67± 2.0 mg/ml) in a concentration-dependent manner (80% ethanol) according to EC_{50} value, in comparison to normal control (IL-1β 5.67± 0.91 mg/ml, IL-6 63.10±2.028 mg/ml) ($P < 0.05$) as represented in Fig. (5).

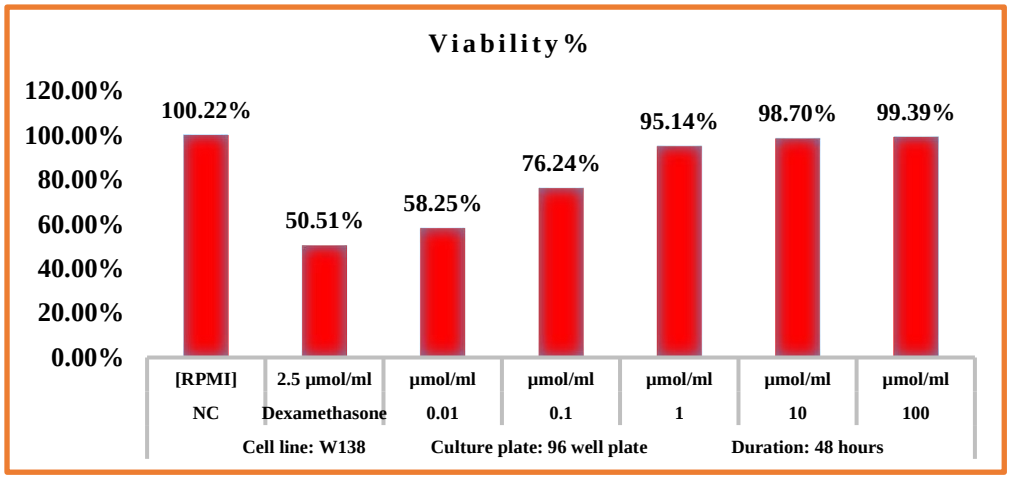

Fig. (3). Cell viability % of *Moringa oleifera* bioactive ethanolic extract.

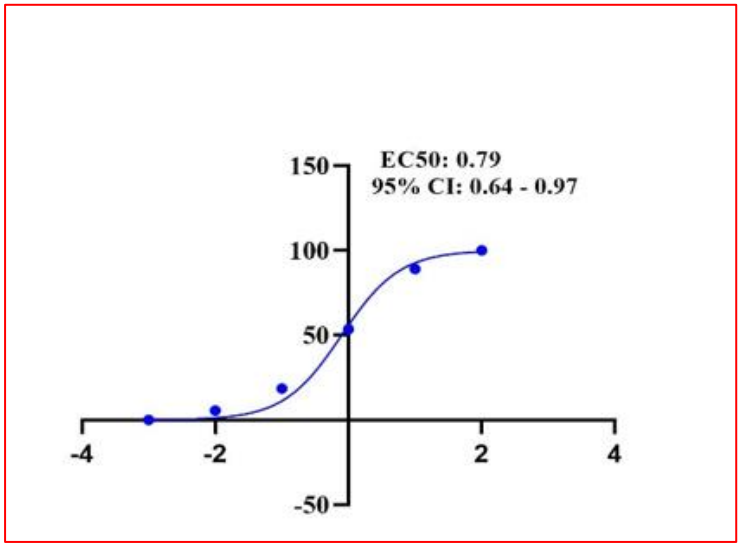

Fig. (4). EC_{50} of *Moringa oleifera* bioactive ethanolic extract.

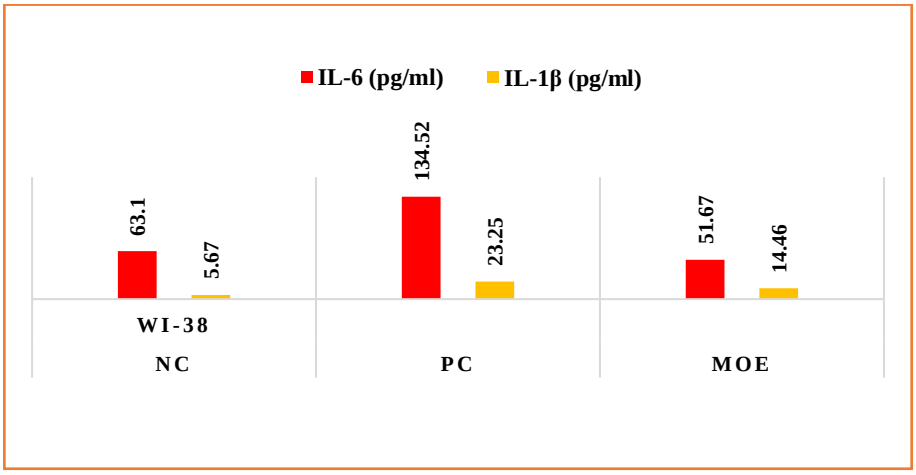

Fig. (5). The effect of *Moringa oleifera* ethanolic leaves extract on lipopolysaccharide-induced inflammatory cytokines (NC: Normal control, PC: Positive control, MOE: *M. oleifera* ethanolic leaf extract).

It is well known that LPS, a component of gram-negative bacteria membranes that is recognized by toll-like receptor 4 (TLR4) on the cell membrane of infected cells, can activate the release of pro-inflammatory mediators via NF-B signaling pathways, which mediate host harmful injury. LPS-stimulated lung inflammations are thus used as a model to study inflammation and the mechanisms of action of anti-inflammatory agents (Zhang et al., 2012). Recently, there has been an increase in the use of natural products as a treatment for a variety of acute and chronic diseases (Sellamuthu et al., 2013).

The obtained results indicated that *M. oleifera* extract could effectively improve LPS-induced inflammatory response by inhibiting the secretions of pro-inflammatory cytokines and its underlying mechanism might be associated with the inhibition of IL-1β and IL-6 inflammatory signaling pathway activation. The anti-inflammatory activity of *M. oleifera* extract might related to the relatively high contents of flavonoids and phenolic acids. These results is matching with that of Chimedza et al. (2017), who indicated that *M. oleifera* extract inhibited mRNA expression and concentrations of interleukine-6 (IL-6), similarly, showed anti-inflammatory activity in LPS-stimulated inflamed cells by decreasing production of NO and inflammatory gene expression (iNOS, IL-1β, and IL-6).

Plant extraction with the appropriate solvent concentration is critical in the field of natural product drug discovery for identifying and isolating pharmacological compounds from medical plants. Fakurazi et al. (2008)

previously reported the hepato-protective properties of 80% ethanolic *M. oleifera* leaf extract in a hepatotoxicin-induced animal model. Accordantly, the 80% ethanolic *M. oleifera* leaf extract was evaluated in this study for cell-specific toxicity and anti-inflammatory properties.

Cell viability assays for *M. oliefera* ethanolic 80% concentration bioactive leaves extract revealed the highest cell viability, with more than 90% viable cells at 1 mol/ml, 10 mol/ml, and 100 mol/ml values. It could be due to the increased availability of nutrition and certain compounds for cells. This finding agrees with a previous study by Fakurazi et al. (2008), who identified 80% ethanolic solvent as the best concentration. It may digest leaf tissue, releasing antioxidants and nutritionally important active compounds from *M. oliefera* leaves.

Pro-inflammatory cytokines, such as IL-1β and IL-6, are primary inflammatory mediators produced by inflamed cells during the inflammatory process. Stimulation of IL-6, IL-1β, and other inflammatory factors, results in a variety of physiological functions such as septic shock, inflammation, and cytotoxicity. Furthermore, it plays a role in the pathogenesis of many inflammatory-related acute/chronic diseases such as cancer, obesity, and cardiovascular disease. Inflamed lung cells produce IL-6 in response to LPS via NF-B activation (Muangnoi et al., 2012).

The IL-1 family is important in both immune defense and inflammation. To date, 11 members of this family have been identified. Both IL-1α and IL-1β are pro-inflammatory cytokines that are synthesized as precursor molecules by a variety of cell types. IL-1 is a key mediator of inflammation that plays an important role in tissue repair and protection against microbial pathogens. Systemic responses to this cytokine are responsible for the beneficial effects in these conditions, which include cellular infiltration and neutrophil mobilization, respectively. However, an excess of this cytokine may have negative effects on a variety of cells and tissues (Allantaz et al., 2007).

IL-6, which has been originally defined as a B-cell differentiation factor is regarded as a multifunctional cytokine that regulates immune responses, hematopoiesis, acute phase response, and inflammation. Increased IL-6 amount in many cases is correlated with several diseases which include rheumatoid arthritis, chronic arthritis, osteoporosis, psoriasis, Crohn's disease, and encephalomyelitis. Therefore, inhibitors of IL-6 could possibly be beneficial in treating inflammatory autoimmune diseases (Antonelli et al., 2009). In the present study, *M. oleifera* ethanolic bioactive leaves extract exhibited significant inhibition in LPS-induced IL-1 β, and IL-6 production. These results recommended that *M. oleifera* leaves may possess anti-inflammatory characteristics and help to reduce some inflammatory associated disorders. These anti-inflammatory properties of *M. oleifera* bioactive leaves extracts might be related to the presence of various

active polyphenols compounds which were identified through HPLC identification analysis.

CONCLUSION

The current study found that 80% ethanolic extract of *M. oleifera* leaves had a remarkable anti-inflammatory effect on LPS-induced inflammation in lung fibroblast cells. *M. oleifera* bioactive extract effectively reduced the production of pro-inflammatory cytokines IL-1 and IL-6. Furthermore, this study suggested that the anti-inflammatory activity of bioactive polyphenols found in *M. oleifera* ethanolic leaves extract can promote effective treatment for pneumonia-related inflammatory disorder.

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تأثير مستخلص أوراق المورينجا أوليفيرا (*Moringa oleifera* Lam.) في علاج الالتهابات الرئوية

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تلعب المورينجا .Moringa *oleifera* Lam دورًا مهمًا في علاج الإلتهاب الحاد المرتبط بالرئة. المورينجا هي شجرة غنية بمجموعة متنوعة من المواد الكيميائية النباتية ذات الفوائد الصحية. من بين الفوائد الصحية، من بين الفوائد الصحية المعروفة عنها خصائصها المضادة للأكسدة والإلتهابات. الغرض من هذه الدراسة هو التحقق مما إذا كان المستخلص الإيثانولي لأوراق المورينجا المجففة سيقاوم التهاب الرئة الحاد في خلايا W138 الناجم عن LPS في النماذج المختبرية. توفر الدراسات الكيميائية النباتية لأوراق المورينجا معلومات عن الإمكانات العلاجية لهذه النبتة ذات الفوائد الطبية. تم استخدام الطرق القياسية لتقييم الخصائص الكيميائية للنبات تقييمًا نوعيًا. سلط الفحص الكيميائي النباتي الضوء على وجود التانينات، والصابونين، والفلافونويد، والأحماض الفينولية، والقلويدات، والتربينويدات، والستيرودات. حدد تحليل HPLC نسبة عالية من البوليفينول النشط بيولوجيًا في المستخلص الإيثانولي للأوراق (٧ أحماض فينولية و٥ فلافونويد) وفقًا للمعايير المستخدمة. أظهر المستخلص الإيثانولي لأوراق المورينجا قابلية مرتفعة لبقاء الخلايا حية بنسبة أكثر من ٨٥٪ عند قيم ١ ميكرو مول / مل و١٠ ميكرو مول / مل و١٠٠ ميكرو مول / مل مقارنةً بالديكساميثازون كدواء قياسي مضاد للالتهابات ($P < ٠.٠٥$). كانت قيمة EC_{50} للمستخلص الإيثانولي لأوراق المورينجا هو ٠.٧٩ ميكرولتر/ مل وقمعت بشكل ملحوظ مستوى إنتاج IL-1β (١٤.٤٦ ± ١.٩٢ مجم/ مل) و IL-6 (٥١.٦٧± ٢ مجم / مل) مقارنةً بمجموعة التحكم الطبيعي ($P < ٠.٠٥$). تدعم هذه النتائج الاستخدام التقليدي للمركبات النشطة بيولوجيًا لنبتة المورينجا أوليفيرا كمصدر واعد كمضادات الأكسدة ضد الأمراض / الاضطرابات المرتبطة بالالتهابات وخاصة التنفسية منها .