

A comparative study of GC-MS analysis of aqueous-methanolic extract from the leaf and fruit of *Morinda citrifolia* (Noni)

Chaitali Kundu, Monalisa Paul, Merina Yashmin, Sandip K Sinha*

ABSTRACT

Background: *Morinda citrifolia* (Noni) has been recognized as an important medicinal plant, used to treat various physiological disorders worldwide, and belongs to the family Rubiaceae. The *M. citrifolia* (Noni) is commonly known as Ach, Bartundi, Hurdi, and Surangi in Bengali. Traditionally, it is used for various purposes of food and medicine. The leaves and fruit of *M. citrifolia* consist of several nutrients and phytochemicals. **Aims and objectives:** The objective of the present study is to identify the phytochemicals from the aqueous-methanolic extract of the leaves and fruit of noni plants using GC-MS analysis. **Materials and Methods:** The Medicinal plant *M. citrifolia* was collected from the forest area of "Jungle-Mahal" of Bishnupur, Bankura district, West Bengal, India, in May to July in 2018. In the present study, the aqueous-methanolic extract of the fruit and leaves of *M. citrifolia* was analyzed by GC-MS, and the mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology (NIST) library. **Results and Discussion:** The aqueous-methanolic leaf and fruit extracts of *M. citrifolia* were analyzed by GC-MS to determine chemical constituents. GC-MS revealed the presence of seventeen different phytochemicals in the aqueous-methanolic extract of the leaf of *M. citrifolia*. The highest peak area of 20.83% for cyclohexane, 1-ethenyl-1-methyl-2,4-bis [1-1-methylethenyl] – [1s- (1a', 2a',4a)] was identified in the leaf of *M. citrifolia*. The aqueous-methanolic fruit extract of *M. citrifolia* showed the presence of 27 different phytochemicals. The highest peak area of undec-10-ynoic acid butyl ester, peak area percentage is 37.71%. The isolation of the probable bioactive compound from the leaves and fruit of *M. citrifolia* would be useful for finding some useful new drugs.

Keywords: *Morinda citrifolia* (Noni), fruit and leaves, Aqueous-methanolic Extract, Phytochemicals, GC-MS analysis.

Indian Journal of Physiology and Allied Sciences (2025);

DOI: 10.55184/ijpas.v77i03.316

ISSN: 0367-8350 (Print)

INTRODUCTION

India is one of the most biodiverse countries in the world and has 15 different climate zones. Ayurveda, Unani, Siddha, and homeopathy are among the accepted medical systems that use approximately 7000 of the 17,000–18,000 flowering plant species for therapeutic purposes (AYUSH System of Medicine). Our Hindu culture's "Rig-Veda" demonstrates that, since the beginning of civilization, people have used herbal plants as traditional medicine. On a global basis, these medicinal herbs are also highly significant commercially. Nearly two thousand years ago, the father of medicine, Hippocrates, advised us to "let medicine be your food and let food be your medicine."

This medicinal component has a long history in folk medicine, having been utilized by our ancestors to treat a wide range of illnesses and environmental impacts.¹ According to the World Health Organisation, traditional medicine will remain essential to providing healthcare, as over 80% of people in third-world nations follow it. *Morinda citrifolia* (MC) is a prominent medicinal plant of the Rubioideae subfamily of the Rubiaceae family. It is also known in Bengali as Noni, Ach, Bartundi, Hurdi, and Surangi. Historically, it has been used for several culinary and therapeutic purposes. It is a little evergreen tree that grows to a height of 3 to 11 meters when fully grown. It might be plants or trees.

The MC plant species differ from one another in terms of height, fruit size, leaves, and scent. MC (Noni) flowers are around 2 to 4 cm long, white, fragrant, tubular, and have three to five lobed petals. Five-lobed, white bloom; 20 to 45

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How to cite this article: Kundu C, Paul M, Yashmin M, Sinha SK. A comparative study of GC-MS analysis of aqueous-methanolic extract from the leaf and fruit of *Morinda citrifolia* (Noni). *Indian J Physiol Allied Sci* 2025;77(3):45-54.

Conflict of interest: None

Submitted: 15/07/2025 **Accepted:** 25/08/2025 **Published:** 17/09/2025

cm long leaves; peduncles: 10 to 30 mm. The fruit is large, fat, and oval. They are 3 to 4 cm in diameter and 5 to 10 cm long when ripe, becoming spongy and mushy. They are edible and creamy-white when fully grown, but their taste and aroma are unpleasant.² It is an effective medicinal herb that can be used to treat various illnesses. Noni has been used as a traditional folk treatment in Polynesia for over two millennia. Numerous portions of the MC plant have been shown to contain more than 200 phytochemicals, many of which have multiple biological effects. The differences in the health benefits of the various parts of MC (Noni) may be due to this plant's several bioactive compounds.³ Traditional medicine in Tahiti, Southeast Asia, Australia, and Hawaii utilizes a variety of plant parts, including fruit, root, leaves, bark, and stems. The plant is native to Southeast Asia but has historically spread throughout a large area, from eastern Polynesia to

India. It grows erratically in the wild and in small individual growing plots and plantations.⁴

Numerous minerals and phytochemicals, which can be roughly categorized into primary and secondary metabolites based on their functions in plant metabolism, are abundant in the leaves and fruits of the MC plant. Primary metabolites comprise common carbohydrates, amino acids, proteins, and chlorophylls, while secondary metabolites comprise alkaloids, saponins, steroids, flavonoids, tannins, and other compounds.⁵ MC is a plant that has gained worldwide recognition for its ability to address a range of physiological issues. More than 160 phytoconstituents have been identified in various parts of the noni plant. Among these, 120 exhibit biological activity.⁶

A literature review has discovered several phytocompounds from extracts of *M. citrifolia* (Noni) fruits, leaves, and roots in a range of solvents, including ethanol, aqueous-methanol, ethanol-aqueous, N-hexane, ether, and methanol, among others.⁷ When the extracted solution was examined, several important bioactive compounds from the leaves were found, such as damnacanthal, quercetin-3-O- β -D-glycopyranoside (which has anti-inflammatory properties), scopoletin (which has antitumor and anticancer properties), and asperulosidic acid (which has hypotensive properties).⁸

These substances are all very helpful in preventing various illnesses. Secondary metabolites comprise most of the active mechanisms found in many medications found in medicinal plants. Natural antioxidant chemicals play a significant role in slowing the production of free radicals and lipid peroxidation in biological systems, thereby protecting cells from damage. Therefore, it is crucial to investigate the phytocompounds in the extract for their primary bioactive components to produce new drugs in the future. Before component analysis, extraction techniques are a crucial stage in recovering and separating bioactive phytochemicals from medicinal plant materials.

The bioactive component of noni fruits is quercetin, which has anti-inflammatory and antioxidant qualities.⁹ Scopoletin and rutin have antipsychotic effects, and xeronine has been shown to have anticancer activity.^{10,11} Among the bioactive substances included in noni roots are 1, 3-hydroxy-6-methylanthraquinone (anticancer activity),¹² quercetin's anti-dyslipidemic, and antioxidant qualities.^{13,4} These bioactive plant components have previously been shown to induce a wide range of biological actions, including anti-inflammatory, anti-addictive, antifungal, antibacterial, antipsychotic, anticancer, anti-depressant, and immune-boosting effects, among others.

People use these plants as their main source of medicine in various countries, including our own India, especially in the "Jungle Mahal" region. The MC (Noni) plant is found in West Bengal's Jungle Mahal region, which includes the districts of Midnapore, Bankura, Purulia, and Birbhum. Historically, a wide range of meals and medicines have been made with it. There are many different ways that people use MC. This

medicinal plant has long been used by common people, particularly in the Jangal Mahal region, to treat a wide range of ailments, including diabetes, fever, stomach ulcers, boils and carbuncles, jaundice, tuberculosis (TB), and loss of appetite.

It also treats human vitamin A insufficiency (leaves), neurological disorders, immunological deficiencies, chemical sensitivity, attention disorders, addiction, and cardiovascular diseases.² In addition to being an essential resource for traditional medicine and herbal businesses, a significant portion of the Indian population relies on medicinal plants for their livelihood and health security. Traditional medicine has utilized a diverse range of plant species to treat a broad spectrum of human ailments. Even compounds made from plant extracts may be useful therapeutic drugs in primary healthcare in many modern countries.¹⁴

GC-MS, or gas chromatography-mass spectrometry, is the most dependable analytical method¹⁵ to recognize and measure organic compounds in their complex biological matrices and combinations. A much finer level of substance identification is made possible by combining GC and MS components than when used separately. Mass spectrometry and gas chromatography alone are insufficient for precisely identifying and studying a chemical. As a result, GC-MS analyses are now frequently employed to examine plant extracts, particularly those used medicinally. This approach has been demonstrated to be effective for studying lipids, fatty acids, volatile essential oils, and non-polar components.¹⁶

The extraction of noni fruit and leaves has been studied in several different solvents.⁷ Still, prior research has not addressed the aqueous-methanolic (30:70%) solvent ratio for GC-MS analysis. Therefore, we are attempting to identify other compounds from the aqueous-methanolic extracts of our current study. Therefore, the main objectives of this study are to identify and characterize the phytocompounds from the leaf and fruit aqueous-methanolic extract of noni plants using GC-MS analysis. Research on the biological activities of the leaf and fruit components of this alcoholic extract is currently lacking. Thus, the primary objective of this study is to extract novel phytochemicals from the leaves and fruits of the MC plant, which could be useful for future drug development and physiological treatment.

MATERIALS AND METHODS

Plant material collection and preparation

M. citrifolia, a medicinal herb, was collected from the Bishnupur forest in the Bankura district of West Bengal, India. After being removed individually, each fruit and leaf was thoroughly cleaned under running water to remove any dust from its surface, left to dry in the shade for additional study, and then ground into a fine powder. The powdered leaves and fruits were stored in two sealed containers.¹⁷

Plant Sample Preparation from Extraction

Next, a cold extraction and sonication technique was employed to create a methanolic extract from MC leaves and shade-dried fruits. Water (30 mL), methanol (70 mL), and 25 g of powdered, dried noni fruit were combined in a sterile glass container and allowed to sit for one day. A water bath sonicator was then used to sonicate the mixture for an additional four hours at 30°C. The sample was filtered twice to obtain a pure, dry extract, and the remaining solution was vacuum-evaporated using a rotary evaporator. The air-dried extract was then ready for GC-MS analysis after being stored in non-reactive containers.^{10,18,19}

GC-MS Analysis of Noni Leaf and Fruit Extracts

A GC-MS (GC Trace GC ultra, MS-POLARISQ, Thermo Scientific India Pvt. Ltd) autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a capillary column (TR-WAXMS, 30 m × 0.25 mm [ID] × 0.25 µm film thickness) were used to perform GC-MS analysis of the methanolic extracts of various parts of MC. An electron ionization system operating in electron impact mode, with an ionization energy of 70 eV and a scan range of 50 to 750 a.m.u. (mass-to-charge ratio), was used for GC-MS detection. A split ratio of 18: 1 was utilized with an injection volume of 1-µL and a constant flow rate of 0.9 µL/min of helium gas (99.999%). The ion source was kept at 230°C, while the injector was at 260°C.

The oven was set to begin at 70°C (isothermal for 2 minutes), then increase by 20°C per minute to 110°C, followed by a 5°C per minute rise to 200°C, and conclude with a 10-minute isothermal hold at 260°C. At 70 eV, full scan mode and fragments from 50 to 750 a.m.u. were used to get mass spectra. The GC-MS ran for 31 minutes overall, with a solvent delay of 0 to 2 minutes. The relative percentage amount was determined by comparing the average peak area of each component to the total area. The TQ Quadrupole Mass Spectrometer was the mass detector employed in this investigation, and the MS Work Station was the software used to manage the mass spectra and chromatograms.^{20,21}

Identification of Components

The relative percentage quantity of each component was found by comparing the average peak area of each component to the total area. Bioactive substances are analysed using the library of the National Institute of Standards and Technology (NIST). Dr. Jim Duke of the Agricultural Research Service developed Dr. Duke's Phytochemicals and Ethnobotanical Databases, which were used to analyze the biological activity of various substances. A database containing over 62,000 patterns from the NIST library was used to interpret the GC-MS. A comparison was made between the unknown compound's spectra and the known components contained in the NIST library. The test compound's component names and molecular formulas were determined.²²

RESULTS

Chromatogram for GC-MS Analysis

In nature, various types of medicinal plants are available around the world, and they have many nutrients. Some phytochemicals, such as phenols and flavonoids, are important substances responsible for several medicinal properties, including anticancer, antioxidant, antifungal, antibacterial, and antipsychotic activity. The identification of the phytocompounds was carried out based on their retention times and molecular formulas. Figures 1, 2, and Tables 1 and 2 show the GC-MS results of the MC (Noni) leaves and fruits. The names of identified compounds in the fruit and leaves of MC (Noni) with their retention time (RT), molecular formula (MF), molecular weight (MW), and peak area percentage are given in Tables 1 and 2.

According to the current study's findings, GC-MS analysis of the aqueous-methanol extracts from MC leaves and fruits revealed the presence of numerous phytochemicals. They carry out several biological functions, as listed in Tables 1 and 2. The fruit and leaves of noni are rich sources of bioactive substances, including terpenoids and esters. The use of this plant to treat various health issues is justified by the presence of many bioactive components and the therapeutic validation of these substances.

The respective chromatogram (Figure 1) was developed by plotting time (in minutes) against relative abundance (%). The maximum height of the peak appearing in the chromatogram is denoted as the parent peak, and the percentage will be 100%. Regarding this parent peak, the percentage of other peaks is measured.

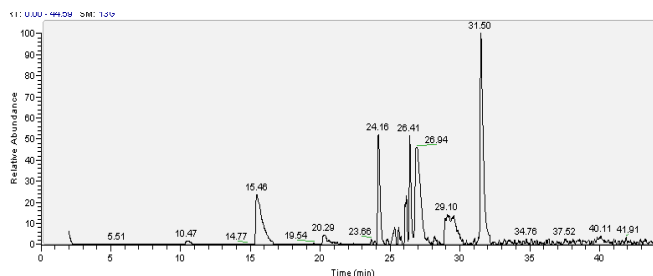


Figure 1: Chromatogram for GC-MS analysis of aqueous-methanolic leaf extract of *Morinda citrifolia* (Noni)

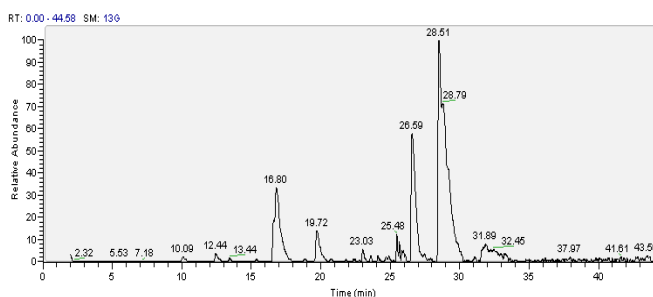
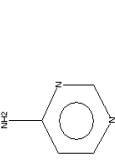
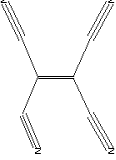
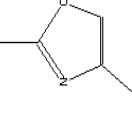
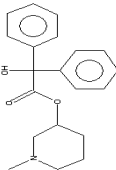
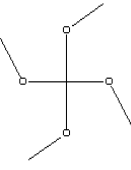
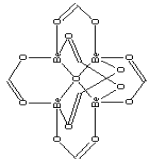
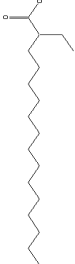
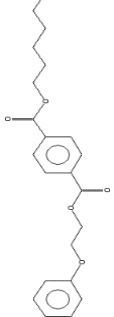


Figure 2: GC-MS analysis of phytochemicals in aqueous-methanolic fruit extract of *Morinda citrifolia*

Table 1: GC-MS analysis of *Morinda citrifolia* (Noni) Methanolic leaf extract (TRWXMMS/27092018)

S. No.	Rt	Compound name	M.F	M.W	Peak area%	Structure of compound	Nature	Activity
1.	10.47	4-Aminopyrimidine.	$C_4H_5N_3$	95	0.56		Aminopyrimidine Derivative.	
2.	15.45	Tetracyanoethylene.	C_6N_4	128	8.04		Tetracyanoethylene derivative.	
3.	20.29	Oxazole,2,4-dimethyl.	C_5H_7NO	97	1.26		Substituted oxazole.	
4.	20.61	Benzeneacetic acid, α'-hydroxy-α'-phenyl-1-methyl-3-piperidinyl ester.	$C_{20}H_{23}NO_3$	325	0.2		Piperidinyl ester.	
5.	21.21	Tetramethyl orthocarbonate.	$C_5H_{12}O_4$	136	0.1		Tetramethyl orthocarbonate.	
6.	23.9	Beryllium, hexakis [ae-((format-o: o')]ac ⁴ oxotetra.	$C_6H_6Be_4O_3$	322	0.17		Beryllium complex.	
7.	24.16	Hexadecanoic acid(2-ethyl-methyl ester.	$C_{19}H_{38}O_2$	298	10.13		Hexadecanoic acid ester.	
8.	24.80	Terephthalic acid, hexyl 2-phenoxyethyl ester.	$C_{22}H_{26}O_5$	370	0.39		Terephthalic acid ester.	

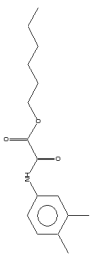
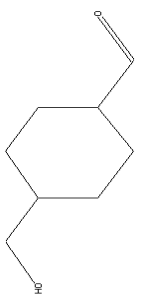
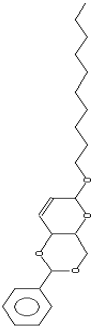
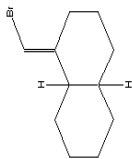
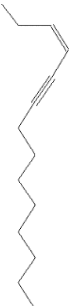
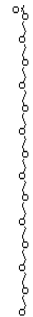
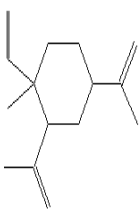
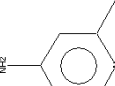
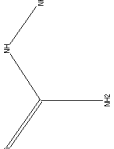
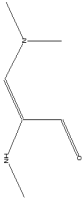
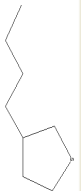
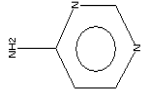
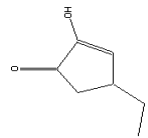
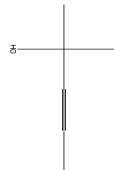
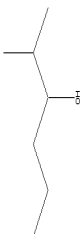
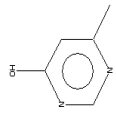
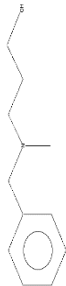
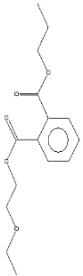
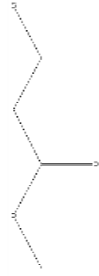
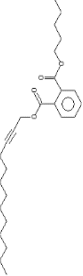
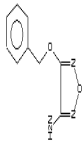
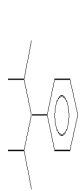
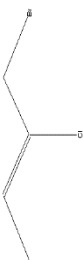
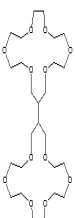
9.	25.33	Oxalic acid, monoamide, N-(3,4-dimethylphenyl)-hexyl ester.	$C_{16}H_{23}NO_3$	277	1.85		Amide ester of amino acid.
10.	25.61	Cyclohexeneoicarboxaldehyde-4- (hydroxy methyl)-	$C_8H_{14}O_2$	142	0.91		Cyclohexeneoicarboxaldehyde.
11.	25.79	2,4,7-Trioxabicyclo[4,4,0] deo-9- ene, 8-decyloxy-3-phenyl-	$C_{23}H_{34}O_4$	374	0.38		Trioxabicyclo Compound.
12.	26.17	1-Bromomethylenedecahydronaphthalene.	$C_{11}H_{17}Br$	228	4.14		decahydro naphthalene.
13.	26.41	3-Tetradecen-5-yne, (Z)-	$C_{14}H_{24}$	192	6.88		Ene-yne compound
14.	26.87	Methyl 11-methyl-dodecanoate.	$C_{14}H_{28}O_2$	228	15.93		Dodecanoate Ester.
15.	27.45	Propionic acid, 3- (allythio)-,ethyl ester.	$C_8H_{14}O_2S$	174	0.04		Propionic acid Ester.
16.	29.1	Tridecaethylene glycol monomethyl ether, acetate.	$C_{29}H_{58}O_{15}$	646	5.77		
17.	31.5	Cyclohexene,1-ethenyl-1-methyl-2,4-bis[1-methylethenyl]-[1S-(1a',2a',4a')]-	$C_{15}H_{24}$	204	20.83		Terpenoid.

Table 2: Identification of phytochemicals from aqueous-methanolic fruit extract of *M. citrifolia* by GC-MS analysis.

S. No	Rt	Name of compound	M.F	M.W	Peak area %	Structure	Nature of compound	*Activity
1	12.44	4-Pyrimidinamine, 6 methyl-	$C_5H_7N_3$	109	1.08		Pyrimidine derivative	Catechol-O-methyltransferase inhibitor
2	13.44	Hydrazinecarbothioamide	CH_3N_3S	91	0.3		Amide	
3	15.38	2-Propenal,3-(dimethylamino)-2-(methylamino)	$C_8H_{12}N_2O$	128	0.16			
4	16.79	3-n-Butylthiolane	$C_8H_{16}S$	144	12.82			Anti-tumour, Nicotinic, increases NK cell activity
5	18.81	4-Aminopyrimidine	$C_4H_5N_3$	95	0.27		Pyrimidine derivative	
6	19.72	4-Ethyl-2-hydroxycyclopent-2-en-1-one	$C_7H_{10}O_2$	126	3.73		Pyran ester	Decrease endothelial platelet adhesion.
7	21.82	2-Methyl-3-pentyn-2-ol	$C_6H_{10}O$	98	0.12			Catechol-O-methyltransferase inhibitor
8	22.76	3-Hexanol,2-methyl	$C_7H_{16}O$	116	0.07		Alcohol	Methyl Donor
9	23.03	4(1H)-Pyrimidinone,6-methyl	$C_5H_6N_2O$	110	1.19		Pyrimidine derivative	The HDL gene suppresses HMG-CoA reductase activity.

10	23.61	1,3-Dioxolane,2-methyl-2-pentyl	$C_9H_{18}O_2$	158	0.45		Catechol-O-methyltransferase inhibitor
11	24.13	3-(Benzyl(methylamino))-1-propanol	$C_{11}H_{17}NO$	179	0.34		
12	24.71	Phthalic acid, 2-ethoxyethyl propyl ester	$C_{15}H_{20}O_5$	280	0.41		Increase aromatic amino acid decarboxylase activity.
13	24.90	Propanoic acid, 3-chloro-methyl ester	$C_4H_7ClO_2$	122	0.48		Increase aromatic amino acid decarboxylase activity. Decrease NE production
14	25.25	2,4,7-Trioxabicyclo(4,4,0) dec-9-ene,8-decyloxy-3-phenyl	$C_{23}H_{34}O_4$	374	0.06		
15	25.48	Cyclohexanecarboxaldehyde,4-(hydroxymethyl)	$C_8H_{14}O_2$	142	1.67		
16	25.67	Phthalic acid, pentyl tridec-2-yn-1-yl ester	$C_{26}H_{38}O_4$	414	1.02		Uric acid inhibitor
17	25.85	10-Pentadecen-5-yn-1-ol(E)	$C_{15}H_{26}O$	222	1.63		Erythro-cytogetic, ionic channel opener.
18	26.37	1,2,5-Oxadiazol-3-amine,4-(phenylmethoxy)	$C_9H_9N_3O_2$	191	0.02		
19	26.58	Dodecanoic acid,2-methyl-	$C_{13}H_{26}O_2$	214	17.86		Methyl Donor
20	27.48	Benzene,[2-methyl-1-(1-methyl ethyl) propyl]	$C_{13}H_{20}$	176	0.27		Methyl Donor
21	27.78	1,3-Dioxolane,2-methyl-2-(phenyl)methyl	$C_{11}H_{14}O_2$	178	0.12		Methyl-Guanidine inhibitor
22	28.50	Undec-10-ynoic acid, butyl ester	$C_{15}H_{26}O_2$	238	37.71		Inhibit uric acid production.

23	28.79	(Z)6,(Z)9-Pentadecandien-1-ol	$C_{15}H_{28}O$	224	0.11		Provide Zinc
24	29.21	1-Cyclohexyl-1-pentyne	$C_{11}H_{18}$	150	1.18		
25	29.89	2-Butene,1-bromo-2-chloro	C_4H_6BrCl	168	0.16		
26	30.09	18-18-Bi 1,4,7,10,13,16-hexaoxacyclononadecane	$C_{26}H_{50}O_{12}$	554	0.25		Crown ether
27	31.89	Isobutyl 2,5,8,11-tetraoxatridecan-13-yl carbonate	$C_{14}H_{28}O_7$	308	2.6		Increase Vita-D, K bioavailability, and Bilirubinolytic. Antioxidant

*Dr. Dukes' Phytochemical and Ethnobotanical Databases.

Through this percentage analysis of the study, we can conclude the content of the ingredients in the sample. In the chromatogram (Figure 1), selected peaks are 10.47, 15.46, 20.29, 23.66, 24.16, 26.41, 26.94, 29.10, and 31.50. If these Retention time (Minutes) denotes different products then the percentage study will be like this. 10.47--0.72; 15.45--10.36; 20.29--1.62; 20.61--0.25; 21.21--0.12; 23.9--0.21; 24.16--13.03; 24.8--0.50; 25.33--2.38; 25.61--1.17; 25.79--0.48; 26.17--5.33; 26.41--8.86; 26.87--20.53; 27.45--0.05; 29.10--7.43; 31.50--26.84. In (Figure 1) the highest content is RT 31.50 minute and the lowest content is RT 23.66 minutes.

The selected peaks in the chromatogram are 12.44, 13.44, 15.38, 16.79, 18.81, 19.72, 21.82, 22.76, 23.03, 23.61, 24.13, 24.71, 24.90, 25.25, 25.48, 25.67, 25.85, 26.37, 26.58, 27.48, 27.78, 28.50, 28.79, 29.21, 29.89, 30.09, and 31.89.

If these retention time (Minutes) denotes different products, then the percentage study will be like this 12.44--1.27; 13.44--0.35; 15.38--0.18; 16.79--15.18; 18.81--0.31; 19.72--4.4; 21.82--0.14; 22.76--0.08; 23.03--1.40; 23.61--0.53; 24.13--0.40; 24.71--0.48; 24.90--0.56; 25.25--0.07; 25.48--1.97; 25.67--1.2; 26.37--0.02; 26.58--21.14; 27.48--0.31; 27.78--0.14; 28.50--44.65; 28.79--0.13 29.21--1.39; 29.89--0.18; 30.09--0.29; 31.89--3.07.

The aqueous-methanolic extract of noni fruit and leaf contained a variety (approximately 27 compounds), Tables 1 and 2, of phytochemicals that were screened for in our current investigation. Our earlier published study (23) already covered several types of biological activity and their effects on lipid profile measurements, histological tissue sections, and haematological parameters. Therefore, we only intended to look at the phytochemical names from the extract of noni fruit and leaves in this study.

DISCUSSION

According to a summary of the current investigation, many bioactive components were discovered in the aqueous-methanolic extract of MC fruits and leaves using GC-MS analysis. Tables 1 and 2 provide the chemical structures of these entities. Numerous extractions using various solvents have been carried out in the plant's prior investigations. Numerous studies have previously documented the discovery of diverse phytochemicals from noni plants (fruit, leaves, and root) in the form of distinct chemical solvents. According to previous literature, MC fruit extract may contain several compounds, such as 1-butane carboxylic acid, butyric acid, semicarbazone, etc., present in 99% methanolic extract.²¹ However, octadecanoic acid, methyl ester, and methyl stearate of the methanolic extract were found in MC leaves.²⁴ The noni fruit's ethanolic extract contains 9, 12, and 1-and 5-cyclodecadine and octadecanoic acid. On the other hand, sigma sterol, campestral, and gamma-tocopherol are present in the ethanolic extract of noni leaves.²⁵ Palmitic acid and E-phytol are visible in the noni leaf aqueous extract.²⁶ 2HBenzopyran2one7hydroxy6methoxy, 4Hpyran4one, 2, 3dihydro3, 5dihydroxy6methyl, and hexadecenoic acid

were found from the aqueous extraction of noni fruit.²⁷ The noni leaf hexane extract recovered compounds such as 5-hydroxymethylfurfural, α -tocopherol, ethyl-decanoate, and others. Noni fruit hexane extract contains phytochemicals such as squalene, 2-methylpentanoic acid, and 5-hydroxymethyl furfural.²⁸

Numerous reports indicate that various compounds have been identified using varying extraction percentages or ratios. According to the literature on solvent extraction, researchers have identified phytochemicals and conducted various types of solvent extraction on noni plants (leaf and fruit). They found numerous chemicals from MC (Noni). Our GC-MS analysis of the aqueous-methanolic (30:70%) extract of noni leaf and fruits revealed the presence of 27 phytochemicals from the fruits and 17 phytochemicals from the leaves. Most of these are likely novel phytochemicals derived from noni, distinct from previously discovered aqueous-methanolic extraction chemicals.

Our findings indicate that several bioactive phytochemicals from GC fractions are present in the aqueous-methanolic extraction of noni leaves and fruits, as determined by GC-MS analysis. Here, Dr. Jim Duke of the Agriculture Department's phytochemicals and ethnobotanical databases was used to anticipate the compounds. In our GC-MS analysis, we found 27 phytochemicals, a few of which are not previously mentioned in different extractions of Noni fruit and leaves. Most of them are different than previous data from GC-MS analysed, including 4-Aminopyrimidine Tetracyanoethylene (8.04%), Oxazole, 2,4-dimethyl (1.26%), Benzeneoetic acid, α' -hydroxy- α' -phenyl-1-methyl-3-piperidinyl ester (0.2%), Tetramethyl orthocarbonate (0.1%), and others, which were revealed by our studies of noni leaves Aqueous-Methanolic (AM) extract (Table 1). Among them, Cyclohexene,1-ethenyl-1-methyl-2,4-bis[1-methylethenyl]-[1S-(1a',2a',4a')] compound has Anti-proliferative, Anti-radical property and Anti-Alzheimer's activity.

However, 27 phytochemicals were also detected in the aqueous-methanolic extract of MC fruits. Among them, a few are the most effective compounds against different physiological hazards like 4-pyridinamine, 6-methyl- (1.08%), hydrazinecarbothioamide (0.3%), 2-propenal, 3-(dimethylamino)-2-methylamino (0.16%), 3-n-butylthiolane (12.82%), and 4-aminopyrimidine (0.27%). 4-(3.73%) ethyl-2-hydroxycyclopent-2-en-1-one 1-(1H)-Pyrimidinone,6-methyl (1.19%) etc. Owing to the distinct soil composition and climate in our Jungalmahal area, the compounds mentioned above differ from those previously documented. Methyl 11-methyl-dodecanoate, acetate, tridecaethylene glycol monomethyl ether, cyclohexene, 1-ethenyl-1-methyl-2,4-bis [1-methylethenyl] [1S-(1a',2a',4a')] Hydrogenase carboxylamide and noni leaf include phytochemicals such as hexadecenoic acid, 2-ethyl methylester, protonic acid, and 3-(allythio)-ethyl ester. Noni fruits contain more active compounds such as 2-Propenal, 3(dimethylamino)2methylamino, 3nButylthiolane, 4(1H) Pyrimidinone, 6-methyl, Phthalic acid, pentyltridec2yn1ylester, (Z) 6, (Z) 9Pentadecandienol,

and Isobutyl2,5,8,11-tetraoxatridecan-13-ylcarbonate. A few of them, like 4(1H)-Pyrimidinone,6-methyl phytochemicals, have an HDL gene suppressant role and HMG-CoA reductase activity, which we have found from noni fruit sample extraction. In contrast to earlier studies, our GC-MS study of the aqueous-methanolic extract of noni fruits and leaves identified several kinds of phytochemicals. Changes in climate, soil characteristics, or environmental conditions could trigger it. Thus, it may be said that we identified several new phytochemicals by our aqueous-methanol extraction of noni. It is possible to infer that these phytochemicals possess medicinal properties. Since noni fruit and leaves likely contain additional bioactivity, they could become important medicinal agents. This could benefit the pharmaceutical sector and provide a valuable drug to help people stay healthy.

The phyto-constituents found in the MC aqueous-methanolic leaf extract and fruit extract are distinct. The utilization of novel bioactive compounds derived from plants, which are important for health and have the potential to be isolated, remains a highly fruitful region for the development of new medications to advance medical care in specific specialties. The present study's findings demonstrated that several phytochemicals were detected by GC-MS analysis in the methanol (70%) extracts of MC (Noni) leaves and fruits. They might be engaged in a variety of biological functions. The fruit and leaves of noni are a good source of bioactive substances, including terpenoids and esters, which may have some ameliorative effects on various physiological disorders. The plant's ability to produce a wide range of bioactive compounds and their therapeutic applications justify its use in treating various health issues. Further research required the HPLC technique to investigate the specific isolation and separation of the above-specified bioactive component from the leaves and fruit of *M. citrifolia*. During this study, we encountered some instrumental issues, which caused our planned work to be delayed for a few days. Additionally, learning about novel drug development and potential biopharmaceutical markers for various diseases and their treatments will be beneficial.

ACKNOWLEDGMENT

The authors gratefully acknowledge the contributions of Prof. Sujoy Kumar Dasgupta, Chairman, and Smriti Ranjan Maji, Technical Officer, C.I. F (P.D.Lab), Bose Institute, Centenary Campus, Kolkata-54, for providing the laboratory facilities for this study.

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PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.