INVESTIGATING HERBAL MEDICINE AS A POTENTIAL TREATMENT FOR MULTIPLE SCLEROSIS

Kirti C. Godse[1]*, Sonali Madane[2], Shraddha Mandale[3]
[1, 2, 3,] Department of Pharmacology, Arvind Gavali College of Pharmacy, Jaitapur, Satara 415004, India

ABSTRACT
Multiple Sclerosis (MS) is a chronic, inflammatory autoimmune disorder of the central nervous system (CNS) characterized by demyelination and neurodegeneration. Current pharmacological treatments are primarily focused on managing symptoms and modifying the disease course, but they often come with side effects and limited efficacy. In recent years, herbal medicine has garnered attention as a potential alternative or complementary approach for the treatment of MS due to its anti-inflammatory, neuroprotective, and immune-modulating properties. This investigation aims to explore the therapeutic potential of various herbal remedies in managing MS. *Citrullus lanatus* (watermelon), a common fruit known for its rich content of antioxidants, amino acids, and anti-inflammatory compounds, has shown promise in reducing oxidative stress and inflammation key factors in MS pathogenesis. This study investigates the potential role of watermelon in the treatment of MS, focusing on its bioactive components such as lycopene, citrulline, and flavonoids. Phytochemical screening and invitro evaluation such as GC-MS method, MTT assay, antioxidant method by DPPH method were conducted for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in watermelon. Furthermore, the feasibility of incorporating watermelon as an adjunct to conventional MS therapies is discussed, with attention to its safety, efficacy, and synergy. Further studies are needed to isolate active principle of the extract as well as to elucidate their exact mechanism of action in various disorders.

Key Words: Neuroprotective, multiple sclerosis, *Citrullus Lanatus*, anti-inflammatory, phytochemical screening, antioxidant.

How to cite this article: Godse KC, Madane S, Mandale S. Investigating herbal medicine as a potential treatment for multiple sclerosis. Int J Drug Deliv Technol. 2026;16(73s): 119-128. DOI: 10.25258/ijddt.16.73s.14

INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disease that targets the central nervous system (CNS), resulting in the loss of myelin and impaired nerve signal transmission. It arises when the immune system mistakenly attacks the myelin sheath that insulates nerve fibers, leading to a variety of neurological symptoms including muscle weakness, vision problems, cognitive decline, and fatigue. Although the exact cause of MS is not fully understood, it is thought to stem from a combination of genetic predisposition and environmental influences, such as viral infections. MS is categorized into four primary types: relapsing-remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive-relapsing MS (PRMS), with RRMS being the most prevalent form.[1-3]

The condition mainly affects young adults, with women being around three times more likely to develop MS than men. MS is a progressive illness that leads to accumulating disability over time, especially in those with PPMS and SPMS. While significant progress has been made in unraveling the disease's underlying mechanisms, the precise biological triggers and pathways of MS initiation and progression remain under investigation. The central immune mechanism involves activated T-cells initiating an inflammatory cascade that damages myelin and leads to neurodegeneration.[4]

Currently available pharmacological treatments, known as disease-modifying therapies (DMTs), are designed to reduce relapses, slow disease advancement, and alleviate symptoms. However, these treatments often carry notable side effects and may be less effective, particularly in the later disease stages.[5] This has spurred growing interest in alternative therapeutic approaches, including herbal medicine, which is recognized for its anti-inflammatory, antioxidant, and neuroprotective benefits. Herbal therapies may offer a complementary or supportive role in MS treatment, partly due to their lower side-effect profiles and natural immune-modulating properties.[6]

This review aims to examine the potential role of herbal remedies in MS management, evaluating their pharmacological properties, safety, mechanisms of action, and possible synergistic interactions with conventional treatments. By identifying promising herbal interventions and their underlying mechanisms, this review seeks to contribute valuable insights to the expanding research on alternative therapies for MS.[7]

Pathophysiology

Multiple sclerosis (MS) is characterized by the

development of plaques within the central nervous system (CNS), accompanied by inflammation, demyelination, axonal injury, and eventual axonal loss. These plaques are found in various regions of the brain and spinal cord, particularly in white matter near the ventricles, optic nerves and tracts, corpus callosum, cerebellar peduncles, long tracts, as well as the subpial areas of the spinal cord and brainstem, and even in gray matter. Plaques are present across all MS subtypes includes primary progressive, secondary progressive, and relapsing-remitting although their distribution and characteristics evolve over time, reflecting significant variability in the patterns of demyelination and oligodendrocyte damage between relapsing-remitting and progressive forms of MS (Figure 1).

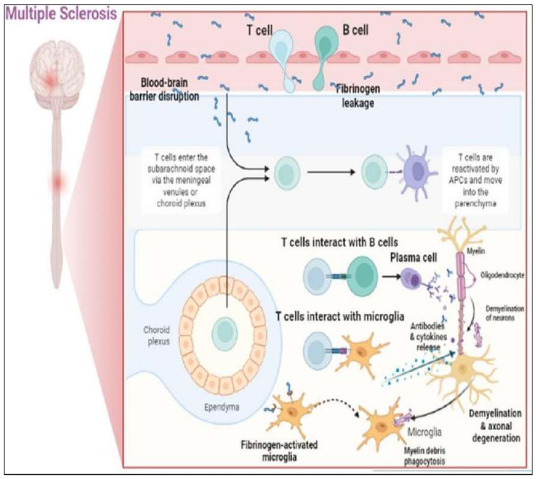


Figure 1. Multiple sclerosis

MS is classified as an autoimmune disorder driven by self-reactive immune cells that cross the blood-brain barrier (BBB) and attack the CNS. Normally, these autoreactive immune cells are eliminated during development in the thymus or bone marrow through a process known as central tolerance in B cells. While some autoreactive cells may escape this deletion and enter circulation, peripheral tolerance mechanisms typically prevent them from triggering disease. However, MS may result when peripheral tolerance fails, due to dysfunctional regulatory T cells or autoreactive T cells that resist suppression. A complex interplay of genetic and environmental factors is thought to influence the activation and function of these autoreactive cells, contributing to disease onset.

Key T cell populations involved in MS include CD8+ T cells, CD4+ Th1 cells, and Th17 cells. These autoreactive T cells produce cytokines such as interferon-gamma, IL-17, and granulocyte-macrophage colony-stimulating factor, all of which are implicated in MS pathogenesis. Elevated immunoglobulin levels in cerebrospinal fluid (CSF) indicate a role for B cells in the disease. A hallmark diagnostic feature of MS is the intrathecal synthesis of oligoclonal immunoglobulins, known as oligoclonal bands (OCBs). In MS, most B cells found in the CSF and brain parenchyma are CD27+ memory B cells. These memory B cells are clonally expanded, exhibit somatic hypermutation, and express class-switched immunoglobulin transcripts within the CNS. The overlap between immunoglobulin proteins in the CSF and the immunoglobulin transcript profiles of B cells provides strong evidence that antibody-producing cells derived from these clonally expanded B cells are a major source of the excessive intrathecal immunoglobulin production, as reflected by OCBs in the CSF.

In multiple sclerosis (MS), inflammatory B cell infiltrates are found in the meninges, and a higher density of these cells is associated with more severe cortical lesions, increased neurodegeneration, and greater clinical disability. B cells are also thought to act as reservoirs for Epstein-Barr Virus (EBV). Following EBV infection, B cells are transformed into antigen-presenting cells, enhancing their ability to process and present antigens. Research has shown that recombinant human myelin oligodendrocyte glycoprotein can be taken up and cross-presented by EBV-infected B cells, which are then effectively targeted by cytotoxic CD8+ T cells. Additionally, B cells from individuals with MS show elevated surface expression of CD40, suggesting that they are more efficient at presenting antigens.

In patients with relapsing-remitting MS (RRMS), increased expression of B cell activation markers has been linked to greater neurodegeneration, reflected by a higher number of T1 hyperintense lesions and reduced brain volume. Beyond B cell-mediated mechanisms, impaired function of effector T cells also contributes to MS progression. Normally, in healthy individuals, CD8+ cytotoxic T cells help control EBV infection by eliminating EBV-infected lymphoblastoid cells. These specific cytotoxic T cells, which recognize and destroy cells expressing EBV latent proteins, are referred to as ―latency-specific T cells. During MS relapses, the population of EBV-specific T cells expands, and latency-specific CD8+ T cell activity increases. However, as MS advances, these latency-specific CD8+ T cells become exhausted and lose their ability to suppress the proliferation of latently infected cells. This dysfunction creates a feedback loop where the growing number of infected cells undermines immune regulation and further depletes T cells. Consequently, repeated relapses may stem from inadequate control of EBV reactivation, leading to increased infection of naive B cells and further viral replication.

B cells also contribute to MS pathology by presenting antigens to T cells and releasing factors that can damage oligodendrocytes. Moreover,

microglia and macrophages release various pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, which promote neurodegeneration by triggering cytokine-induced cell death, impairing astrocytic glutamate uptake, and inducing dysfunctional RNA-binding proteins. These immune cells also release glutamate, which may cause excitotoxicity and contribute to neuronal injury. Additionally, reactive oxygen and nitrogen species produced by microglia and macrophages can cause oxidative stress and mitochondrial dysfunction, further exacerbating neurodegeneration. However, microglia are also capable of adopting anti-inflammatory roles that support remyelination.[8] The image provides a visual summary of the main signs and symptoms of Multiple Sclerosis (MS), organized by the body systems they affect (Figure 2).[9-11]

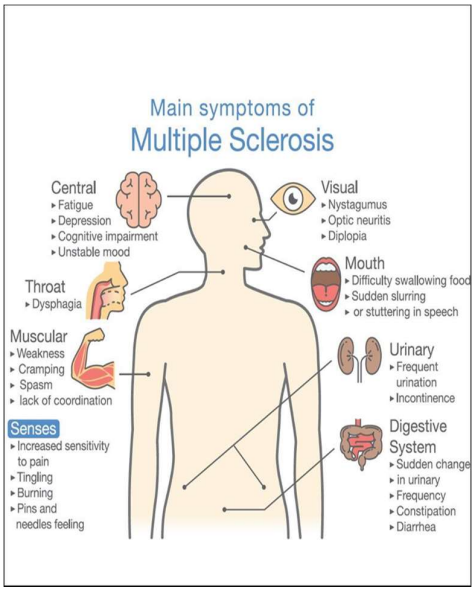


Figure 2. Signs and symptoms of Multiple Sclerosis

Watermelon (Citrullus lanatus)

Watermelon (*Citrullus lanatus*) is a widely enjoyed fruit known for its juicy pulp and abundant seeds. Despite its popularity and global export, the seeds and rinds are often discarded, even though the seeds are rich in both nutritional and functional components.[12]Watermelon is highly adaptable to various climates and soil conditions, contributing to its international commercial importance.[13] Its pleasant taste and nutritional profile make it a valuable source of bioactive compounds beneficial to human health. The fruit contains glucose, fructose, and a range of essential vitamins, including A, D, C, K, E, and several B- complex vitamins.[14] Notably, watermelon seeds are packed with biologically active ingredients like tocopherols, phospholipids, and sterols, making them important contributors to a healthy diet.[15] Both the pulp and seeds exhibit several health-promoting properties, such as anti-inflammatory, blood sugar-lowering, antibacterial, immunogenic, and antioxidant effects. *Citrullus lanatus*, the scientific name for watermelon, refers to a fruit-bearing vine that belongs to the Cucurbitaceae family. It primarily propagates through seeds and thrives in warm, sunny environments, requiring a minimum temperature of 25°C for optimal growth. Watermelon grows best in well-drained, nutrient-rich soils with slightly acidic pH levels. It is commonly cultivated in regions like the coastal areas of Ghana, forest zones, and particularly near rivers in the Northern Savannah.[16] The fruit earns the name "watermelon" due to its high-water content—approximately 93%. Its large, round shape and sweet, pulpy flesh resemble other melons. The term "Citrullus" is derived from the word citrus, while lanatus comes from Latin, meaning "woolly," a reference to the fine hairs found on the plant's stems and leaves. According to a 1977 WHO report, infectious diseases were responsible for around 50% of deaths in tropical regions.[17]

Citrulline

Watermelon is a rich source of citrulline, an amino acid that acts as an osmolyte and radical scavenger, helping plants cope with stress. It contains 0.7–3.6 mg/g of citrulline, with an average of 3.1 mg/g across 56 cultivars. Citrulline enhances nitric oxide production, which plays a key role in cardiovascular health. L-citrulline is converted into L-arginine and nitric oxide, improving endothelial function and blood flow. It has potential benefits for conditions like hypertension, heart failure, muscle metabolism, and diabetes. Additionally, citrulline supports immune function, wound healing, and cardiovascular health. Watermelon supplementation may improve cardiovascular and metabolic health by enhancing L- arginine availability.[18-20]

MATERIALS AND METHODS

Fresh leaves of *Citrullus lanatus* were collected from cultivated watermelon fields located in Solapur district. The collected plant material was botanically identified and authenticated by a taxonomist at the Y.C. Institute of Science, Satara. A voucher specimen was prepared and deposited with the accession number SSM001, SDM001, PMK001.[21-22] Only fresh and undamaged leaves were chosen.[23] The harvested leaves were immediately rinsed to eliminate microbial contaminants. The cleaned leaves were spread on clean trays in a single layer and shade-dried at room temperature (25–30°C) to prevent the degradation of heat-sensitive bioactive compounds. Drying continued for 10–14 days until a constant weight was achieved.[22]The leaves were periodically

turned to ensure uniform drying and to prevent mold growth. Drying was monitored until the leaves were crisp.[23]Once dried, the leaves were crushed with a grinder into fine powder.[24] The powdered leaves were stored in airtight, amber-colored glass bottles to protect from moisture and light, and kept in a cool, dry place until further use.[25]

Extract Preparation

Maceration Method was used for the Extraction of *Citrullus lanatus*. 100g of the powdered leaf material was weighed and soaked in 500mL of 80% methanol. The mixture was kept in a clean, sterile conical flask, covered, and shaken occasionally to enhance extraction. Maceration was allowed to proceed at room temperature for 72 hours. After extraction, the mixture was filtered using Whatman No.1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to yield a semi-solid crude extract. The crude extract was stored in sterile, labeled amber vials at 4°C until used for further phytochemical and pharmacological analysis.[25]

Phytochemical Screening of extract

Qualitative phytochemical tests were conducted using standard methods to detect the presence of phytochemical constituents. Watermelon leaves contain various phytochemical constituents including alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, and glycosides, which contribute to their potential medicinal and antioxidant properties.[26]

In-vitro evaluation study of extract

1. GCMS (Gas Chromatography Mass Spectrometry)

GCMS, or Gas Chromatography-Mass Spectrometry, is a powerful analytical technique that combines two methods to identify and quantify substances in a sample. It's used in various fields like environmental monitoring, food safety, and pharmaceutical analysis. GCMS can also refer to the Global Case Management System used by Immigration, Refugees and Citizenship Canada (IRCC). GCMS as an analytical technique consist of gas Chromatography (GC) which Separates components of a sample mixture based on their boiling points, using a carrier gas and a column with a stationary phase, and other is Mass Spectrometry (MS) which identifies and quantifies the separated components by measuring their mass-to-charge ratio. [27]

2. In vitro Antioxidant activity by DPPH (96 well method)

Principle of Assay: DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical with a deep violet color. Antioxidants reduce DPPH, causing a color change from violet to yellow, which can be measured spectrophotometrically at 517 nm. 96-Well Plate Layout Example in table 1.

Table 1. 96-Well Plate Layout Example

Row	Standard (e.g., Ascorbic Acid)	Test Sample	Control (DPPH only)
Volumes	100 µL standard + 100 µL DPPH	100 µL test sample + 100 µL DPPH	100 µL methanol + 100 µL DPPH

Procedure

Add 100 µL of each test sample or standard into wells in triplicates. (Dissolve samples and standard in methanol to prepare a series of concentrations), (e.g., 250, 500, 1000 µg/mL). Add 100 µL of freshly prepared 0.1 mM DPPH (Dissolve 3.94 mg of DPPH in 100 mL methanol, Store in dark bottle and use freshly) solution to each well (except blank). Add 100 µL methanol to blank wells (no DPPH). Cover plate with aluminium foil and incubate in the dark for 30 minutes at room temperature. Read absorbance at 517 nm using a microplate reader.

3. MTT PROCEDURES (SH-SY-5Y Neuroblastoma Cell Line)

Neuroblastoma cell lines were purchased from the National Centre for Cell Science (NCCS) in Pune and cultured in DMEM. The cell line was grown in a 25-cm tissue culture flask with an antibiotic solution containing the following antibiotics: penicillin (100 U/mL), streptomycin (100 g/mL), and amphotericin B (2.5 mg/mL). The DMEM was supplemented with 10% FBS, l-glutamine, and sodium bicarbonate.

Principle of Assay:

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cell store duce the yellow water-soluble substrate 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) into an insoluble, purple colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Figure 3).

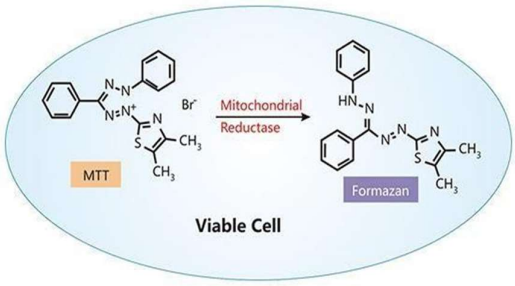


Figure 3. cell viability

Procedure

Cytotoxicity studies using SH-SY5Y cell neuroblastoma cell line, SH- SY-5Yis known as a relevant cellular model for biochemical investigations on Alzheimer's disease (AD). SHSY5Y cells also known as neuro steroid-producing cells which express the chief steroidogenic enzymes. The cell counts of SH-SY5Y, neuroblastoma cell line was adjusted to 1.0 × 105 cells/mL using DMEM medium containing 10% FBS. To each well of a 96 well flat bottom microtitre plate, 100 µL of diluted cell suspension (70–80% confluence) was added. After 24 h, at a sufficient population, the cells were centrifuged, and the pellets were suspended at Volume viz. 100 µL. Then, the plates were incubated for 24 h at 37 °C in a 5% CO2 atmosphere for microscopic examination, and the observations were recorded every 24 h. After 24 h, In order to induce oxidative stress, cells were treated with the concentrations (500 µM) of H2O2 for 90 min.20 µl of MTT (2 mg/mL) was added to MEM-PR (MEM without phenol red) and shaken gently. The plates were further incubated for 2 h at 37 °C in 5% CO2 atmosphere. DMSO (100 µl) was added slowly to the plates and gently shaken to solubilize the formed formazan. Absorbance was read at 570 nm using the microplate reader. Per cent cell viability and concentration of extract required to inhibit cell growth by 50% were obtained from dose-response curve. Triplicate samples were analyzed by measuring the absorbance of each sample by a Elisa micro plate reader (Benesphera E21) at a wavelength of 570 nm.

RESULT

1. Phytochemical Screening of extract

The phytochemical study of extracts from *Citrullus lanatus* leaves revealed a broad variety of phytochemicals. The key phytochemical components, such as alkaloids, flavonoids, saponins, tannins, terpenoids, were present in extracts which contribute to their potential medicinal and antioxidant properties. (Table 2).

Table 2. Phytochemical Screening of extract

Sr.	Constituents	Test	Aqueous
No.			**Extract**
1	Alkaloid	Mayer's reagent	-
		Dragendorff's reagent	-
		Wagner reagent	+
		Hager's	+
2	Carbohydrates	Molisch's Test	-
		Benedict's Test	-
		Barfoed's Test	-
3	Triterpenoids	Salkowski reaction	+
4	Flavonoids	Shinoda Test	+
5	Saponin	Foam test	+
6	Tannin	Gelatin Test Ferric chloride test	+ +

2. GCMS/ Gas Chromatography Mass Spectrometry [28-35]

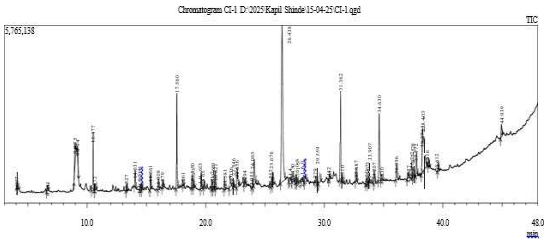


Figure 4. GC-MS chromatogram of extract of *Citrullus lanatus* leaves

Table 3. GC-MS spectral analysis of extract of *Citrullus lanatus* leaves

Sr. No.	Name of compound	Retention time	Peak area	Activity	% Area
1	1,2,3,5-Cyclohexanetetrol, (1α,2β,3α,5β)-	26.438	41,332,604	Neuroprotection, myelin support	20.63
2	7-(2-Hydroxypropan-2-yl)- 1,4a-dimethyldecahydronaphthalen- 2(1H)-one	31.362	12,462,325	Anti-inflammatory, antioxidant	6.22
3	9,12,15-Octadecatrienoic acid,	38.403	14,183,077	Immune modulation, anti-inflammatory	7.08
4	n-	34.6	10,8	Modulates	5.

	Hexadecanoic acid	30	02,587	immunity (cautiously), cell membrane support	39		N,N-di(allyl)-	59	8,623	nsmission modulation (potential)	51
5	3,6-Octadienoic acid, 3,7-dimethyl-, methyl ester	17.560	10,482,513	Neuroprotection, anti-inflammatory	5.23	7	Silane, [(1,1-dimethyl-2-propenyl)oxy] dimethyl-	24.085	24.085	Drug delivery/ penetration enhancement	2.25
6	Nonylamine,	29.5	5,02	Neurotra	2.						
8	1H-Imidazole-4-ethanamine, β,β-dimethyl-	33.907	33.907	Histaminergic modulation, immune interaction	2.05						

a) 1,2,3,5-Cyclohexanetetrol (Inositol Derivative)

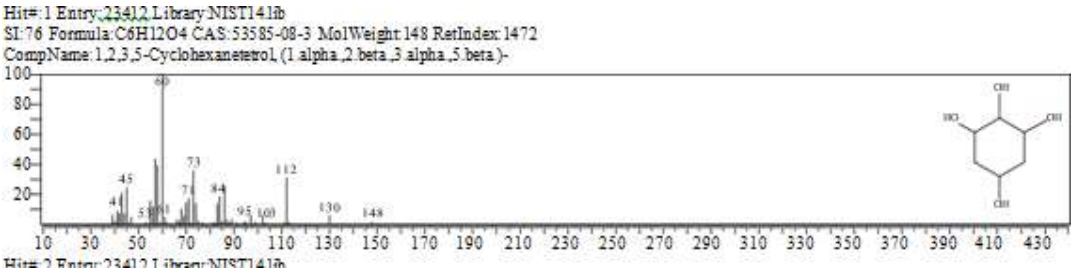


Figure 5. GC-MS chromatogram of 1,2,3,5-Cyclohexanetetrol

- Biological role: Related to inositol isomers, which are important for cell signaling and nerve function.
- Relevance to MS: Inositol compounds can modulate neurotransmission, aid in myelin repair, and may help reduce neuroinflammation.
- Potential: May act as a neuroprotective agent.

b) 7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-2(1H)-one

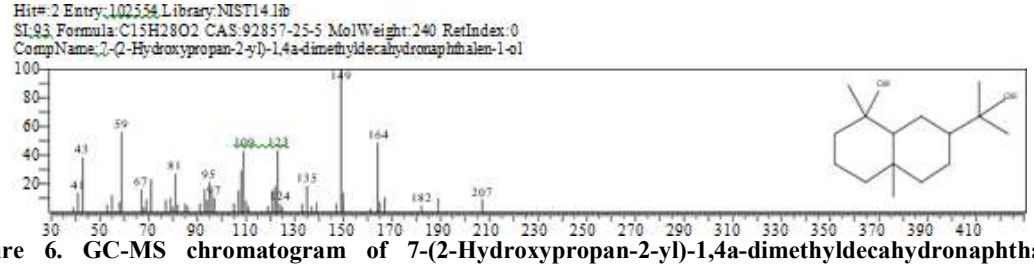


Figure 6. GC-MS chromatogram of 7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-2(1H)-one

- Compound class: Likely a terpenoid.
- Relevance to MS: Terpenoids are known for anti-inflammatory and antioxidant effects, which may help reduce the immune-mediated damage in MS.
- Potential: Could modulate immune activity and protect neurons from oxidative stress.

c) 9,12,15-Octadecatrienoic Acid (α-Linolenic Acid, ALA)

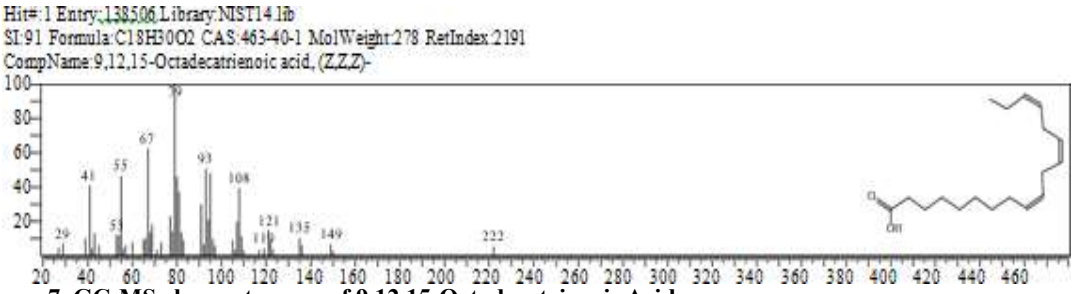


Figure 7. GC-MS chromatogram of 9,12,15-Octadecatrienoic Acid

- Compound class: Omega-3 fatty acid.
- Relevance to MS: Has strong anti-inflammatory effects. Shown to modulate cytokine production and immune cell behavior. Some studies suggest ALA may help reduce relapse rate in MS patients.
- Potential: Widely researched for neurodegenerative and autoimmune diseases.

d) n-Hexadecanoic Acid (Palmitic Acid)

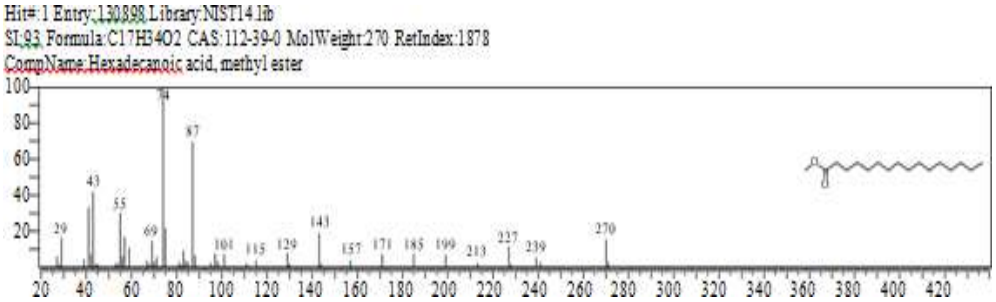
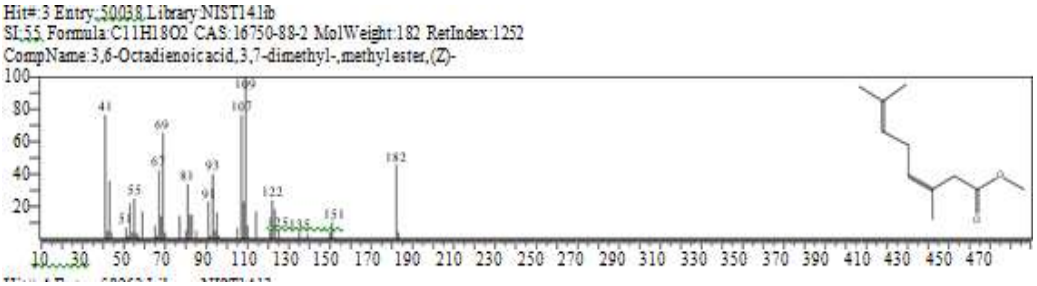


Figure 8. GC-MS chromatogram of n-Hexadecanoic Acid

- Compound class: Saturated fatty acid.
- Relevance to MS: Though traditionally associated with pro-inflammatory pathways, some derivatives of palmitic acid have been shown to have immunomodulatory roles.

e) 3,6-Octadienoic Acid, 3,7-dimethyl-, Methyl Ester

Figure 9. GC-MS chromatogram of 3,6-Octadienoic Acid, 3,7-dimethyl-, Methyl Ester



- Compound class: Monoterpene derivative.
- Relevance to MS: Terpenes have shown antioxidant, neuroprotective, and anti- inflammatory activities.
- Potential: May support myelin integrity and suppress microglial activation.

f) Nonylamine, N,N-di(allyl)

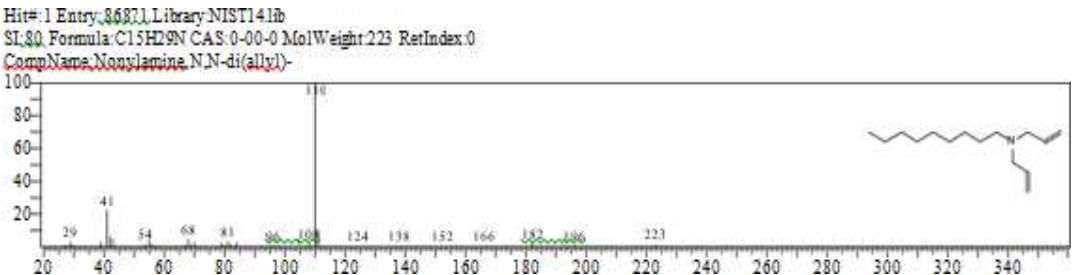


Figure 10. GC-MS chromatogram of Nonylamine, N,N-di(allyl)

- Compound class: Alkylamine derivative.
- Relevance to MS: Amines can act as neurotransmitter analogues or neuromodulators.
- Potential: Could influence synaptic signaling, though direct evidence in MS is limited.

g) Silane, [(1,1-dimethyl-2-propenyl) oxy] dimethyl-

- Compound class: Organosilicon compound.
- Relevance to MS: Some organosilicon compounds have been explored for drug delivery and blood-brain barrier penetration.
- Potential: May act as a carrier or enhancer of neuroactive compounds.

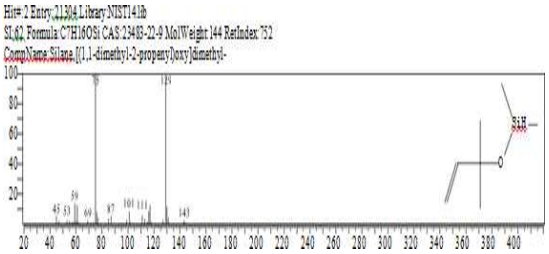


Figure 11. GC-MS chromatogram of Silane, [(1,1-dimethyl-2-propenyl) oxy] dimethyl-

h) 1H-Imidazole-4-ethanamine, β, β-dimethyl-

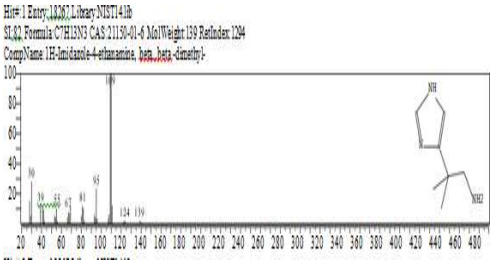


Figure 11. GC-MS chromatogram of 1H-Imidazole-4-ethanamine, β,β-dimethyl-

- Structure similarity: Related to histamine derivatives.
- Relevance to MS: May interact with histamine receptors, which play a role in immune modulation and neuroinflammation. Histamine receptor modulation has been explored as a therapeutic strategy in MS.
- Potential: Could influence neuroimmune signaling.

3. In vitro Antioxidant activity by DPPH (96 well method) [36]

The DPPH free radical scavenging assay was used to evaluate the antioxidant potential of plant extract (Watermelon leaf extract) compared with the standard antioxidant, Ascorbic acid, at concentrations of 250, 500, and 1000 µg/ml (Table 4).

- Standard (Ascorbic Acid)

Exhibited strong dose-dependent antioxidant activity. % Inhibition increased from 77.40% (250 µg/ml) to 86.17% (1000 µg/ml). Serves as a positive control confirming assay validity.

- Sample (Watermelon Leaf Extract)

Showed moderate antioxidant activity. % Inhibition increased with concentration:43.01% (250 µg/ml), 45.39% (500 µg/ml), 49.32% (1000 µg/ml). Indicates a concentration-dependent radical scavenging potential.

Sr. No.	Sample code	Conc.	OD			Mean	Percent Inhibition
1	Control	-	1.456	1.564	1.485	1.501	
2	Standard (Ascorbic Acid)	250	0.334	0.337	0.347	0.339	77.40
		500	0.315	0.316	0.303	0.311	79.26
		1000	0.213	0.201	0.209	0.207	86.17
3	Sample –A1(watermelon leaf Extract)	250	0.856	0.855	0.856	0.855	43.01
		500	0.823	0.825	0.812	0.82	45.39
		1000	0.756	0.755	0.772	0.761	49.32

Table 4. Antioxidant Activity of Sample by DPPH Method (96-Well Plate)

4. MTT PROCEDURES (SH-SY-5Y Neuroblastoma Cell Line) [37-38]

The MTT assay demonstrated that Sample A1 Watermelon leaves extract offer protective effects against H_2O_2-induced cytotoxicity in SH-SY5Y neuroblastoma cells in a dose- dependent manner. H_2O_2 treatment significantly reduced cell viability to 6.95%, indicating strong oxidative stress-induced cytotoxicity. Sample (Watermelon extract) increased cell viability from 40.46% (10 µl/ml) to 47.52% (100 µl/ml), showing moderate cytoprotection (table 5).

Table 5. Effects of Sample against SH-SY-5Y (Neuroblastoma Cell Line) by MTT assay

Sr.no	concentration (µl/ml)	Absorbance (O D)				Cell viability (%)
		1	2	3	Average	

1.	Control	2.107	2.114	2.11	2.110	
2.	H2o2	0.175	0.181	0.167	0.174	6.95
3.	Sample-A1 WM 10	0.854	0.856	0.852	0.854	40.46754
	40	1.003	1.002	1.004	1.003	40.08259
	100	1.225	1.226	1.229	1.22666667	47.52804

CONCLUSION

Medicinal plants, which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. The findings of this study provide preliminary yet promising evidence supporting the potential of herbal medicine in the treatment of Multiple Sclerosis (MS). Through comprehensive phytochemical screening, various bioactive compounds—including alkaloids, flavonoids, terpenoids, and phenolics—were identified in the selected plant that is watermelon leaves extract, many of which are known for their anti-inflammatory, antioxidant, and neuroprotective properties. In vitro assays further demonstrated that these extracts possess significant biological activity relevant to MS pathology, including inhibition of pro-inflammatory mediators and protection against oxidative stress-induced cellular damage. While these results underscore the therapeutic potential of herbal formulations, further investigations involving advanced pharmacological evaluations, in vivo studies, and clinical trials are essential to validate efficacy, elucidate mechanisms of action, and ensure safety. This study lays a foundational step towards integrating phytomedicine into the broader scope of MS management and highlights the need for continued research in this promising domain. From this study it can be concluded that the *Citrullus lanatus* may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds.

REFERENCES

1. Sato, H., Otsuka, T., & Tanaka, M. (2018). Citrulluslanatus and its effects on oxidative stress and inflammation in diseases. Journal of Nutritional Biochemistry.
2. Olatunde, A. O., & Olaleye, M. T. (2021). Therapeutic potential of plant-based antioxidants in neuroinflammatory diseases. Frontiers in Pharmacology.
3. Lassmann, H., Brück, W., & Lucchinetti, C. F. (2012). The immunopathology of multiple sclerosis: an overview. Brain Pathology.
4. Thompson, A. J., Baranzini, S. E., & Geurts, J. J. (2018). Multiple Sclerosis. Lancet.
5. Hauser, S. L., & Cree, B. A. (2020). Treatment of Multiple Sclerosis: A Review. American Journal of Medicine.
6. Cree, B. A., Houtchens, M., & Goodin, D. S. (2016). Multiple Sclerosis Therapy: Current Landscape and Future Perspectives. Therapeutic Advances in Neurological Disorders.
7. Vallée, A., Lecarpentier, Y., & Guillevin, R. (2017). Natural and synthetic substances as potential candidates for neuroprotection in multiple sclerosis. Molecules.
8. Epidemiology and pathophysiology of multiple sclerosis. Ward M, Goldman MD. Continuum (Minneap Minn) 2022 [DOI] [PubMed] [Google Scholar]
9. Cleveland Clinic. (2025). Multiple Sclerosis (MS). Retrieved May 12, 2025, from https://my.clevelandclinic.org/health/diseases/17248-multiple-sclerosis
10. Mayo Clinic. (2025). Multiple sclerosis: Symptoms and causes. Retrieved May 12, 2025,from https://www.mayoclinic.org/diseases-conditions/multiple- sclerosis/symptoms-causes/syc-20350269
11. National Multiple Sclerosis Society. (2025). MS Symptoms. Retrieved May 12, 2025, from https://www.nationalmssociety.org/Symptoms-Diagnosis/MS-Symptoms
12. Lakshminarayana, R., Urooj, A., & Siddhuraju, P. (2020). Nutritional potential and bioactive components of watermelon (Citrullus lanatus) seeds. LWT - Food Science and Technology, 123, 109123. https://doi.org/10.1016/j.lwt.2020.109123
13. Food and Agriculture Organization of the United Nations (FAO). (2017). Nutritional contribution of watermelon and its by-products in food systems. Retrieved from http://www.fao.org
14. Yahia E. M., & Campos, M. G. (2019). Bioactive compounds and functional properties of watermelon: A review. Food Reviews International, 35(2), 118–142. https://doi.org/10.1080/87559129.2018.1517114
15. Jarret, R. L., Levy, I. J., & Merrick, L. C. (2019). Seed composition and oil content in watermelon germplasm. Journal of Food Composition and Analysis, 84, 103289. https://doi.org/10.1016/j.jfca.2019.103289
16. Agbagba, O. C., Ndukwe, I. C., & Anyaegbunam, H. N. (2020). Production and marketing of watermelon in Nigeria: A review. African Journal of Agricultural Research, 15(8), 1192–1201.

https://doi.org/10.5897/AJAR2020.14910
17. World Health Organization (WHO). (1977). Tropical disease research: Progress 1975–1976. Retrieved from https://apps.who.int/iris/handle/10665/149384
18. Erhirhie EO, Ekene NE. Medicinal values on Citrullus lanatus (watermelon): pharmacological review. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2013;4(4):1305-1312.
19. Alka G, Anamika S, Ranu P. A review on watermelon (Citrullus lanatus) medicinal seeds. Journal of Pharmacognosy and Phytochemistry. 2018;7(3):22222225.
20. Nkoana DK, Mashilo J, Shimelis H, Ngwepe RM. Nutritional, phytochemical compositions and natural therapeutic values of citron watermelon (Citrullus lanatus var. citroides): A Review. South African Journal of Botany. 2022;145:65-77.
21. Martin, G. J. (2004). Ethnobotany: A Methods Manual. Earthscan Publications Ltd.
22. World Health Organization (WHO). (2003). WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. https://apps.who.int/iris/handle/10665/42783
23. Wallis, T. E. (1985). Textbook of Pharmacognosy (5th ed.). CBS Publishers & Distributors.
24. Khandelwal, K. R. (2008). Practical Pharmacognosy: Techniques and Experiments (19th ed.). Nirali Prakashan.
25. Emeje, M. O., Ofoefule, S. I., & Onyishi, I. V. (2012). Standardization and stability studies of herbal formulations. African Journal of Pharmacy and Pharmacology, 6(22), 1644–1649. https://doi.org/10.5897/AJPP11.641
26. Harborne, J. B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis (3rd ed.). Chapman & Hall.
27. Aguilar, F. J., et al. (2016). Biological significance of inositol and inositol phosphates in human diseases. Pharmacological Research, 104, 1–14. https://doi.org/10.1016/j.phrs.2015.12.016.
28. Wagner, K. H., & Elmadfa, I. (2003). Biological relevance of terpenoids: Overview focusing on mono-, di-, and tetraterpenes. Annals of Nutrition and Metabolism, 47(3– 4), 95–106. https://doi.org/10.1159/000070030
29. Simopoulos, A. P. (2002). Omega-3 fatty acids in inflammation and autoimmune diseases. Journal of the American College of Nutrition, 21(6), 495–505. https://doi.org/10.1080/07315724.2002.10719248
30. Natarajan, R., et al. (2016). Palmitic acid metabolism and its role in inflammation and chronic diseases. Lipids in Health and Disease, 15(1), 1–11. https://doi.org/10.1186/s12944-016-0283-2
31. Russo, P., et al. (2013). Protective role of terpenes in neurodegenerative diseases: Focus on Alzheimer's disease. CNS & Neurological Disorders - Drug Targets, 12(3), 239–246. https://doi.org/10.2174/18715273113120300006
32. Levy, R. B., & Ziv, N. E. (2012). Amines and neurotransmitter analogs: Emerging roles in synaptic function and plasticity. Frontiers in Cellular Neuroscience, 6, 21. https://doi.org/10.3389/fncel.2012.00021
33. Raza, A., et al. (2020). Recent advances in organosilicon compounds for drug delivery: Bridging the gap between organic and inorganic chemistry. European Journal of Medicinal Chemistry, 207, 112745. https://doi.org/10.1016/j.ejmech.2020.112745
34. Thurmond, R. L., et al. (2008). Histamine and its receptors: Recent insights into histamine signaling and immune modulation. Nature Reviews Drug Discovery, 7(1), 41–53. https://doi.org/10.1038/nrd2465
35. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. J Food Sci Technol. 2015 Feb;52(2):1212-7. doi: 10.1007/s13197-013-1105-9. Epub 2013 Jul 24. PMID: 25694742; PMCID: PMC4325072.
36. Gaikwad, A., Harshad, R., & Patil, S. B. (2024). Formulation, Evaluation, and Antioxidant Properties of Herbal Soap for Anti-Acne Treatment. International Journal of Novel Research and Development, 9(8), August 2024. ISSN: 2456-4184. Retrieved from www.ijnrd.org.
37. Pavithra Mettupalayam Kaliyannan Sundaramoorthy and Kannan Kilavan Packiam, In vitro enzyme inhibitory and cytotoxic studies with Evolvulus alsinoides (Linn.) Linn. Leaf extract: a plant from Ayurveda recognized as Dasapushpam for the management of Alzheimer's disease and diabetes mellitus. BMC Complementary Medicine and Therapies (2020) 20:129
38. Doaa M. Hanafy, Neuroprotective Activity of Mentha Species on Hydrogen Peroxide-Induced Apoptosis in SH- SY5Y Cells, Nutrients 2020, 12, 1366,1-17.