Biological Forum – An International Journal **14(4): 1209-1220(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Phytochemical characterization of moringa (*Moringa oleifera* Lam.) using Gas Chromatography Mass Spectrometry (GC-MS)

Shubhada Tayade[1*], G.J. Janavi[2], T. Arumugam[3], B. Rajagopal[4] and P. Geetharani[5]
[1]Research Scholar, Department of Vegetable Science,
Horticultural College and Research Institute, Periyakulam (Tamil Nadu), India.
[2]Professor and Head, Department of Vegetable Science,
Horticultural College and Research Institute, Periyakulam (Tamil Nadu), India.
[3]Dean, Horticultural College and Research Institute, Periyakulam (Tamil Nadu), India.
[4]Associate Professor (ABT), Department of Fruit Science,
Horticultural College and Research Institute, Periyakulam (Tamil Nadu), India.
[5]Professor (SS&T), Department of Vegetable Science, Periyakulam (Tamil Nadu), India.

(Corresponding author: Shubhada Tayade)*
(Received 09 September 2022, Accepted 29 October, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The family Moringaceae includes the significant tropical vegetable and herb known as Moringa (*Moringa oleifera* Lam), which is valued for its therapeutic, dietary, industrial, agricultural, and socioeconomic properties. This genus is indigenous to India and contains 13 species that have been raised in the wild for their diverse virtues across Asia, Africa, and other parts of the world. Each part of moringa plant is a clandestine source of nutrients that are essential for human growth and development, yet this virtuous plant is not completed exploited in this area of application. Even after moringa being the cradle of many more biochemicals and nutrients, majority of studies in moringa has been done with regards to only Quercetin and Vitamin- C. Moringa also exists in many wild and undomesticated forms in India which needs to discovered. This study exploits the potential of ten moringa genotypes raised at Horticultural College & Research Institute, Tamilnadu Agricultural University, Tamil Nadu, for biochemical characterization, which was performed by employing Gas Chromatography Mass Spectrometry. The result of this investigation validates the presence of biochemical compounds in the leaves of *Moringa oleifera*, which are of high medicinal and therapeutic value and approve its position among the Superfoods. The biochemical compounds like Vitamin C, Quercetin, Kaemferol, Phytol, Tryptophan, Nicotinamide, Serotonin, Chlorogenic Acid, Niazimin, β- Sitosterol, IAA and Gallic Acid have been reported to prevent and alleviate may chronic conditions are found to the present in the moringa leaves, among which Vitamin C and Quercetin were found to be predominant in these genotypes, followed by Tryptophan and Kaemferol in the genotypes studied. Moringa genotypes PKM MO 48 and PKM MO 47 have been found to have significantly more biochemical compounds in them.

Keywords: Moringa, superfood, GC-MS, phytochemical compounds, PKM.

INTRODUCTION

Moringa has long been recognized to boost one's health, as moringa was utilized by kings and queens to increase their alertness and preserve good skin quoted Azhagu Madhavan (2021). During conflict, Indian soldiers were fed with *M. oleifera* leaves to boost their vitality and ease pain and tension. Skin infections, nervousness, asthma, cuts, fever, diarrhoea and sore throats are among the other traditional uses of the genus, summarized Gopalakrishnan *et al.* (2016). Alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes are among the phytoconstituents found in moringa species, researched Ma *et al.* (2020). The ethanolic leaf extract of *Moringa oleifera* proved to be a reservoir of bioactive compounds identified by GC-MS which could prove effective in the treatment of various diseases as reported by Bhattacharya *et al.* (2018).

The biological features of this genus, particularly *M. Oleifera* have been the subject of several studies, anti-inflammatory, antioxidant, anticancer, and antidiabetic properties of this plant are now widely recognized. Moringa species are very nutritious, offering daily nutritional supplements and enhancing people's immune systems, according to Caceres *et al.* (1992). Moringa leaves include Vitamin C, Vitamin A, and high levels of vital amino acids, according to studies. The antioxidant, phytochemical, and antibacterial profiles of *M. oleifera* cultivars were discovered to vary.

Gas Chromatography-mass spectroscopy (GC-MS) is a hyphenated system that is a very compatible technology and the most often used technique for identification and quantification of bioactive and phytochemical

components in plants according to Thanikachalam and Jayaraj (2021). (GC-MS) is often utilised for the direct examination of chemical components found in herbal remedies. According to Balabhaskar and Vijayalakshmi (2021), medicinal plants contain several bioactive components that may be detected at concentrations as low as 1.0 nanogram utilizing GC-MS analysis.

*Moringa oleifera*is a plant with lots of therapeutic properties that are said to be able to treat up to 300 ailments. Its roots, bark, leaves, flowers, pods, and seeds are all utilised in ethnobotanical medicine to treat a range of diseases. Healing properties include anticancer, antidiabetic, antibacterial, anti-stress, antioxidant, and anti-inflammatory, to name a few. The current section explores the numerous biomolecules found in *M. oleifera* leaf extract using gas Chromatography mass spectrometry. GCMS assay was used to validate the rich biochemical and nutritional nature of *Moringa oleifera,* quoted Enerijiofi *et al.* (2021). Gas Chromatography-mass spectrometry results revealed presence of 19 phytoconstituents in hexane extract, 6 in ethyl acetate and 7 compounds in methanolic extract. Methanol extract was found to contain the highest phenolic content and flavonoids, founded Ai Owaisi *et al. (*2014). Aqueous extract was made from a *Moringa oleifera* grown in home garden, GCMS analysis, amino acid diagnostics, flavonoids diagnosis, and vitamin C analysis were done. The leaf aqueous extract included sixteen chemical components, the most prominent of which were quercetin (245.7 ppm), kaemferol (299.6 ppm) and vitamin C content (237.3 ppm), Younis Khalaf *et al.* (2021) founded. The results of the GC-MS analysis led to the identification of a number of chemicals in the *Moringa oleifera* plant's methanol extract. The chromatogram from the GC-MS revealed 100 peaks, indicating the presence of 100 chemicals along graphically and area quoted Kadhim and AL-Shammaa (2014).

Moringa oleifera has been examined for its health benefits, which are ascribed to the several bioactive components found in substantial concentrations in various parts of the plant, such as vitamins, phenolic acids, flavonoids, isothiocyanates, tannins, and saponins. The leaves of *Moringa oleifera* have been demonstrated to be effective in a variety of chronic illnesses, including hypercholesterolemia, hypertension, diabetes, insulin resistance, non-alcoholic liver disease, cancer, and general inflammation.

In the present investigation, ten most diverse moringa genotypes selected on the basis of molecular characterization were chosen for confirming the presence of biochemical compounds viz. Vitamin C, Quercetin, Kaemferol, Phytol, Tryptophan, Nicotinamide, Serotonin, Chlorogenic Acid, Niazimin, β- Sitosterol, IAA & Gallic Acid.

MATERIALS AND METHOD

Collection of Plant materials. Fresh *Moringa oleifera* leaves of 10 most distinct genotype were hand-picked from the Moringa genetic resource garden (Fig. 1), HC&RI, Tamilnadu Agricultural University Periyakulam.

Fig. 1. Moringa Genetic Resource Garden, HC& RI, Periyakulam, TNAU.

Plant material preparation. *Moringa oleifera* leaves were separated, cleaned thoroughly with distilled water to eliminate contamination, and chopped into smaller pieces before being shade dried at room temperature (28°C) for three days. The dried leaves were ground into a fine powder using an electric blender and the powdered components were kept in airtight polyethene bags out of direct sunlight until needed.

Plant sample extraction. Forty gram of dried leaf powder (Fig. 2) was taken and placed overnight in a sealed bottle with 100 mL of 40% methanol and intermittent shaking at room temperature (28°C). The sample filtered through Watman No. 1 filter paper (three times) to ensure purity of filtrate. Using a rotating vacuum evaporator, the filtrate (Fig. 3) was condensed under low pressure at 40°C for 10 minutes. The extract was stored in the refrigerator at 4°C.

Fig. 2. Moringa Leaf Powder.

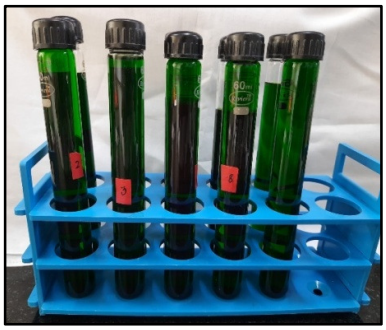


Fig. 3. Moringa GCMS Samples.

Analysis by Gas Chromatography Mass Spectrometry (GCMS). A CH-GCMS MS-02 (Model: 7250 GC/Q-TOF, Agilent Technologies) at SITRA Laboratories, Coimbatore with an acquisition rate of 1 to 50 spectra/second independent of mass resolution was used for investigation of phytochemical compounds in moringa samples. Detector Microchannel plate/scintillator/PMT aids in identifying compounds through high-resolution, accurate-mass data and sensitive detection. The GCMS cycle took 38 minutes to complete with peak area normalisation and area accommodation.

Identification of Phytocompounds. Phytochemicals were identified using PubChem database which has chemical compounds and their biological test activities. The National Centre for Biotechnology Information (NCBI) is part of the National Library of Medicine, which is supported by National Institutes of Health in the United States (NIH). PubChem is available for free via web user interface which allows to download millions of compound structures and descriptive information for free. Multiple substance descriptions and tiny compounds with less than 100 atoms and 1000 bonds are included and supported by more than 80database vendors. 293 million substance entries, including mixes, extracts, complexes, unidentified chemicals and 1.25 million bioassay & bioactivity findings. Table 1 depicts the list of important phytochemical compounds found in moringa genotypes.

Table 1: List of important phytochemical compounds found in moringa genotypes.

Sr. No.	Compound Name	IUPAC Name	CAS No.	Molecular Formula
1	**Vitamin C**	(2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one	50-81-7	C6H8O6
2	**Quercetin**	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	117-39-5	C15H10O7
3	**Kaemferol**	3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one	520-18-3	C15H10O6
4	**Phytol**	(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	150-86-7	C20H40O
5	**Tryptophan**	(2S)-2-amino-3-(1H-indol-3-yl)propanoic acid	73-22-3	C11H12N2O2
6	**Nicotinamide**	Pyridine-3-carboxamide	98-92-0	C6H6N2O
7	**Serotonin**	3-(2-aminoethyl)-1H-indol-5-ol	50-67-9	C10H12N2O
8	**Chlorogenic Acid**	(1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid	202650-88-2	C16H18O9
9	**Niazimin**	O-ethyl N-[[4-(3,4,5-trihydroxy-6-methyloxan-2-yl)oxyphenyl]methyl]carbamothioate	147821-49-6	C16H23NO6S
10	**β- Sitosterol**	17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	2308-85-2	C45H80O2
11	**IAA**	2-(1H-indol-3-yl)acetic acid	87-51-4	C10H9NO2
12	**Gallic Acid**	3,4,5-trihydroxybenzoic acid	149-91-7	C7H6O5

RESULTS

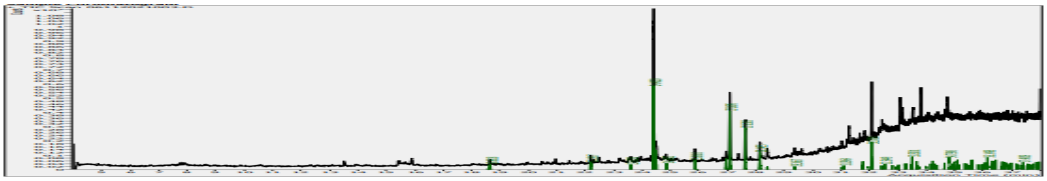
Table 2 shows the presence of the following biochemical compounds graphically. The absence of the biochemical compound is represented by (-) in the table. The Gas Chromatography Mass Spectrometry Chromatograms for all the ten moringa samples used for biochemical characterization, which were generated by using Mass Hunter Agilent Software. Table 3 shows the area covered by each phytochemical in each sample of moringa genotypes.

Table 2: Phytochemicals detected in GCMS assay performed for 10 moringa genotypes.

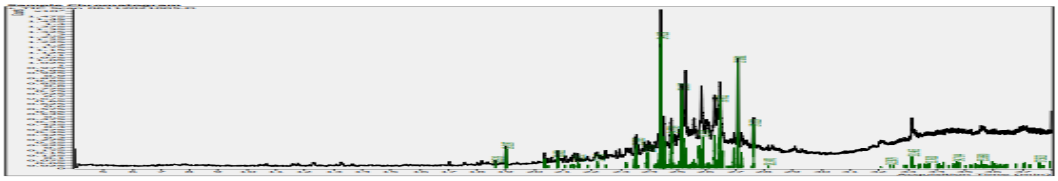
Sr. No.	Phytochemical Compound	PKM 1	PKM 2	PKM R	PKM MO 15	PKM MO 27	PKM MO 43	PKM MO 48	PKM MO 49	PKM MO 65	PKM MO 53
1	Vitamin C	24.402	25.1241	18.6393	24.4022	24.4802	24.4806	24.3965	24.4105	24.3976	24.5786
2.	Quercetin	24.4129	23.5565	24.4028	27.0868	24.3964	25.8605	26.9907	25.1853	25.8619	-
3.	Kaemferol	24.4022	27.6329	27.6286	20.4771	22.1897	27.092	22.4762	27.0865	25.8557	23.8911
4.	Phytol	25.861	20.8021	32.5302	25.8539	35.654	29.3363	18.7495	-	22.9746	-
5.	Tryptophan	27.0876	27.0881	27.6321	-	32.0547	28.6244	26.9957	25.8617	27.0876	-
6.	Nicotinamide	28.3971	25.1387	33.2106	-	37.6004	36.5951	33.1464	-	28.6847	25.8655
7.	Serotonin	36.844	19.0197	18.6427	18.6456	18.6427	23.1989	13.8695	18.755	13.8999	-
8.	Chlorogenic Acid	33.5072	26.4779	35.3295	-	-	33.147	24.9134	-	32.3122	-
9.	Niazimin	27.6303	26.5643	27.0835	-	27.6292	-	25.8603	-	-	-
10.	B Sitosterol	32.0701	24.7789	24.4134	-	-	-	29.1134	-	36.9513	-
11.	IAA	24.3865	24.5236	25.8671	24.4712	27.0908	24.4149	23.2173	22.4846	16.7391	-
12.	Gallic Acid	26.9497	24.4039	22.1976	37.994	29.063	24.4806	28.6156	24.4105	35.3929	24.5786

Table 3: Area covered by the phytochemicals in moringa genotypes as shown in the GCMS chromatogram**.**

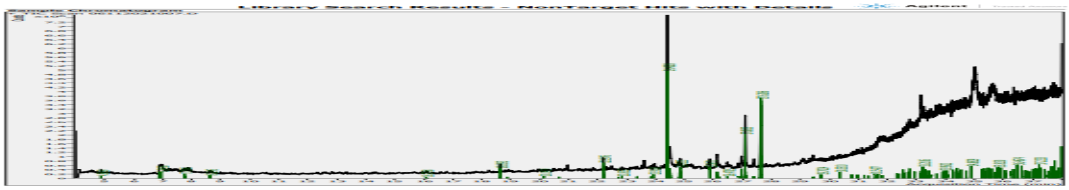
Sr. No.	Compound Name	PKM 1	PKM 2	PKM R	PKM MO 15	PKM MO 44	PKM MO 46	PKM MO 48	PKM MO 47	PKM MO 65	PKM MO 53
1	Vitamin C	11540417	3926024.4	550963.9	56340764	7020050.5	29110030	54754430.7	30992129	14577411.1	268506421
2	Quercetin	1008227	1745254.5	9535724.9	10014276.9	6457077.7	17368843.9	41597849.6	6003793.8	5244641.1	-
3	Kaemferol	11359086.8	3225584.1	6427815.8	1812895.1	444616.1	77964414.1	2394174.5	16222851.7	4107421.9	66132221.9
4	Phytol	1339740.8	1547531.1	318236.3	918251.6	13798050	14024906.7	10711501.6	-	267451.1	-
5	Tryptophan	8464520.5	20300881.8	5923987.8	-	3931414.6	12261024	41597849.6	70457088.1	21238804.4	-
6	Nicotinamide	149390.1	6450488.8	367610.4	-	10701243.8	10830678.8	11746012	-	1562115.9	54617955.2
7	Serotonin	226537.7	1638840.5	587881.3	1718980.5	275070.5	3251435.8	13430826.3	17788570.9	987769.2	-
8	Chlorogenic Acid	508383.7	15853892.6	405851.5	-	-	2066228.2	8904695.5	-	176287.2	-
9	Niazimin	5079909.5	2138654	1281468.3	-	5282891.8	-	8179067.4	-		-
10	B Sitosterol	996512.5	39.09105.8	2039003.5	-	-	-	21915749.2	-	602983.6	-
11	IAA	1773869.7	2240234.6	293425.6	2738111.6	8593689.4	7775834	1110086.1	30356906.9	468327.4	-
12	Gallic Acid	820366.9	24065743.6	781763.1	712113.9	433623.3	-	1058109.9	-	421048.3	-



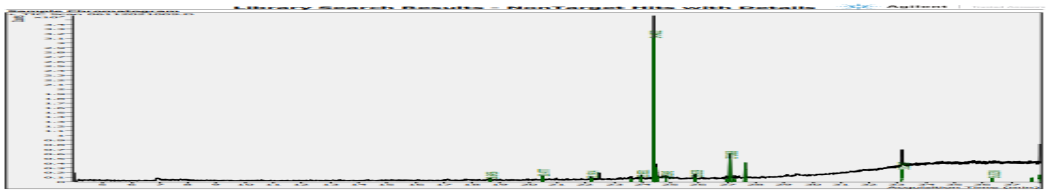
Graph 1. GCMS Chromatogram showing presence of phytochemicals in PKM 1.



Graph 2. GCMS Chromatogram showing presence of phytochemicals in PKM 2.

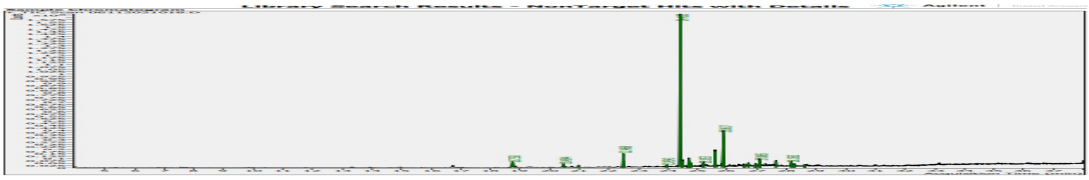


Graph 3. GCMS Chromatogram showing presence of phytochemicals in ROHIT.



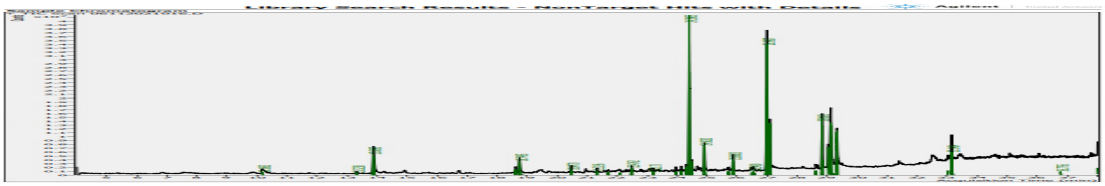
Graph 4. GCMS Chromatogram showing presence of phytochemicals in PKM MO 15.

Graph 5. GCMS Chromatogram showing presence of phytochemicals in PKM 44.

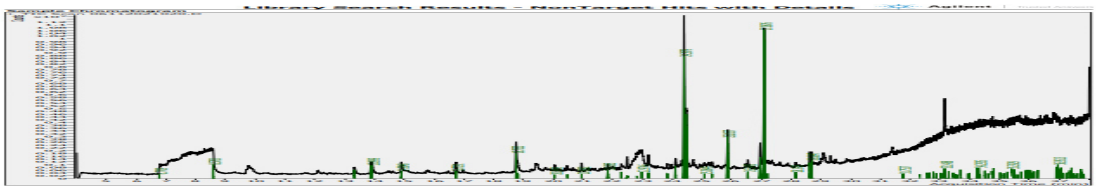


Graph 6. GCMS Chromatogram showing presence of phytochemicals in PKM MO 46.

Graph 7. GCMS Chromatogram showing presence of phytochemicals in PKM MO 48.



Graph 8. GCMS Chromatogram showing presence of phytochemicals in PKM MO 47.



Graph 9. GCMS Chromatogram showing presence of phytochemicals in PKM MO 65.

Graph 10. GCMS Chromatogram showing presence of phytochemicals in PKM MO 53.

Vitamin C. Vitamin C is a potent antioxidant which is present abundantly in *Moringa oleifera*, which may neutralize damaging free radicals and helps to manage infections and repair wounds. Vitamin C deficiency leads to scurvy, it is well known for helping in curing the effects of scurvy by taking it orally or adding Vitamin C rich food into the diet Bendich *et al.* (1986). Vitamin C plays an important role in synthesis of collagen which is a fibrous protein found in connective tissue that is woven across the body's many systems, including the nervous, immunological, bone, cartilage, blood, and others and can be obtained from *Moringa oleifera* leaves and pods quoted, Mahdi *et al.* (2017). This vitamin aids in the production of numerous hormones and chemical messengers that are important in the brain and nerves.

The above evidences claimed *Moringa oleifera* has Vitamin C content. In this investigation vitamin c was detected in following genotypes at the respective retention time; PKM 1(18.6393), PKM 2 (24.4022), ROHIT (24.4802), PKM MO 15 (24.402),PKM MO 44 (25.1241), PKM MO 46 (24.4806), PKM MO 48 (24.3965), PKM MO 47(24.4105), PKM MO 65 (24.3976), PKM MO 53 (24.5786) (Fig. 4).

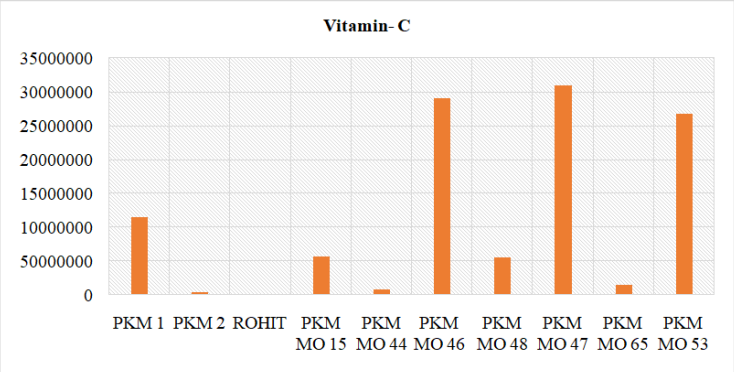


Fig. 4. Graphical representation of presence of Vitamin- C in 10 genotypes of moringa.

Quercetin. Quercetin (also known as 3, 3′, 4′5, 7-pentahydroxyflavone) is a flavonoid present in a variety of vegetables like kale, moringa and broccoli, where it is conjugated with residual sugars to create quercetin glycosides, quoted Younis Khalaf *et al.* (2021). The reliable source of quercetin is moringa leaves which is an effective antioxidant and also gives anti-arthritis effects. It may have anticancer characteristics, which might help prevent malignant cells from spreading and tumor formation, concluded Ai Owaisi *et al.* (2014). Quercetin is also said to help in the prevention of neurodegenerative illnesses like Alzheimer's and Parkinson's as it prevents histamine cell production, quercetin might be an efficient antihistamine which can be found in moringa species researched, Ai Asmari *et al.* (2015). The *Moringa oleifera* (Moringaceae) plant was studied in Mozambique by Marrufo *et al.* (2013). The flavonoids quercetin (126µg/g) were discovered by GC-MS investigation of the chemical components. Moringa is a proven source of quercetin, Fig. 5 describes the presence of quercetin in moringa genotype graphically; PKM 1 (24.4129), PKM 2 (23.5565), ROHIT (24.4028), PKM MO15 (27.0868), PKM MO 44(24.3964), PKM MO 46 (25.8605), PKM MO 48 (26.9907), PKM MO 47 (25.1853), PKM MO 65 (25.8619).

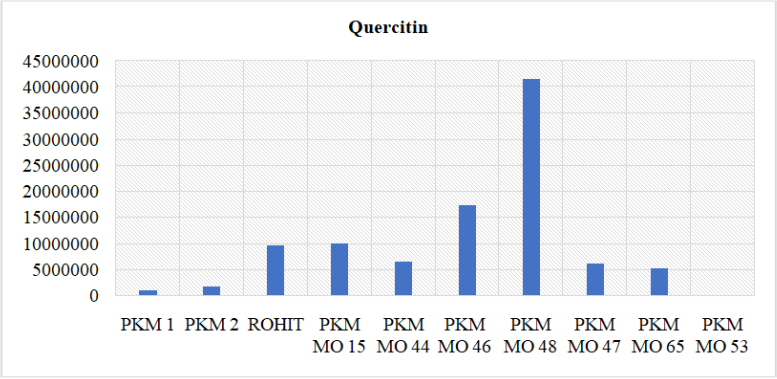


Fig. 5. Graphical representation of presence of Quercetin in 10 genotypes of moringa.

Kaemferol. Kaemferol, a naturally occurring dietary flavonoid, is a powerful cancer chemo preventive agent which is physiologically active molecule with wide range of pharmacological properties, including antioxidant, anti-inflammatory, antibacterial, antidiabetic, and cancer-fighting properties, quoted Imran *et al.* (2019). Kaemferol has been detected in methanol extract of Moringa oleifera leaf shown to have anticancer properties in cancer cells from a variety of tissues, including breast, ovarian, gastric, lung,

pancreatic, and blood malignancies, briefed Wong *et al.* (2019). The pathophysiology of many illnesses, particularly inflammatory disorders, is influenced by oxidative stress and help with osteoporosis, summarized Kaur *et al.* (2020). These accounts of moringa being a rich and natural source of Kaemferol, Fig. 6 shows the presence of Kaemferol in moringa genotypes with their respective retention time; PKM 1 (24.4022), PKM 2 (27.6329), ROHIT (27.6286), PKM MO 15 (20.4771), PKM MO 44 (22.1897), PKM MO 46 (27.092), PKM MO 48 (22.4762), PKM MO 47 (27.0865), PKM MO 65(25.8557) and PKM MO 53(23.8911).

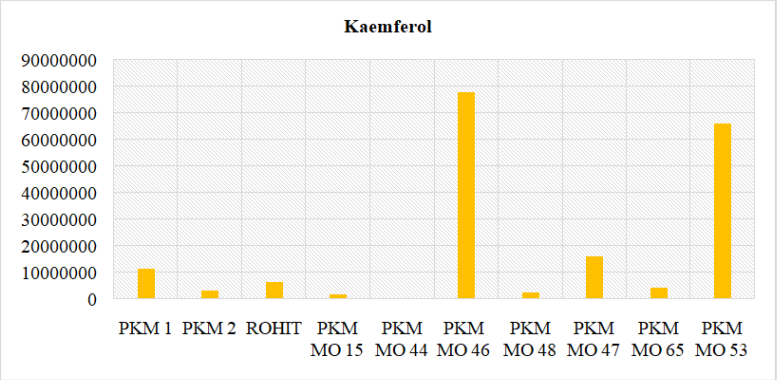


Fig. 6. Graphical representation of presence of Kaemferol in 10 genotypes of moringa.

Phytol. Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is a diterpene that belongs to the category of unsaturated alcohols with branched chains, it is a by-product of plant chlorophyll metabolism and abundant in nature, quoted Hansen, (1980). Phytol has been shown to prevent Staphylococcus aureus development as well as counteract the teratogenic effects of retinol, researched Mach, (2015). Study carried out by Aja *et al.* (2014) supported the effects of phytol on the central nervous system and it has been proposed to possess both metabolic and anti-inflammatory characteristics. Six common chemicals were found by GCMS analysis of secondary metabolites in *M. oleifera*, utilising methanol, ethanol, and acetone as solvents. Methanol extract contains a high amount of phytol (25.09) and vitamin E (27.97), which function as antioxidants, analgesics, anti-inflammatory, and antipyretic agents, summarized Devi *et al.* (2020). The results of this study (Fig. 7) confirm the presence of phytol in moringa genotype PKM 1 (25.861), PKM 2 (20.8021), ROHIT (32.5302), PKM MO 15(25.8539), PKM MO 44 (35.654), PKM MO 46 (29.3363), PKM MO 48 (18.7495) and PKM MO 65 (22.9746).

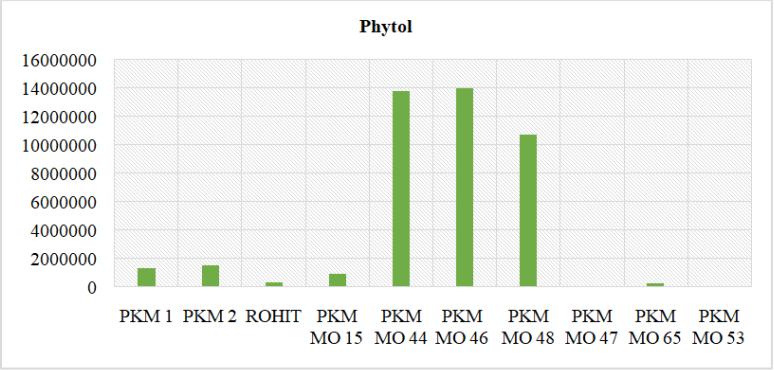


Fig. 7. Graphical representation of presence of Phytol in 10 genotypes of moringa.

Tryptophan. Tryptophan is an amino acid that is required for proper newborn growth as well as the formation and maintenance of proteins, muscles, enzymes, and neurotransmitters in the body, quoted Moffett and Namboodiri (2003). It's a necessary amino acid as body is unable to make itnaturally, it must be obtained from food or medicinal sources like *Moringa oleifera* leaves Aderinola *et al.* (2020). Tryptophan is used by the body to create melatonin and serotonin which regulates the sleep-wake cycle, whereas serotonin aids in the regulation of hunger, sleep, mood, also can be used by the liver to make niacin (vitamin B3), which is required for energy metabolism and DNA synthesis, briefed Miller *et al.* (2018). In this study, profuse presence of Tryptophan was found (Fig. 8) in moringa genotype PKM 1(27.6321),ROHIT(32.0547), PKM MO 15 (27.0876), PKM MO 44 (27.0881), PKM MO 48 (28.6244), PKMMO 49 (26.9957), PKM MO 65 (25.8617) and PKM MO 53(27.0876).

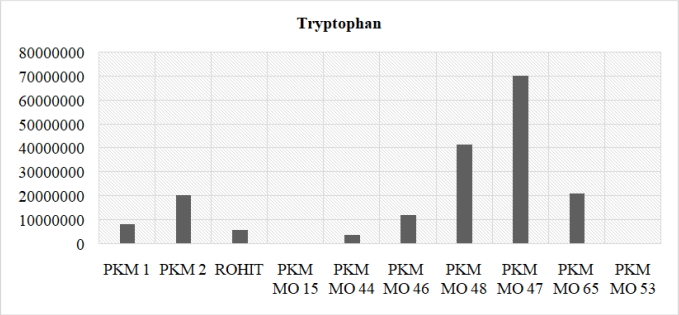


Fig. 8. Graphical representation of presence of Tryptophan in 10 genotypes of moringa.

Nicotinamide. Niacinamide (nicotinamide) is a derivative of vitamin B3 (niacin) which is water soluble and used to prevent and cure niacin deficiency (pellagra). Diarrhea, disorientation (dementia), tongue redness/swelling and peeling red skin are all symptoms of niacin insufficiency can be alleviated by consumption of nicotinamide by natural sources like green peas, potato, moringa and anchovies, briefed Bogan and Brenner, 2008. Many pharmaceutical compositions are prepared using nicotinamide which is synthesized chemically. The Fig. 9 shows the presence of nicotinamide in moringa genotype; PKM 1 (28.3971), PKM 2 (25.1387), ROHIT (33.2106), PKM MO 44 (37.6004), PKM MO 46 (36.5951), PKM MO48 (33.1464), PKM MO 65 (28.6847) and PKM MO 53 (25.8655).

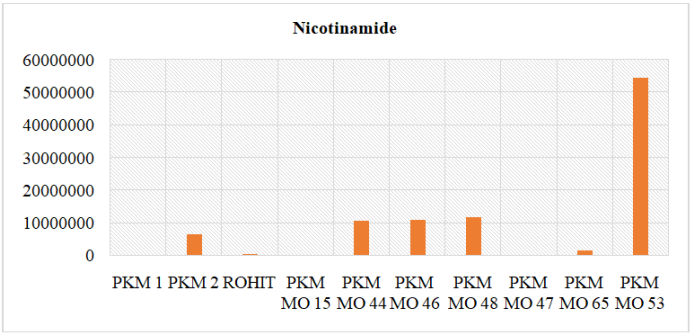


Fig. 9. Graphical representation of presence of Nicotinamide in 10 genotypes of moringa.

Serotonin. Serotonin (also known as 5-hydroxytryptamine or 5-HT) is a naturally occurring neurotransmitter that transports impulses between nerve cells (called neurons) all over your body. It is necessary for the proper functioning of the central nervous system (CNS)quoted Mohammad Zadeh *et al.*(2008). Serotonin assists with mood regulation and memory, sleep, sexual function, bone health, and blood coagulation researched, Berger *et al.* (2009). In the human body, serotonin frequently referred to as the "happy chemical" since it promotes happiness and well-being, its mostly found in the brain, intestines, and blood platelets and communicate between nerve cells. It regulates sleep-wake cycles and the biological clock as a precursor to melatonin, briefed Lucki, (1998). Presence of serotonin in moringa genotypes samples in this study signals towards the possible reason of aphrodisiac nature of moringa quoted, Posmontier, (2011). GCMS analysis performed in *Moringa oleifera* identified 8 compounds including flavinoids, glucosides, amino acids, quercetin, serotonin, dopamine and vitamin E and vitamin C, summed up Mahdi *et al.* 2017. The Fig. 10 revealed the presence of serotonin detected in moringa genotype PKM 1 (36.844), PKM 2(19.0197), ROHIT (18.6427), PKM MO 15 (18.6456), PKM MO 44 (18.6427), PKM MO 46 (23.1989),PKM MO 48 (13.8695), PKM MO 47 (18.755) and PKM MO 65 (13.8999).

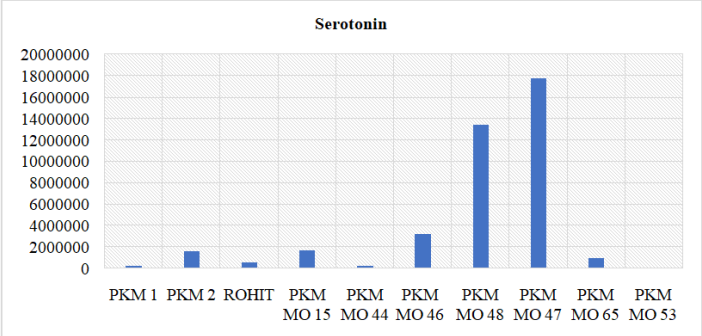


Fig. 10. Graphical representation of presence of Serotonin in 10 genotypes of moringa.

Chlorogenic Acid. Chlorogenic Acid (CGA, 3-CQA) is the most common isomer among the caffeoyl-quinic acid isomers (3-, 4-, and 5-CQA), and is now recognised as 5-CQA according to IUPAC rules.). CGA found in moringa leaves is a bioactive dietary polyphenol that has antibacterial, hepatic-protective, cardio-protective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, antiviral, anti-microbial, anti-hypertension, free radical scavenger, and CNS stimulant properties, quoted Lagurin *et al. (*2015). Furthermore, CGA has been discovered to alter lipid metabolism and glucose in genetically and healthy metabolic diseases and can be obtained from sources like coffee, tea, eggplants, moringa, potato, artichokes, kiwi, grapes, pears etc. summed up, Santana Galvez *et al*. (2017). CGA is thought to have a key role in lipid and glucose metabolism control, which might aid in the treatment of a variety of illnesses including hepatic steatosis, cardiovascular disease, diabetes, and obesity, summarized Olthof *et al. (*2001). *Moringa oleifera* L. was grown in Iraq in study done by Ai Shammaa, (2014)Al using soxhlet extraction, and the extract was studied using GC-MS with reference standards to identify the phenolic compounds in each portion of the plant, based on mass fragmentation behaviour, base peak, and retention duration (TR), results proved the presence of phenolic acids like (Chlorogenic acid, caffeic acid and coumaric acid) in moringa leaves. The above studies confirmed the presence of chlorogenic acid in moringa supported the results of this investigation. Chlorogenic acid was detected (Fig. 11) in moringa variety PKM 1 (33.5072), PKM 2 (26.4779), ROHIT (35.3295), PKM MO 46 (33.147), PKM MO 48 (24.9134) & PKMMO 65 (32.3122).

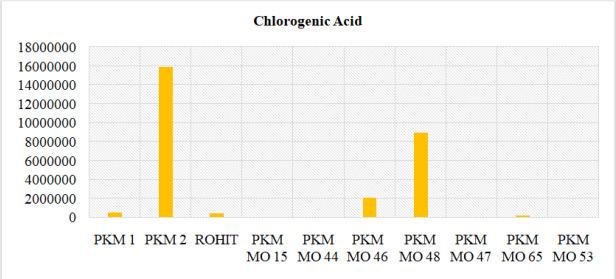


Fig. 11. Graphical representation of presence of Chlorogenic Acid in 10 genotypes of moringa.

Niazimin. Niazimin from *M. oleifera* has significant antioxidant properties and might be used as a natural antioxidant to prevent diabetic atherosclerosis and shown to be an effective free radical scavenger, quoted Pandey *et al. (*2019). Niazimin inhibited the growth of vascular smooth muscle cells produced by high glucose levels (VSMC). In chemical carcinogenesis, niazimin is thought to be a powerful chemo preventive agent summed up, Faizi *et al.* (1994); Jattan *et al.* (2021) extracted two nitrile glycosides, niazirin and niazirinin, from an ethanolicextract of *Moringa oleifera* leaves. Niazirinin is a novel chemical among these nitrile glycosides which is found only in *Moringa oleifera*. 4-[(4'-O-acetylalpha-Lrhamnosyloxy) benzyl] isothiocyanate, niaziminin A, and niaziminin B were also identified which are novel products found in moringa. Niazimin-A substances isolated from *Moringa oleifera* ethanolic leaf extract have been shown to have significant anti-cancer properties quoted, Pangastuti *et al.* (2016). The Fig. 12 shows the presence of Niazimin in moringa genotype; PKM 1 (27.6303), PKM 2 (26.5643), ROHIT (27.0835), PKM MO 44 (27.6292), PKM MO 47 (25.8603).

Beta-sitosterol. Beta-sitosterol is one of numerous plant-derived sterols (phytosterols) with a structure similar to cholesterol. Beta-sitosterol is thought to help decrease cholesterol and lessen the risk of some malignancies, quoted Saeidnia *et al.* (2014). BPH (benign prostatic hyperplasia) symptoms are believed to be relieved by it. It also aids in maintaining thecholesterol metabolism and carries anti-inflammatory and anti-cancer properties, summed up Awad *et al.* (1996). "Plant sterol ester," is used component used in pharmaceuticals contains betasitosterol derived from plant based sources like avocado, pistachio nuts, almonds, beetroots, brussel's sprouts, moringa and oranges briefed Duester (2001). Moringa has Beta Sitosterol content which can be a natural and healthy source of this bio compound sounded Raafat and Hdaib (2017). The GCMS result (Fig. 13) depicts the presence of beta sitosterol detected in moringa variety graphically, PKM 1 (32.0701), PKM 2 (24.7789), ROHIT (24.4134), PKM MO 48 (29.1134) and PKM MO 65 (36.9513).

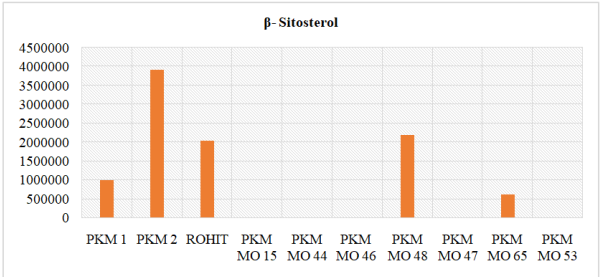


Fig. 12. Graphical representation of presence of Naizim in 10 genotypes of moringa.

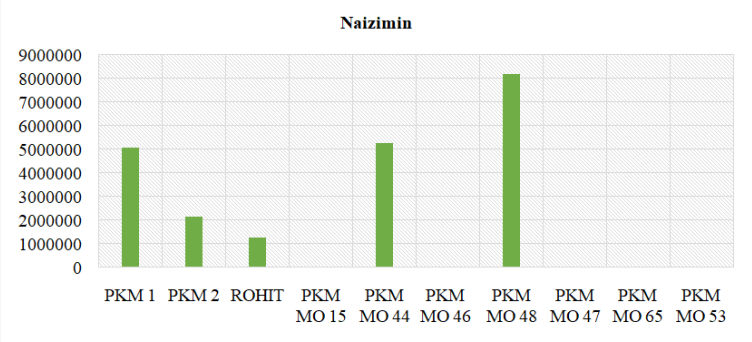


Fig. 13. Graphical representation of presence of β- Sitosterol in 10 genotypes of moringa.

IAA. The plant hormone indole-3-acetic acid (IAA), is renowned for a variety of actions, including cell proliferation, stimulation and antioxidant properties quoted, Pence and Caruso, 1987. Auxins are plant hormones that regulate growth, development, and wound healing, among other things summarized, Gayathri *et al.* (2015). The IAA (indoleacetic acid) content was checked by using GC-MS in moringa leaves and pods, IAA was found in the range of 11.50 ± 0.77 µg/ml in leaves and 38.80 ± 1.35 µg/ml in pods. The GCMS results of this study (Fig. 14) shows the presence of IAA in *Moringa oleifera* genotype, PKM 1 (24.3865), PKM 2 (24.5236), ROHIT (25.8671), PKM MO 15 (24.4712), PKM MO 44 (27.0908), PKM MO 46 (24.4149), PKM MO 48 (23.2173), PKM MO 47 (22.4846) and PKM MO 65 (16.7391).

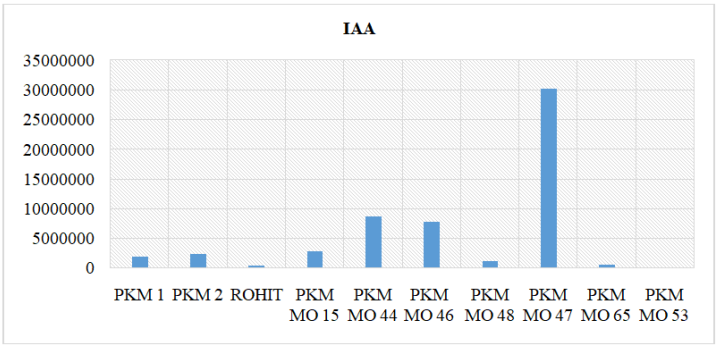


Fig. 14. Graphical representation of presence of IAA in 10 genotypes of moringa.

Gallic Acid. Gallic acid is a well-known natural antioxidant that is a polyphenolic secondary metabolite and is also an Ayurvedic herb, quoted Ow and Stupans, (2003). Gallic acid has a number of health benefits, including antioxidant researched Kim, (2007), anti-inflammatory founded Kroes *et al. (*1992), and anti-cancer briefed, Verma *et al.* (2013) qualities. The anti-hyperlipidemic activity of gallic acid in a high-fat diet resulted in a decrease in triglycerides and low density lipoprotein cholesterol, also increase in high-density lipoprotein cholesterol quoted Hsu and Yen, (2007). Presence of gallic acid was detected in GCMS assay of moringa genotypes PKM 1 (26.9497), PKM 2 (24.4039), ROHIT (22.1976), PKM MO 15(37.994), PKM MO 44 (29.063), PKM MO 46 (24.4806), PKM MO 48 (28.6156), PKM MO 47 (24.4105), PKM MO 65 (35.3929), and PKM MO 53 (24.5786) (Fig. 15).

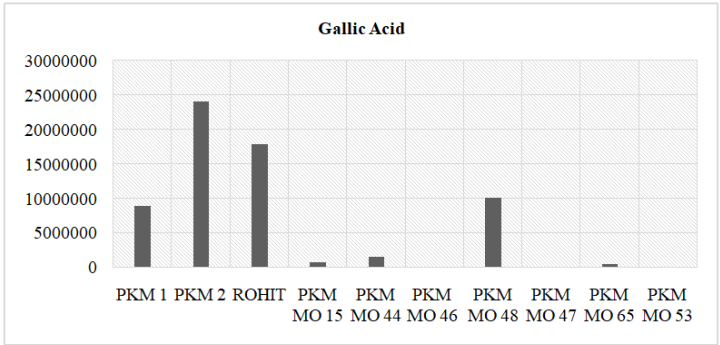


Fig. 15. Graphical representation of presence of Gallic Acid in 10 genotypes of moringa.

DISCUSSION AND CONCLUSION

Ten most diverse moringa genotypes were chosen for GCMS assay to validate the rich biochemical and nutritional nature of *Moringa oleifera* species. The bioactive compounds *viz*., Vitamin C, Quercetin, Kaemferol, Phytol, Tryptophan, Nicotinamide, Serotonin, Chlorogenic Acid, Niazimin, β- Sitosterol, IAA & Gallic Acid were selected for characterizing the moringa genotypes. The GCMS Chromatogram revealed the presence of the selected twelve biochemical compounds in genotypes PKM MO 48, PKM 49 and PKM MO 46 making them wholesome source of these nutritional compounds.

Moringa genotype PKM MO 48 graphically covered larger area in terms of all biochemical compounds followed by moringa genotype PKM MO 47 and PKM MO 46. The Chromatogram showed the presence of all the biochemical compounds in variety PKM 1, PKM 2, ROHIT and genotype PKM MO 65 making them wholesome source of these nutritional compounds. Moringa includes more than 20,000 chemical compounds according to GC-MS assay done in this investigation and therefore study of those compounds is highly encouraged to bring a medical revolution which is aided by moringa species also, safer and natural plant based medicines can be derived from it.

FUTURE SCOPE

Presence of these twelve medicinally important biochemical compounds found in moringa definitely proves the importance of moringa and its name as *superfood*. Despite such high phytochemical richness in this plant, only a few species including *M. concanensis, M. peregrina, M. stenopetala,* and *M. oleifera,* have been explored and studied from a phytochemical point of view, also the majority of the research focusing on the leaves. Hence, more species and parts of moringa plant is recommended to study and explored for better understanding and more refined investigation in the future.

Acknowledgement. I consider it a privilege to express my heartfelt gratitude and sincere appreciation to my esteemed Chairperson, Dr. G. J. Janavi, Professor and Head, Department of Vegetable Science, Horticultural College and Research Institute, Periyakulam, and Chairperson of my Advisory Committee, for her clear practical guidance & constant supervision throughout the course of my investigation. I'd like to express my sincere thanks to the Vice-Chancellor and Dean (SPGS) TNAU for providing a dynamic and friendly atmosphere to pursue my studies.

Conflicts of Interest. None.

REFERENCES

Aderinola, T. A., A. M. Alashi, I. D. Nwachukwu, T. N. Fagbemi, V. N. Enujiugha, and Aluko, R. E. (2020). In vitro digestibility, structural and functional properties of *Moringa oleifera* seed proteins. *Food Hydrocolloids, 101*, 105574.

Ai Asmari, A. K., S. M. Albalawi, M. T. Athar, A. Q. Khan, H. Ai Shahrani and Islam, M. (2015). *Moringa oleifera*as an anti-cancer agent against breast and colorectal cancer cell lines. *PloS one, 10*(8), e0135814.

Ai Owaisi, M., N. Ai Hadiwi, and Khan, S. A. (2014). GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. *Asian Pacific Journal of Tropical Biomedicine, 4*(12), 964-970.

Ai Shammaa, D. A. S. (2014). Phytochemical Investigation of the most important phenolic compounds in *Moringa oleifera* L. cultivated in Iraq.

Aja, P., Nwachukwu, N., Ibiam, U., Igwenyi, I., Offor, C. and Orji, U. (2014). Chemical constituents of *Moringa oleifera* leaves and seeds from Abakaliki, Nigeria. *American Journal of Phytomedicine and Clinical Therapeutics, 2*(3), 310-321.

Awad, A., Chen, Y. C., Fink, C. and Hennessey, T. (1996). Beta-Sitosterol inhibits HT-29 human colon cancer cell growth and alters membrane lipids. *Anticancer research, 16*(5A), 2797-2804.

Azhagu Madhavan, S. (2021). Evaluation of Potential and In-Vitro Antioxidant Activity of Mangrove Leaves *Avicennia marina* Ethanolic Extract.

Balabhaskar, R., and Vijayalakshmi, K. (2021). Identification of Secondary Metabolites from the Ethanol extract of the leaves of *Bauhinia tomentosa* by GC-MS Analysis. *Research Journal of Pharmacy and Technology, 14* (5), 2735-2741.

Bendich, A., Machlin, L., Scandurra, O., Burton, G. and Wayner, D. (1986). The antioxidant role of vitamin C. *Advances in Free Radical Biology & Medicine, 2*(2), 419-444.

Berger, M., Gray, J. A. and Roth, B. L. (2009). The expanded biology of serotonin. *Annual review of medicine, 60*, 355-366.

Bhattacharya, A., Tiwari, P., Sahu, P. K. and Kumar, S. (2018). A review of the phytochemical and pharmacological characteristics of *Moringa oleifera*. *Journal of pharmacy & bio- allied sciences, 10*(4), 181.

Bogan, K. L. and Brenner, C. (2008). Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD+ precursor vitamins in human nutrition. *Annu. Rev. Nutr., 28*, 115-130.

Caceres, A., Saravia, A., Rizzo, S., Zabala, L., De Leon, E. and Nave, F. (1992). Pharmacologic properties of *Moringa oleifera*. 2: Screening for antispasmodic, anti- inflammatory and diuretic activity. *Journal of ethnopharmacology*, *36*(3), 233-237.

Devi, G., T. N. Suryadevara, G. Divyabharathi, L. V. S. A. R. Velaga, and P. Ponmurugan (2020). Efficacy of *Moringa oleifera's* Therapeutic Compounds and its Antimicrobial Activity. *Research Journal of Pharmacy and Technology, 13*(8), 3867-3872.

Duester, K. C. (2001). Avocado fruit is a rich source of beta-sitosterol. *Journal of the Academy of Nutrition and Dietetics, 101*(4), 404.

Enerijiofi, K. E., F. H. Akapo, and J. O. Erhabor (2021). GC–MS analysis and antibacterial activities of *Moringa oleifera*leaf extracts on selected clinical bacterial isolates. *Bulletin of the National Research Centre, 45* (1), 1-10.

Faizi, S., B. S. Siddiqui, R. Saleem, S. Siddiqui, K. Aftab, and A. U. H. Gilani. (1994). Isolation and structure elucidation of new nitrile and mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. *Journal of Natural Products, 57*(9), 1256-1261.

Gayathri, M., P. S. Kumar, A. M. L. Prabha, and G. Muralitharan (2015). *In vitro* regeneration of *Arachis hypogaea* L. and *Moringa oleifera* Lam. using

extracellular phytohormones from *Aphanothece* sp. MBDU 515. *Algal Research, 7*, 100-105.

Gopalakrishnan, L., K. Doriya, and D. S. Kumar (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness, 5*(2), 49-56.

Hansen, R. (1980). Phytol: its metabolic products and their distribution. A review. *New Zealand Journal of Science, 23*(3), 259-275.

Hsu, C. L., and G. C. Yen (2007). Effect of gallic acid on high fat diet-induced dyslipidaemia, hepatosteatosis and oxidative stress in rats. *British Journal of Nutrition, 98*(4), 727-735.

Imran, M., B. Salehi, J. Sharifi-Rad, T. Aslam Gondal, F. Saeed, A. Imran, M. Shahbaz, P. V. Tsouh Fokou, M. Umair Arshad, and H. Khan (2019). Kaempferol: A key emphasis to its anticancer potential. *Molecules, 24* (12), 2277.

Jattan, M., N. Kumari, R. Kumar, A. Kumar, B. Rani, D. Phogat, S. Kumar, and P. Kumar (2021). Moringa (*Moringa oleifera* L.): An underutilized and traditionally valued tree holding remarkable potential. *Journal of Horticultural Sciences, 16*(1), 1-13.

Kadhim, E. J., and D. A. AL-Shammaa (2014). Phytochemical characterization using GC-MS analysis of methanolic extract of *Moringa oleifera* (Family Moringaceae) plant cultivated in Iraq. *Chem Mater. Res., 6*(5), 9-26.

Kaur, N., D. S. Arora, N. Kalia and M. Kaur (2020). Antibiofilm, antiproliferative, antioxidant and antimutagenic activities of an endophytic fungus *Aspergillus fumigatus* from *Moringa oleifera*. *Molecular biology reports, 47*(4), 2901-2911.

Kim, Y. J. (2007). Antimelanogenic and antioxidant properties of gallic acid. *Biological and Pharmaceutical Bulletin, 30*(6), 1052-1055.

Kroes, B. V., A. Van den Berg, H. Q. Van Ufford, H. Van Dijk, and R. Labadie. (1992). Anti-inflammatory activity of gallic acid. *Planta medica, 58*(06), 499-504.

Lagurin, L. G., M. Galingana, J. Magsalin, J. Escaño, and F. Dayrit (2015). Chemical profiling of Philippine *Moringa oleifera* leaves. *International Symposium on Moringa,* 1158.

Lucki, I. (1998). The spectrum of behaviors influenced by serotonin. *Biological psychiatry, 44*(3), 151-162.

Ma, Z., J. Ahmad, H. Zhang, I. Khan, and S. Muhammad (2020). Evaluation of phytochemical and medicinal properties of Moringa (*Moringa oleifera*) as a potential functional food. *South African Journal of Botany, 129*, 40-46.

Mach, J. (2015). Phytol from degradation of chlorophyll feeds biosynthesis of tocopherols. *American Society of Plant Biologists*.

Mahdi, H. J., N. Khan, R. Mahmud, M. Asmawi, A. Vikneswaran, and L. Murugaiyah (2017). LC/MS, GC/MS screening and *in vivo* anti-inflammatory activity of Malaysian *Moringa oleifera* Lam leaf extracts and fractions against carrageen an-induced paw oedema in rats. *J. Innov. Pharm. Biol. Sci., 4*, 48-54.

Marrufo, T., F. Nazzaro, E. Mancini, F. Fratianni, R. Coppola, L. De Martino, A. B. Agostinho, and V. De Feo. (2013). Chemical composition and biological activity of the essential oil from leaves of *Moringa oleifera* Lam. cultivated in Mozambique. *Molecules, 18*(9), 10989-11000.

Miller, G. J., R. Embalabala, C. Bennett, J. Buck, B. Russell, E. Holder, H. Blanchard, and L. Henry (2018). *Moringa oleifera*, Miracle Tree and Superfood: Antibacterial evidence and nutritional benefits.

Moffett, J. R. and M. A. Namboodiri (2003). Tryptophan and the immune response. *Immunology and cell biology, 81*(4), 247-265.

Mohammad Zadeh, L., L. Moses, and S. Gwaltney Brant. (2008). Serotonin: a review. *Journal of veterinary pharmacology and therapeutics, 31*(3), 187-199.

Olthof, M. R., P. C. Hollman, and M. B. Katan (2001). Chlorogenic acid and caffeic acid are absorbed in humans. *The Journal of nutrition, 131*(1), 66-71.

Ow, Y. Y., and I. Stupans (2003). Gallic acid and gallic acid derivatives: effects on drug metabolizing enzymes. *Current Drug Metabolism, 4*(3), 241-248.

Pandey, V., V. Chauhan, V. Pandey, P. Upadhyaya and O. R. Kopp (2019). *Moringa oleifera* lam.: a biofunctional edible plant from India, phytochemistry and medicinal properties. J. *Plant Stud., 8*(10).

Pangastuti, A., I. F. Amin, A. Z. Amin, and M. Amin (2016). Natural bioactive compound from *Moringa oleifera* against cancer based on in silico screening. *Journal Teknologies, 78*(5).

Posmontier, B. (2011). The medicinal qualities of *Moringa oleifera. Holistic nursing practice, 25*(2), 80-87.

Raafat, K., and F. Hdaib. (2017). Neuroprotective effects of *Moringa oleifera*: Bio-guided GC-MS identification of active compounds in diabetic neuropathic pain model. *Chinese journal of integrative medicine*, 1-10.

Saeidnia, S., A. Manayi, A.R. Gohari, and M. Abdollahi. (2014). The story of beta-sitosterol-a review. *European Journal of Medicinal Plants*, 590-609.

Santana Galvez, J., L. Cisneros Zevallos, and D.A. Jacobo Velazquez. (2017). Chlorogenic acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. *Molecules, 22*(3), 358.

Thanikachalam, V. and I. A. Jayaraj. (2021). Phytochemistry of *Amaranthus viridis*: GC-MS Analysis. *Int. J. Cur. Res. Rev., 13*(07), 162.

Verma, S., A. Singh, and A. Mishra (2013). Gallic acid: molecular rival of cancer. *Environmental Toxicology and Pharmacology, 35*(3), 473-485.

Wong, S. K., K. Y. Chin, and S. Ima-Nirwana (2019). The osteoprotective effects of kaempferol: The evidence from in vivo and in vitro studies. *Drug design, development and therapy, 13*, 3497.

Younis Khalaf, H., A.A. Thaker, and M. Salih Shalal (2021). Study of the chemical composition, amino acid, Flavonoids and Vitamin C of *Moringa oleifera* Leaves Extract Grown in AL-Ramadi-Iraq. *Indian Journal of Forensic Medicine & Toxicology, 15*(3).

How to cite this article: Shubhada Tayade, G.J. Janavi, T. Arumugam, B. Rajagopal and P. Geetharani (2022). Phytochemical characterization of moringa (*Moringa oleifera* Lam.) using Gas Chromatography Mass Spectrometry (GC-MS). *Biological Forum – An International Journal, 14*(4): 1209-1220.