Review Article

The Multifaceted Benefits and Applications of *Moringa oleifera*: A Comprehensive Review

Abstract

Moringa oleifera, commonly known as the Drumstick tree, is a multifaceted plant having extensive medicinal and nutritional applications. Various species of the plant are distributed across different tropical and sub-tropical areas around the world for diverse usage. It has also been recognized in Ayurveda as well as Unani systems of medicine for prevention and treatment of different diseases along with applications in environmental management, such as water purification and biopesticides. The plant is rich in essential nutrients, including vitamins, minerals, amino acids, and various bioactive compounds like flavonoids, tannins, and saponins. Its leaves, pods, and flowers are used as dietary supplements, offering significant health benefits, particularly in underdeveloped countries. It is known for a wide range of pharmacological properties, such as antioxidant, immunomodulatory, anti-microbial, and anti-cancer activities, making it a valuable resource in traditional and modern medicine. Various scientific studies have highlighted its potential in reducing cholesterol, boosting immune responses, and inhibiting the growth of various pathogens as well as cancer cells. This review highlights the multifaceted utility of *Moringa oleiferai.e.*, nutritional value of *Moringa oleifera*, its phytochemical richness and certain therapeutic properties such as antioxidant, immunomodulatory, anti-microbial and anti-cancer properties thus showing its versatility.

Keywords: anti-cancer, anti-microbial, antioxidant, immunomodulatory, *Moringa oleifera*

Introduction

Moringa has various species distributed across the different tropical and sub-tropical areas around the world for diverse usage.There are about 12 varieties of Moringa species out of which some are *Moringaovalifolia*, *Moringadrouhardii*, *Moringalongituba*, *Moringaoleiferaetc.*[1].*Moringaoleifera*, commonly known as Drumstick, benzoil tree, ben oil tree, Moringa,Shajmah, Sanjna or Sohanjna,is a commonly grown tree in India which belongs to Family Moringaceae. It is considered as a ‘Magical tree’ or ‘Miracle

Vegetable'because it is both a medicinal and a functional food.It is aperennial, deciduous soft wood tree with an average height of 10-12 m and has been used in traditional medicine and different industriesfor centuries**[2,3,4]**. It has application in many fields as most of the plant parts are edible and is having important medicinal properties **[5]**.It has also been recognized in Ayurveda as well as Unani systems of medicine for prevention and treatment of different diseases, for *e.g.*, fatigue, bronchitis, hay fever, gastric ulcers and skin diseases. The leaves, immature pods and flowers of Moringa can be used as high nutritive supplement having various important pharmacological properties **[6]**. It contains several nutrients such as vitamins, minerals and fatty acids **[7]** and various active ingredients such as steroids, tannins, triterpenoids, flavonoids **[8]**.Leaves of Moringa are highly nutritious and a very good and cheap source of protein, vitamins A, B, C and E,β-carotene, folic acid, pyridoxine, riboflavin, niacin, different minerals, amino acids and different phenolic compounds **[9]** and it was also found to be rich in gallic acid, quercetin glycosides, kaempferol and chlorogenic acid **[10]**. Apart from this it has other applications such as animal forage, alley cropping, blue dye, fertilizer, biogas, green manure, gum, honey and sugar cane juice clarifier, bio-pesticide, honey, tannin for tanning hides, pulp, ropes, water purification*etc.***[12]**. Its seed can be used in waste water treatment **[11]**.Thus, *Moringa oleifera* represents an important multitasking crop. It is consumed by humans in very diverse ways, as the leaves are known to be rich in tocopherols, ascorbic acid, carotenoids and have high nutritional value thus, the powder of the Moringa leaves is traditionally consumed by pregnant and lactating women in under-developed countries **[13]**. It has also been used for the treatment of bronchitis, asthma, diarrhoea, epilepsy, diabetes, anaemia, skin infections, *etc.***[14]**.It is also found to be very useful feed supplement in animals because leaves of the plant are of high nutritive value **[15]**. The present review aims to amalgamate the extensive uses of *Moringa oleifera*, highlighting its significance as a versatile plant with immense medicinal and nutritionalapplications. A holistic potential of *Moringa oleifera* is discussed along with its phytochemistry showing its antioxidant, immunomodulatory, antimicrobial and anticancer properties.

Phytochemistry of *Moringa oleifera*

Moringa oleifera isfound to be rich in various compounds like glucosinolates and isothiocyanates**[16]**, alkaloids such as Moringinine and Moringine are present in stem bark **[17]**, pigments such as alkaloids, kaempferol, rhamnetin, isoquercitrin and kaempferitrin are present in flowers **[18]**. In a study, it was reported that maceration of the dried leaves of *Moringa oleifera* with 70% ethanol provided highest yield of the extract of 40.50% w/w. In the HEK-293 (Human Embryonic Kidney-293) cells the median effective dose of 70%

ethanolic extract of Moringa was found to be 378.36 µg/ml**[19]**.Ethanolic extract of the leaves of Moringa is found to be rich in secondary metabolites such as flavonoids, tannins, saponins, alkaloids, steroids and anthraquinonewhich may be responsible for the therapeutic potential of the Moringa leaves**[20]**.The methanolic extract of *Moringa oleifera*showed the presence of highest number of phytochemicals as compared to extracts prepared in other solvents namely alkaloids, tannins, phenolics, carbohydrate, amino acids and terpenoids**[21]**.The plant is rich in rhamnose, glucosinolates and isothiocyanates **[22]**. GC-MS analysis of aqueous extract of *Moringa oleifera* leaves showed the presence of various phytochemicals *i.e.*,1,3-dihydroxyacetone dimer;acetic acid;4(1H)-pyrimidinone, 2,6-diamino-; 4H-pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl-; 2-hexynoic acid; butanedioic acid, 2-hydroxy-2- methyl-, (S)-; 3,3'-iminobispropylamine; 1-hexanamine; 1,3-dioxolan-2-one, 4,5-dimethyl-; 2-butenethioic acid, 3-(ethylthio)-, s- (1-methylethyl) ester; propanamide, N,N-dimethyl-; 2-isopropoxyethyl propionate; D-mannoheptulose; azetidin-2-one 3,3-dimethyl-4-(1- aminoethyl)-; carbonic acid, butyl 2-pentyl ester; tetra acetyl-d-xylonic nitrile; alpha-D-glucose; 1H-cyclopenta[c]furan-3(3aH)-one, 6,6a-dihydro-1-(1,3-dioxolan-2- yl)-, (3aR,1-trans,6a-cis)-; 3-[1-(4-Cyano-1,2,3,4- tetrahydronaphthyl)] propanenitrile; quinolinium, 1-ethyl-, iodide; N-isopropyl-3-phenylpropanamide; propanamide; 1,2-ethanediamine, N-(2- aminoethyl)-; 1,4-benzenediol, 2-methyl- and ethene, ethoxy-**[23]**.The GC-MS analysis of methanolic extract of *Moringa oleifera* leaves revealed majorly the presence of 1,3-propanediol; 2-ethyl-2- (hydroxymethyl)- (21.19%); propionic acid; 2-methyl-, octyl ester (15.02%); ethanamine; N-ethyl-N-nitroso- (5.21%); and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (5.00%). The IC_{50} of aqueous and methanolic extract of Moringa leaveswas found at a concentration of 4.65 µl/ml and 1.83 µl/ml, respectivelyafter an incubation period of 30 minutes. Another important finding was that the methanolic extract of Moringa leaves was found to have higher number of phytochemicals as compared to its aqueous extract. At a concentration of 5 µl/ml the free radicle scavenging potency with DPPH free radical of aqueous extract was found to be 35.8% and that of methanolic extract was found to be 88.5% at initial point of time and this may be due to presence of higher number of polyphenolic compounds isolated in methanolic extract. The majority of phytochemicals present in *Moringa oleifera*leaves are water soluble and show positive results for alkaloids, flavonoids, terpenoids, diterpenes, phytosterols, carbohydrates, glycosides, tannins and coumarine. Ethanolic extract of leaves of Moringa was found positive for polyphenols, glycosides, tannins and terpenoids and chloroform extract of Moringa leaves was found positive for cardiac glycosides and steroids**[24]**.

Comment [J1]: Can't it be quoted in antioxidant analyses later???

Nutritional Value of *Moringa oleifera*

All parts of the plant are enriched with protein, vitamins, minerals, essential amino acids and different phenolic compound. It is also found that the leaves of Moringa contain a negligible amount of anti-nutritional factors such as trypsin inhibitors, saponins, tannins and phytates **[25]**.Moringa leaves have a relatively higher percentage of crude protein which may range from 25% to 32% which is easily digestible as it contains higher proportion of pepsin soluble nitrogen (82-91%) and low proportion (1-2%) of acid detergent insoluble protein. Proximate analysis of Moringa leaf powder was performed on dry matter basis and the nutrient profile was found to be 93.45% dry matter, 29.62% crude protein, 10.23% crude fibre, 37.50% carbohydrate, 14.25% ash, 8.40% ether extract, 2.65% calcium, 0.48% phosphorus, 2034.82 kcal/kg metabolizable energy**[25]**.*Moringa oleifera* leaf powder was used to replace canola meal in different groups of broilers and it was found that with gradual increase in level of Moringa leaf powder as replacement of canola meal, a higher feed intake was observed but with similar body weight gain resulting in poor FCR..The poor FCR indicated that there might be a problem with digestibility or availability of protein and other nutrients due to presence of anti-nutritional factor in *Moringa oleifera* leaf powder**[26]**. Leaves of the plant may contain tannin levels upto 2% which interferes with biological utilization of protein, carbohydrate, and lipids in monogastric animals**[27]**. The mineral make-up, vitamin make-up and amino acid make-up of leaves of *Moringa oleifera*is show in Table 1, Table 2 and Table 3, respectively.

Comment [J2]: Define FCR
How can a statement of "poor FCR" is reasonable when neither the range was mentioned nor its association. –Explain???

Table 1. Mineral make-up of *Moringa oleifera* leaves [28]

Minerals	**Leaves (mg/kg)**
Calcium (Ca)	4900
Phosphorous (P)	3600
Potassium (K)	13800
Sodium (Na)	6700
Magnesium (Mg)	2700
Manganese (Mn)	122
Iron (Fe)	415
Zinc (Zn)	47
Copper (Cu)	12

Comment [J3]: Units make them uniform as per standard format suggested by the journal

Table 2. Vitamin make-up of *Moringa oleifera* leaves [29]

Minerals	**Leaves (mg/100 gms)**
Vitamin A	18.9
Thiamin (B1)	2.02-2.64
Riboflavin (B2)	20.5-21.3
Niacin (B3)	7.6-8.2
Cynocobalamin (B12)	2.64

Comment [J4]: Units make them uniform as per standard format suggested by the journal

Vitamin C	15.8-17.3
Vitamin D	ND
Vitamin E	10.8
Vitamin K	ND

Table 3. Amino acid make-up of *Moringa oleifera* leaves [30]

Essential amino acids	**Leaves (mg/100 gms)**
Arginine	1780
Histidine	716
Isoleucine	1177
Leucine	1960
Lysine	1637
Methionine	297
Phenylalanine	1640
Threonine	1357
Tyrosine	2650
Tryptophan	486
Valine	1413
Non-essential amino acids	
Alanine	3033
Serine	1087
Cysteine	10
Proline	1203
Glycine	1533
Glutamic acid	2530
Aspartic acid	1430

Anti-nutritional factors present in *Moringa oleifera*

Anti-nutritional factors can be defined as substances which are generated in natural feed ingredients *via* normal metabolism of plants, which interact with the chemical composition or interfere with digestion or metabolic processes in the body by different mechanisms, and pose an effect contrary to optimum nutrition but it also depends on the digestive process of the ingesting animal **[31]**. These anti-nutritional factors may be responsible for inhibiting protein digestion and utilization, energy utilization, anti-vitamin factor or may also disrupt the immune function. The majority of factors responsible for anti-nutritional effects on animals include, plant lectins, polyphenols, phytic acid, protease inhibitors, tannins*etc*. **[32]**. Tannins are phenolic compound that interact with enzyme trypsin or amylase or their substrate to form indigestible complex thus making it less palatable and thus reducing feed intake. Percentage of tannins can be reduced by the process of drying, fermentation or silaging upto15-30%**[33]** as the content of tannins in Moringa leaves ranges from 12.0 to 20.6 mg/g **[34]**, thus in

practical application better feeding is seen after processing the leaves by these methods. The phytates and oxalate level in *Moringa oleifera* leaves are lower than those in other household vegetablesand on dry matter basis these are 22.3 mg/g**[35]**and 27.5 mg/g**[36]**respectively.Saponins are also present in the leaves of the plant that are responsible for providing better taste to the plants but on dry matter basis only 4.7–5 g/kg of saponin is present which doesn't cause any adverse effect on livestock **[37]**.Less fibre content is present in leaves of the plant *i.e.*, 5.89% on dry matter basis but it is negligible and have no adverse effects as anti-nutritional factor. Lignin can be used as source of energy in ruminantsas rumen microbes can degrade this into monosaccharides but it is difficult to digest by monogastric animals as they don't secrete enzymes responsible for lignin digestion **[38]** and as the tree grows the amount of lignin present in its leaves increases **[39]**. Thus, the processing of Moringa leaves *via* various methods is found to be beneficial to reduce the levels of certain anti-nutritional factors, and thereby enhancing their nutritional value and palatability for livestock consumption.

Medicinal Properties of *Moringa oleifera*

Beyond its nutritional value, *Moringa oleifera* has been traditionally used in herbal medicine for its diverse therapeutic properties such as antioxidant, immunomodulatory, anti-microbial and anti-cancer which are mentioned in the text below.

Antioxidant properties

The antioxidants are known to produce their effect by directly reacting with reactive oxygen species, chelating the catalytic metal ions and/or quenching the reactive oxygen species **[40]**.Natural antioxidants which include phenolics and flavonoids are bioactive and safer to use as compared to synthetic antioxidants. In a study, it was found that*Moringa oleifera*supplementation caused a significant decrease in triglycerides and total cholesterol levels in heat stressed chickens**[41]**. HPLC–MS analysis on ethanolic extract of *Moringa oleifera*showed presence of quercetin and kaempferol glycosides. It was also found that the total antioxidant activity of extracts from 1 mg *Moringa oleifera* leaf was approximately equal to the total antioxidant activity of 0.95–1.35 mmol $FeSO_4$.The free radical scavenging activity of extracts from 1 mg leaf was approximately equivalent to that of 0.026 mg oligomeric proanthocyanidins (OPC) and IC_{50} value of the Moringa leaf extract was found to be 0.7440 mg/L in DPPHradical scavenging assay **[42]**.The acetone extract of *Moringa oleifera* leaves had a total phenolic content of 120.33 mg tannic acid equivalent/ gm, total flavonoid content of 295.01 mg quercetin equivalent/ gm, total flavonol of 132.74 mg quercetin equivalent/ gm and proanthocyanidin content of 32.59 mg catechin equivalent/gm

Comment [J5]: Can we compare 2 different units and state the equivalence of activity

Comment [J6]: Abbreviation of DPPH

whereas aqueous extract of *Moringa oleifera*leaves contained 40.27, 45.1, 18.10 and 16.91 mg of respective equivalents. The Moringa fed group showed higher level of total phenols and proanthocyanidins, highest DPPH %, ABTS% and highest reducing power. Moringa fed broilers showed highest concentration of GSH, catalase and SOD**[43]**. Aqueous leaf extract of *Moringa oleifera* showed significant increase in DPPH free radicle scavenging activity, superoxide anion and nitric oxide radical scavenging activity and decreased lipid peroxidation and extent of DNA damage. The mature leaves of Moringa were found to have higher level of activities of enzymatic as well as non-enzymatic antioxidants**[44]**. 70% ethanolic extract of the dried leaves of *Moringa oleifera*has a DPPH scavenging activity with EC_{50} 62.94 µg/ml and a Ferric reducing/antioxidant power (FRAP) value of 51.50 mmol $FeSO_4$ equivalent/100 g extract**[19]**. Methanolic extract of *Moringa oleifera* leaves showed a concentration dependent increase in free radical scavenging activity, DPPH radical scavenging activity, hydrogen peroxide scavenging activity and nitric oxide free radical scavenging activity**[45]**.Its supplementation was found to have significant decrease in triglyceride and total cholesterol levels and significantly increase HDL (high density lipoprotein) in heat stressed birds thus showing beneficial effect on the lipid profile of the broilers. There was also a significant increase in blood GSH-Px, and mRNA expression of glutathione peroxidase, superoxide dismutase and catalase and a significant decrease in level of liver and muscle tissue TBARS of heat stressed broilers**[41]**. Moringa leaves were found to have a total phenolic content ranging between 2.1 and 4.6 g Gallic Acid Equivalent/100 g DM and total flavonoid content ranging between 0.9 and 1.8 g catechin equivalent/100 g DM varying in age from 30, 45 and 60 days of leaves.The methanolic extract had the highest amount of total phenolics while ethanolic extract had the highest content of flavonoids.With increase in the age of leaves vitamin C content as well as carotenoid content decreased significantly and was found to be highest in 30-day old leaves whereas tocopherol content increased significantly as the age of the leaves progressed to 60 days.When the DPPH radical scavenging activity of *Moringa oleifera* leaves was measured it was found that ethanolic extract of 60 days old leaves had highest activity 71.1%. The $ABTS^+$ radical scavenging activity was higher in 30-day-old *Moringa oleifera* leaves. The total antioxidant capacity was found highest in aqueous extract of 60 days old leaves. FRAP of Moringa leaves was found to be maximum in the methanolic extract of 45-day-old and was minimum in 60 days old leaves**[46]**.Leaves of Moringa were having two times more total phenolic content and three times more total flavonoid content as compared to the commonly consumes South African vegetables like cabbage, spinach, broccoli, cauliflower and peas, thus exhibiting greater

Comment [J7]: Abbreviation of ABTS

Comment [J8]: Abbreviation of GSH and SOD

Comment [J9]: Correct it as - Ferric reducing antioxidant power (FRAP)

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antioxidant activity as compared to these vegetables.The DPPH free radicle scavenging assay showed that the fresh crushed Moringa leaves had highest scavenging activity followed by Moringa flower, leaves collected from younger trees, leaves collected from older trees and then other vegetables **[47]**. Total phenolic content of *Moringa oleifera* leaf meal was 377 gallic acid equivalents (GAE)/g and antiradical activity was 63%.Broilers fed with Moringa meal were found to have reduced lipid peroxidation in the liver as well as spleen at higher dose rates **[6]**. Ethanolic extract from the flowers, inflorescence rachis, fundamental tissue of stem and leaf tissue of *Moringa oleifera*contained at least three flavonoids and the saline extract from the flowers and leaf tissue revealed at least two flavonoids and the antioxidant activity was stronger in ethanolic extract as compared to the saline extract and also the best scavenging capacity against DPPH radical was found in ethanolic extract of leaves**[7]**. The sample of Moringa plant taken in winter was having higher ash content (except the stalk part), calcium and phenolic components (except the leaf part) and stronger antioxidant activity than the summer samples. The total phenolic component in 100gm of leaf, stem and stalk of Moringa was found to be 181.3-200.0 mg, 71.9-134.4 mg and 68.8-93.8 mg catechin equivalent, respectively. The leaves of Moringa were having the highest antioxidant activity followed by stem and stalk in both winter as well as summer samples. The methanolic extract of Moringa had high DPPH radical scavenging activity, reducing power, hydrogen peroxide scavenging activity and higher superoxide dismutase activity**[39]**. The ethanolic extract of *Moringa oleifera* at a concentration of 100 mg/ml had a total phenolic content of 2.59 mg Gallic Acid Equivalent (GAE)/ml and that of aqueous extract was found to be 1.49 mg GAE/ml and the total phenolic content of ethanolic extract was 3.82 mg QE/ml and that of aqueous extract was 3.75 mg QE/ml. 5 mg/ml concentration of Moringa extract showed a DPPH radical scavenging activity of around 72% for both ethanolic as well as aqueous extract**[24]**.

Immunomodulatory activity

It was found that when canola meal in broiler ration was gradually replaced with 8%*Moringa oleifera* leaf powder the antibody titre against IBDV was highest. The antibody titre against NDVin broilers was also improved with gradual increase in dietary *Moringa oleifera* leaf powder**[26]**. Increased antibody titre in birds fed with *Moringa oleifera* in diet may be due to the presence of lectin in the Moringa leaveswhich is responsible for modulating the defence system of body and thus claimed to be an immune boosting plant**[12]**.In a study**,** the levels of IL-2 and IL-6 of broiler chickenssupplemented with Moringawere better as compared to those without Moringa supplementation in heat stressed or normal grown broilers in which

Comment [J13]: Do all I the studies considered for the review preparation are strictly restricted to Indian subcontinent only or globally???)

Comment [J14]: Abbreviation

Comment [J15]: Abbreviation of NDV

their levels were significantly down-regulated**[41]**. Ethanolic extract of dried seeds of *Moringa oleifera*, was found to inhibit spleen weight as well as circulatory leukocyte and splenocyte countsin mice administeredsheep RBC as the antigen. There was inhibition of delayed-type hypersensitivity reaction as there was decrease in mean foot pad thickness at 48 h interval. There was amelioration in the production of the humoral antibody titre and there was down-regulation of macrophage phagocytosis due to carbon particles**[48]**.A novel polysaccharide, designated as MOP-3, was extracted and isolated from the leaves of *Moringa oleifera*and in the immunomodulatory assay it was suggested that MOP-3 could significantly enhance pinocytic capacity and increase the secretion of reactive oxygen species (ROS), nitric oxide (NO), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α) by up-regulating the corresponding mRNA expression levels in RAW 264.7 cells**[49]**. Moringa is known to inhibit chronic inflammation, such as asthma, ulcerative colitis, and metabolic diseases. It can attenuate physical and chemical irritation-induced immune disorders, such as metal intoxication, drug side effects, or even the adverse effect of food additives. Autoimmune diseases, like rheumatoid arthritis, atopic dermatitis, and multiple sclerosis, can also be inhibited by *Moringa oleifera***[50]**. The antibody titre measured by using haemagglutination inhibition assay against new castle disease was significantly improved in broilers fed with *Moringa oleifera* leaf meal**[51]**. Dietary inclusion of *Moringa oleifera*leaf meal (up to 10%) in feed of *Sparusaurata*(gilthead seabream) showed improvement inhead kidney leucocyte phagocytosis, respiratory burst and peroxidase activities. An inclusion of 5% showed an increase in serum humoral components, including protease, ACH_{50} and lysozyme activities and also an increase in IgM level. An increase in skin-mucosal immunity such as protease, antiprotease, peroxidase and lysozyme activities were also found. It also led to an upregulation of the intestinal mucosal immunity genes (*lyso* and *c3*), tight junction proteins (*occludin* and *zo-1*) and anti-inflammatory cytokines (*tgf-β*) and a downregulation of pro-inflammatory cytokine (*tnf-α*)**[52]**. Methanolic extract of *Moringa oleifera* was found to significantly increase the levels of serum immunoglobulins and also prevented the mortality induced by bovine *Pasteurellamultocida* in mice. There was significant increase in circulating antibody titre in indirect haemagglutination test. The extract also produced significant increase in adhesion of neutrophils, attenuation of cyclophosphamideinduced neutropenia and an increase in phagocytic index in carbon clearance assay.It was also found that the lower dose of the extract was more effective than the higher dose**[53]**. Methanolic leaf extract of *Moringa oleifera*, when given at a dose of 1000 mg/kg body weight in cyclophosphamide treated rats led to significant increase in WBC, lymphocyte, and

Comment [J16]: Abbreviation of MOP

Comment [J17]: abbreviation

Comment [J18]: italics

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Comment [J20]: Full form of ACH

Comment [J21]: Statement to be reframed as it is ambiguous

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neutrophil counts and a significant neutrophil adhesion was found. There was a significant dose-dependent increment in the mean hemagglutination antibody titre to sheep red blood cells (SRBC). Thus, the extract showed significant changes in humoral as well as cell mediated immunity **[54]**.A novel polysaccharide (MOP-2) was extracted and purified from the leaves of *Moringa oleifera* by hot water extraction and chromatographic purification and it was found that it could significantly enhance the proliferation and pinocytic capacity of the RAW264.7 macrophage cells. MOP-2 was able to promote the secretion of ROS, nitric oxide (NO), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α) by activating mRNA expressions of iNOS, IL-6 and TNF-α and thus can be developed as a novel natural immunoregulatory agent in functional foods**[55]**. When the cells from spleen ofBalb/C micewere grown in RPMI medium in presence of aqueous extract of *Moringa oleifera* Lam. leaf, it was found that in flowcytometry assay the extract at low doses was able to increase the cell number of $CD4^+$ and $CD8^+$cells and in higher doses there was significant increase in $B220^+$ cells when compared to the control**[56]**. When leaf extract of *Moringa oleifera*was administered orally to cyclophosphamide treated rats, there was a significant dose-dependent increase in total WBC count and percentages of neutrophil, eosinophil, monocytes, and lymphocytes. There were significantly reduced serum hepatic enzymes (ALT, AST, and ALP) and the administration of *Moringa* extract was able to reverse the effects of cyclophosphamide on blood parameters and hepatic enzymes in a dose dependent manner **[57]**.

Anti-microbialactivity

Crude aqueous and methanolic extract of Moringa at a concentration of 50mg/mlwas found to have antibacterial activity against different pathogenic bacteria of humans namely, *Proteus* spp., MDR of *Escherichia coli*,*Shigella* spp., *Salmonella paratyphi*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Escherichia coli*, *Salmonella typhi*, MDR of *Klebsiella* and *Staphylococcus aureus*. Methanolic and isopropanol extract of Moringa at a concentration of 80mg/ml showed significant anti-fungal activity. Hexane, benzene and isopropanol fractions of *Moringa oleifera*also showed activity against Hepatitis-B virus **[21]**. Itwas found that, the ethyl acetate, ethanol and chloroform extract of Moringa leaves was found to have a significant anti-microbial activity against many bacteria such a *E. coli, P. aeruginosa, Klebsiella spp., S. pneumonia* and *B. cereus* and anti-fungal activity against *A. niger, A. flavus, Trichoderma* sp. and *Candida* sp.**[20]**. It was also found that the ethanolic extract was most effective against majority of the microbes which may be due to higher polarity of ethanol. In a study,it was reported that the aqueous, ethanolic and methanolic extract of

Comment [J23]: abbreviations

Comment [J24]: Do all the studies included in these observations are of same geographical region and same units of concentration used

Comment [J25]: abbreviation

Comment [J26]: italics

Moringashowed varying degree of antibacterial activity against *S. aureus, E. coli* and *B. subtilis* and out of all the extracts methanolic extract showed maximum antibacterial activity against *S. aureus***[45]**. In another study, it was found that the acetone leaf extract of *Moringa oleifera* was found to have a significant inhibitory effect on shedding of oocyst in faeces of broilers which was found to be in a concentration dependent manner *i.e.* 1,2,3,4 and 5 g/kg body weight with an inhibitory effect of 96.4 %, 97.4%, 98.7, 99.1 and 99.8 % inhibition of oocyst shed in the faeces respectively. Similarly, the groups treated in the same sequence has a body weight increase of 20.0, 22.0, 26.0, 29.0 % and 34.0 % respectively and the extract given at the dose rate of 5g/kg body weight showed highest body weight gain**[58]**. Inanother study it was reported that the ethanolic extract of *Moringa oleifera* leaves was found effective against *E. coli, S. aureus, B. subtilis*and *P. aeruginosa*in a decreasing order **[24]**which might be due to presence of high level of terpenoids and tannins in the extract **[59]** as tannins are able to inhibit the cell wall synthesis **[60]** and terpenoids are known to cause dissolution of cell walls of microbes by weakening the membranous tissues **[61]**. Another finding was that the aqueous extract of the plant was found to have antibacterial activity against *P. syringica* and the reason for this may be presence of diterpenes in the aqueous extract which can damage cell wall of gram-negative bacteria that contain phospholipids and lipopolysaccharides as barriers **[62]**.Apolar extracts of seeds of *Moringa oleifera* showed a significant concentration-dependent antimicrobial activity against *S. aureus* and *S. epidermidis*. 4 mg/mlof the seed extract reduced the viability of these bacteria up to 50% and it was associated to the content of specific fatty acids**[63]**. 0.5%, 0.75% and 1% incorporation of *Moringa oleifera* leaves in chicken sausages showed low pH value, decreased TBARS and low Total Plate Count throughout the storage period and thus showing the antioxidant and antimicrobial potential of Drumstick leaves in chicken sausages**[64]**.*Moringa oleifera*leaf extract (8% b/v, 4% b/v and 2% b/v) was able to inhibit the growth of *S. epidermidis* by showing the inhibition zone (14 mm, 10.8mm and 9.3 mm) around the extract**[65]**. Using minimum inhibitory concentration (MIC), the growth inhibition of*P. aeruginosa* and *E. carotovora* were found to be $86 \pm 1\%$ (ethyl acetate extracts of roots) and $79 \pm 0.3\%$ (ethanolic extracts of leaves), respectively, thus showing anti-bacterial activity of various parts of *Moringa oleifera***[66]**. In a study, it was reported that chloroform extraction was the best extraction method to extract antimicrobial compounds from *Moringa oleifera*and bark of the plant showed higher number of antimicrobial compounds compared to leaves and roots of the pant. The extract showed significant activity against *Salmonella enteritica* and *Listeria monocytogenes* was found to be most resistant to all the type of the extract**[67]**. A new

Comment [J27]: oocysts of which organisms were shed??

compound (*Moringa* A) from *Moringa oleifera* seeds was found to inhibit virus replication in host cells and protects infected cells from the cytopathic effect induced byInfluenza A viruses. The EC_{50} and EC_{90} values of *Moringa* A for Influenza A viruses were 1.27µM and 5.30 µM, respectively, when RAW264.7 cells were infected. *Moringa* A was observed to decrease the inflammatory cytokines TNF-*α*, IL-6, IL-1β, and IFN-β in H1N1 infected RAW264.7 cells. *Moringa* A was found to inhibit the expression and nuclear transfer of the cellular protein transcription factor EB (TFEB) and weaken the autophagy in infected cells, which could be an important antiviral mechanism**[68]**.

Anti-cancer effect

Although several advancements have occurred in field of chemotherapeuticsbut it is also associated with various adverse effects such as nausea, anaemia, skin irritation, nephrotoxicity and infertility*etc.***[69]**. So, natural resources are one of the major sources of anti-cancer drugs and more than 60% of the presently available cancer drugs are derived from natural sources. The leaf extract of Moringa has undergone extensive research for its anti-cancer properties. Its leaves are rich in various important phytoconstituents such as polyphenols and flavonoids which are known to possess antioxidant as well as anti-cancer properties **[70]**. Dichloromethane leaf extract of *Moringa oleifera* showed and IC_{50} of 112-133µg/ml at killing cancer cells of various cell lines *i.e.*,HepG2, Caco-2, and MCF-7. The extract also showed chemoprevention as shown in quinone reductase (QR) induction assay as there was significant induction of QR **[71]**.20 µg/ml of *Moringa oleifera* leaf extract showed significant anti-cancer activity against primary leukemia cells harvested from patients with acute myeloidleukemia and acute lymphoblastic leukemia and HepG2 cells in the MTT assay **[72]**. The soluble cold distilled water (4°C) extract of *Moringa oleifera* with a concentration of 300 µg/mlwas able to induce apoptosis, inhibit tumour cell growth, and decrease the level of internal ROS in human lung cancer cells suggesting that treatment with *Moringa oleifera* significantly reduced cancer cell proliferation and invasion. There was higher cytotoxicity against cancer cells as compared to healthy cells. When A549 cells were treated with the extract there was downregulation of many oncogenes and iPS-induction genes when observed with western blot and RT-PCR **[73]**. F1 fraction of *Moringa oleifera* leaves showed potential cytotoxic effects in Hep-2 cell lines with a CTC_{50}value of 12.5±0.5 µg/ml. In the same study it was found that *Moringa oleifera*leaf fraction at the dose rate of 5 mg/kg and 10 mg/kg, orally showed significant reduction in body weight and increase in mean survival time in Dalton's lymphoma ascites model in mice as compared to the control and treatment with the leaf fraction was comparable to 5-Fluorouracil treatment in rats **[74]**. Leaves of Moringa are

Comment [J28]: Mention the Representative cancers

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known to possess cytotoxicity as it contains various flavonoids such as quercetin, kaempferol and myricetin that are known to possess anti-cancer activity. These compounds also possess cytotoxicity because they can induce apoptosis through intrinsic pathways by inhibition of mitogen-activated protein kinase (MAPK), extracellular-signal-regulated kinase 1/2 (ERK 1/2), c-Jun N-terminal protein kinase 1 (JNK), and protein kinase C (PKC) **[75]**. In a study it was reported that *Moringa oleifera* leaf extracts induced the apoptosis of HepG2 cells (human hepatocellular carcinoma) and in hollow fiberassay it was found that the oral administration of the leaf extract significantly reduced (44-52%) the proliferation of the HepG2 cells and A549lung cancer cells **[76]**. n-hexane fraction of *Moringa oleifera* leaves was found to be rich in 10 phenolic compounds *viz*. quercetin, gallic acid, sinapic acid, vanillic acid, 4-hydroxy-3-methoxy benzoic acid, p-coumaric acid, m-coumaric acid, 4-hydroxy-3-methoxy cinnamic acid, caffeic acid, and syringic acid. It showed 50% reduction in Hela cancer cell viability at a concentration of 416 µg/ml**[77]**. GC-MS analysis of aqueous extract of *Moringa oleifera*showed the presence of presence of quinic acid, octadecanoic acid, hexadecanoic acid (palmitic acid), α-tocopherol (Vitamin-E) and ɣ-sitosterol as major bioactive compounds. It was able to reduce the tumour volume and tumour weight and increased their life expectancy. *In vitro* cytotoxicity assays showed that the extract induced dose and time-dependent toxicity in Ehrlich ascites carcinoma and Human laryngeal carcinoma cells. Flow cytometric analysis confirmed significant induction of apoptotic cells by changing the mitochondrial membrane potential in Ehrlich ascites carcinoma cell line**[78]**.

Conclusion

Moringa oleifera stands out as a multifunctional plant with several medicinal and nutritional benefitsso also known as 'Magical Tree'. It has a rich phytochemical composition, including glucosinolates, isothiocyanates, flavonoids, and other bioactive compounds, and these bioactive compounds are responsible for its wide-ranging therapeutic properties. The bioactive compounds present in the plant extract help to mitigate oxidative stress and enhance immune function. Additionally, its antimicrobial properties make it effective against a broad spectrum of pathogens. It also shows hope in cancer treatment through its ability to induce apoptosis and inhibit tumour growth. The application of Moringa extends beyond human consumption to animal feed, water purification, and industrial uses.*Moringa oleifera* stands out as an essential and beneficial crop for diverse applications because of its profound health benefits and multifunctional uses.

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