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Comparative Study of Bioactive Phytochemicals from Hexane Extract of Two Amaranthaceae Leafy Vegetables by GC-MS Method

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Abstract

Amaranthus tricolor Linn and Amaranthus viridis Linn, belonging to the Amaranthaceae family, are popularly consumed leafy vegetables in India and many other countries globally. The bioactive components of Hexane extract from these two plants have been analysed using gas chromatography-mass spectrometry (GC–MS) technique. According to analysis, both species contain a total of nineteen important chemicals. Of these, ten were found in A. tricolour L. and nine in A. viridis L. Their structures were determined through percentage similarity indices from NIST library. The GC-MS analysis reveals that most prevalent secondary metabolite compounds include Tetradecane, Heptadecane, 2-Methyltetracosane, Hexadecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Phthalic acid butyl undecyl ester, Pentadecanoic acid, Nonadecane, Z,E-3,13-Octadecadien-1-ol, cis,cis,cis–7,10,13–Hexadecatrienal, Butyl octyl phthalate, Henicosane, Eicosane and Oleic acid, 3-(octadecyl) propyl ester. Structures are verified through an examination of the classical fragmentation patterns, the base peak and molecular ion peak of the compounds. While some of the chemical compounds are specific to each species, others are common to both plants. The natural occurrence and pharmacological properties of identified secondary metabolites were studied from literature. Results validate the existence of bioactive substances known for their pharmacological effects and therapeutic qualities.

Keywords: Amaranthacea, *Amaranthus tricolor* Linn, *Amaranthus viridis* Linn, Bioactive phytochemicals, GC-MS

INTRODUCTION

Leafy greens are extremely useful for maintaining good the health and aids as a protective food for preventing malnutrition and undernourishment [1, 2]. Amaranth is a crop that is underutilized but has a tremendous yield potential and can thrive in a variety of agroclimatic situations. Recently, this versatile crop has drawn interest from all across the world as a crop of the future. The Amaranthus genus of Amaranthaceae family comprises approximately seventy species, of which species like *Amaranthus tricolor*, *Amaranthus cruentus* and *Amaranthus viridis* are widely consumed leafy vegetables in many countries across the world. The biomass of amaranth, both as green and dried, has high nutritional value and is utilized as a rich source of minerals, fiber, protein, amino acids and vitamins [3-7]. *Amaranthus tricolor Linn* is famous for its tasty, spinach-like leaves that have a beautiful purple-red color. Most of India, particularly Bihar, Jharkhand, South India, West Bengal, and Africa, like other tropical continents, eat it as a staple dish [8]. *Amaranthus viridis Linn* is a green, erect, annual plant and in Greek amaranthus means

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'never-fading flower'. In India, it is referred to locally as 'Chowlai' and is also called pseudocereals. According to review of literatures, different parts of these plants such as leaves, grains, stem and roots are used as raw form, poultice, decoction or as a powder to cure the various medical ailments [9]. In recent decades, both the species have been reported to shows wide spectrum of pharmacological activities such as antioxidant, antinociceptive, anti-inflammatory, antipyretic, antibacterial, hepatoprotective, hematological, hypoglycemic and hypolipidemic [10,11]. Additionally, ancient Indian folk remedies in rural areas used *A. tricolor* and *A. viridis* plants to treat or lessen the severity of a wide range of medical conditions. These included treating piles, blood problems, bladder trouble, toothaches, and dysentery, as well as acting as an astringent, diuretic, haemorrhage, and hepatoprotective agent [12-17]. Despite the extensive use of these plants in traditional medicine and the research on their pharmacological and therapeutic properties, not enough has been done to identify the phytochemicals responsible for these plants' therapeutic qualities. Gas chromatography-mass spectrometry (GC–MS) has been widely used in recent years to identify different bioactive therapeutic compounds found in medicinal plants. With only a small amount of plant extracts needed, GC-MS is one of the best, fastest, and most accurate methods for detecting a wide range of substances, including as alcohols, alkaloids, nitro compounds, long-chain hydrocarbons, organic acids, steroids, esters, and amino acids [18, 19].

In the view of above facts, leaves of *Amaranthus tricolor* L. and *Amaranthus viridis* L. have been examined for non-polar to semi-polar phytochemical constituents, using n-hexane as a solvent. The GC–MS technique was used in this investigation to detect and identify phytochemical substances found in the both medicinal plants.

MATERIALS AND METHODS

Sample Collection and Preparation

The fresh leafy vegetables of *Amaranthus tricolor* and *Amaranthus viridis* were purchased from the local market of Pune City, Maharashtra, India. The leaves were collected separately, air-shade dried under room temperature and dried samples were further milled into a fine powder using a mortar pestle and were stored in an air-tight containers.

Authentication of Plant Material

The collected plant sample were authenticated from Botanical Survey of India (BSI).

Preparation of Plant Extracts

The extracts of both plants were prepared by using weighed quantity of leaf powder in known volume of n-hexane solvent, and stirring on the magnetic stirrer for 3 hours, at room temperature. The resulting plant extracts were filtered and concentrated on rotary-evaporator to get the respective crude extracts, to be used in analysis.

GC-MS Study of n-Hexane Extracts of *A. tricolor* L. & *A. viridis* L.

The n-Hexane extract of leaves of both plants were solvated in analytical grade hexane (100 parts-per-million samples), were analyzed via gas chromatography (GC) and mass spectrometry (MS) using a Shimadzu TQ-8030 GC-MS. The GC-MS chromatograms of the extracts were matched with NIST library. The structures of the compounds identified are defined by the % similarity values and confirmed by genesis.

GC-MS Analysis

Gas chromatography analysis was performed by Agilent 6890N with FID using HP-5 capillary column. GC-MS analysis was performed using a Shimadzu QP 5050A mass spectrometer coupled with a Shimadzu 17A gas chromatograph fitted with a split- spitless injector and a DB-5 fused silica capillary column (30m × 0.25mm i. d., 0.25 μm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 250 ˚C, and the split ratio was 40:1. Oven

temperature programming was done from 50 to 280 ˚C at 10 ˚C/min, and it was kept at 280 ˚C for 5 min. interference temperature was kept at 250 ˚C. Ionization mode was electron impact ionization and the scanning range was from 40 amu to 400 amu. Mass spectra were obtained at 0.5 sec. interval. The spectra of the compounds were matched with NIST and Wiley library. The structures were defined by the % similarity values and confirmed by genesis. The data is incorporated in Table no.1.

RESULT AND DISCUSSION

The hexane extracts of *A. tricolor* and *A. viridis* leaves underwent phytochemical analysis using Gas chromatography-mass spectrometry (GC–MS). The GC-MS profiles of the identified phytochemicals are shown in the GC-MS chromatograms of the hexane extracts of both species (**Figure 1 and 2**).

Following the combination of the chromatogram's peaks, a database of known component spectra was examined. The *A. tricolor* generates twenty-seven peaks, while the *A. viridis* produced twenty-four peaks. The spectra of the compounds were matched with NIST and Willey library.

Their structures were identified by the percentage similarity values with their retention time (**Table 1 and 2**). Further, structures were confirmed by the study of classical fragmentation pattern (**Figure 4**) such as, base peak, molecular ion peak of the compounds. Out of twenty-seven peaks of *A. tricolor* and twenty-four peaks of the *A. viridis*, a total of **nineteen compounds** were identified by GC-MS analysis. The **GC-MS** pattern of non-polar hexane extract of *A. tricolor* Linn (**Figure 1**) and *A. viridis* Linn (**Figure 2**) shows complex nature, containing various non-polar homologues of hydrocarbons along with aldehydes, ketone, acids, esters, phthalates, etc. Aromatic long chain phthalates are common in plant sources. The results showed the presence of various fatty acids, heterocyclic compounds, and esters, among other things.

Among the identified nineteen major compounds, nine are detected to be present in *A. viridis* L. and ten are recognized in *A. tricolor* L. (**Table 1 and 2**).

GC-MS study illustrates the presence of **five common components** in both plant materials as tetradecane (**1**), heptadecane (**2**), 2-methyltetracosane (**3**), hexadecanoic acid (palmitic acid) (**4**) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (**5**).

Phthalic acid butyl undecyl ester (**6**) and pentadecanoic acid (**7**), nonadecane (**8**), Z, E-3,13-octadecadien-1-ol (**9**) and cis, cis, cis–7, 10, 13–hexa decatrienal (**10**) are found to be unique to *A. tricolor* Linn. The structures of some identifies compounds are provided (**Figure 3**).

Table 1. GC-MS Analysis of Hexane Extract of *A. tricolor* Linn.

No.	Retention time (min.)	Name of the Compound	Mol. formula	Peak area (%)	SI	M^+ ion peak (m/z)	Base peak (m/z)
1	13.985	Tetradecane	$C_{14}H_{30}$	6.56	93	198	57
2	16.230	Heptadecane	$C_{17}H_{36}$	16.06	95	240	57
3	18.00	Hexadecanoic acid Palmitic acid	$C_{16}H_{32}O_2$	9.95	91	256	60
4	18.095	Butyloctyl phthalate	$C_{20}H_{30}O_4$	4.44	88	334	149
5	18.260	Henicosane	$C_{21}H_{44}$	12.66	94	296	57
6	19.425	3,7,11,15-Tetra methyl-2-hexa decen-1-ol (Phytol)	$C_{20}H_{40}O$	8.34	90	296	71
7	20.120	Eicosane	$C_{20}H_{42}$	9.26	89	282	57
8	21.820	2-methyl tetracosane	$C_{25}H_{52}$	7.83	82	352	57
9	26.46	Oleic acid, 3-(octa decyloxy) propyl ester	$C_{39}H_{76}O_3$	13.49	75	592	57

SI: Similarity Index

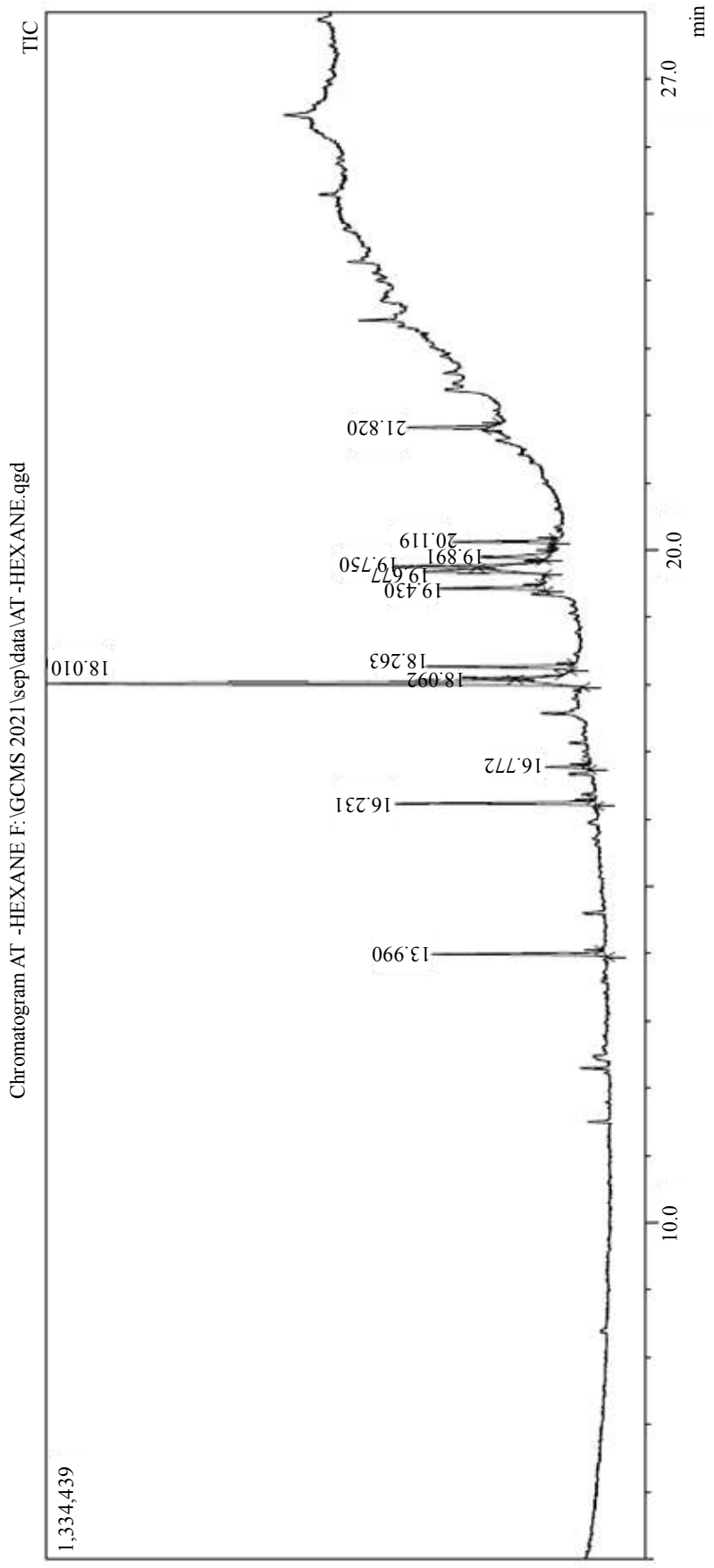


Figure 1. Image if GC-MS Chromatogram of Hexane Extrac *A. tricolor* L

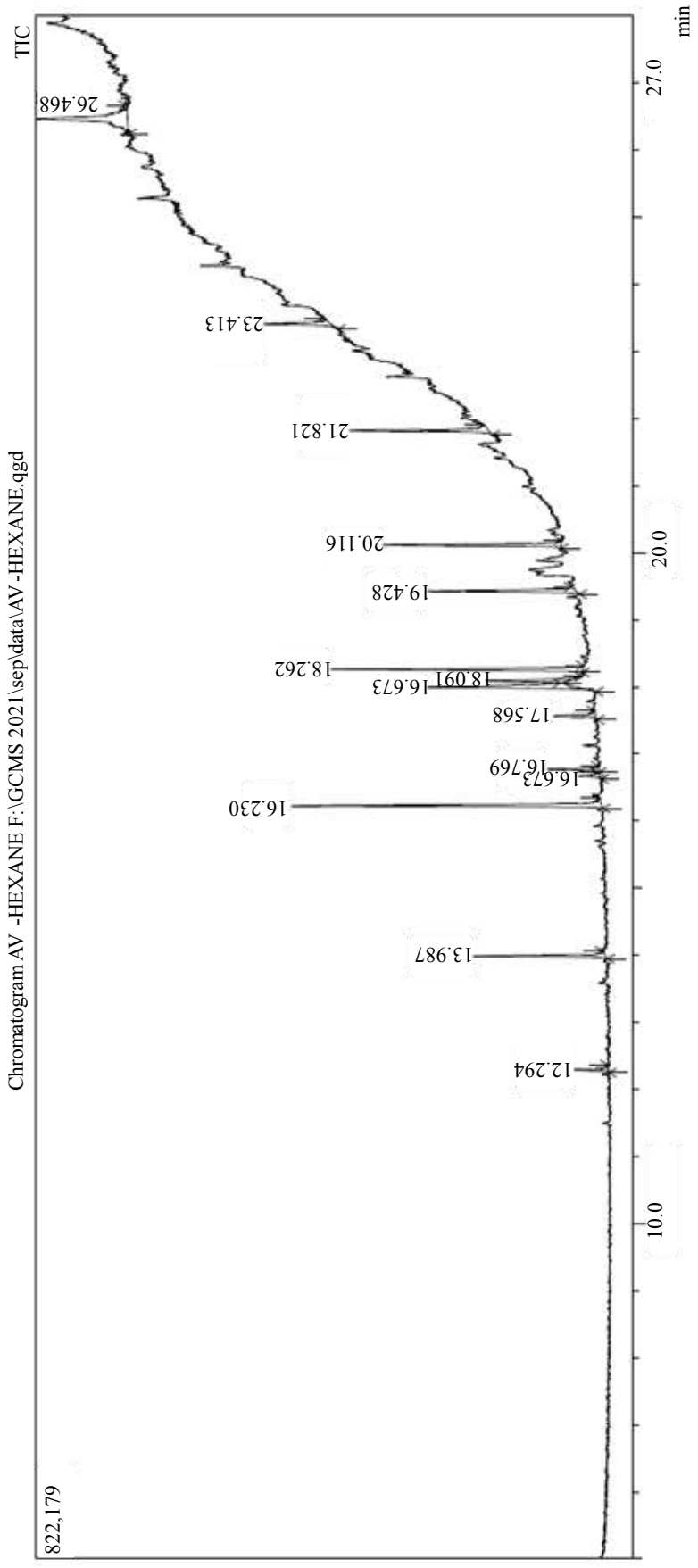


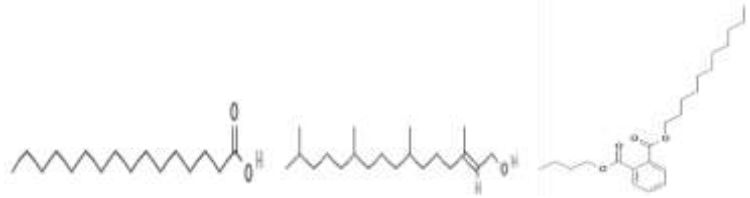
Figure2. Image if GC-M Chromatogram of *Hexane* Extract of *A. virdis* L.

Table 2. GC-MS Analysis of Hexane Extract of *A. viridis* Linn.

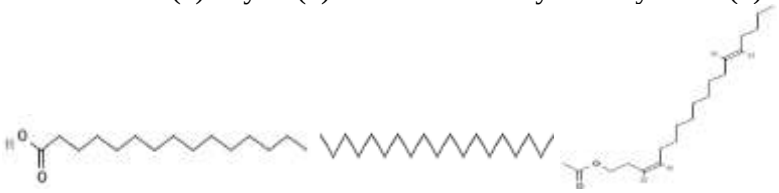
Sr. No.	Retention time (min.)	Name of the Compound	Mol. formula	Peak area (%)	SI	M^+ ion peak (m/z)	Base peak (m/z)
10	13.990	Tetradecane	$C_{14}H_{30}$	8.61	93	198	57
11	16.230	Heptadecane	$C_{17}H_{36}$	10.14	93	240	57
12	18.01	Hexadecanoic acid Palmitic acid	$C_{16}H_{32}O_2$	34.38	91	256	60
13	18.09	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	3.15	86	376	149
14	18.260	Nonadecane	$C_{19}H_{40}$	8.30	89	268	57
15	19.43	3,7,11,15-Tetra methyl-2-hexa decen-1-ol (Phytol)	$C_{20}H_{40}O$	6.28	90	296	71
16	19.675	Z, E-3,13-Octadecadien-1-ol	$C_{18}H_{34}O$	6.52	89	266	57
17	19.755	cis, cis, cis – 7, 10, 13 - Hexadecatrienal	$C_{16}H_{26}O$	5.26	87	234	79
18	19.89	Pentadecanoic acid	$C_{15}H_{30}O_2$	5.6	79	242	57
19	20.115	2-methyltetra cosane	$C_{25}H_{52}$	5.56	88	352	57

SI: Similarity Index

Tetradecane (**1**) Heptadecane (**2**) 2-Methyltetracosane (**3**)

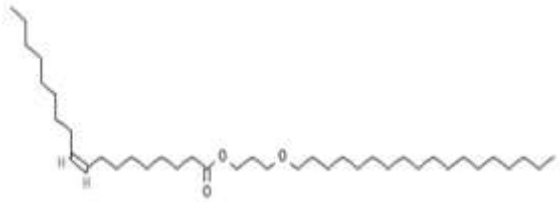


Palmitic acid (**4**) Phytol (**5**) Phthalic acid butyl undecyl ester (**6**)



Pentadecanoic acid (**7**) Nonadecane (**8**) Z, E-3,13-octadecadien-1-ol (**9**)

Butyloctyl phthalate (**11)** Henicosane (**12**) Eicosane (**13**)



Oleic acid, 3-(octa decyloxy) propyl ester (**14**)

Figure 3. Structures of Identified Compounds.

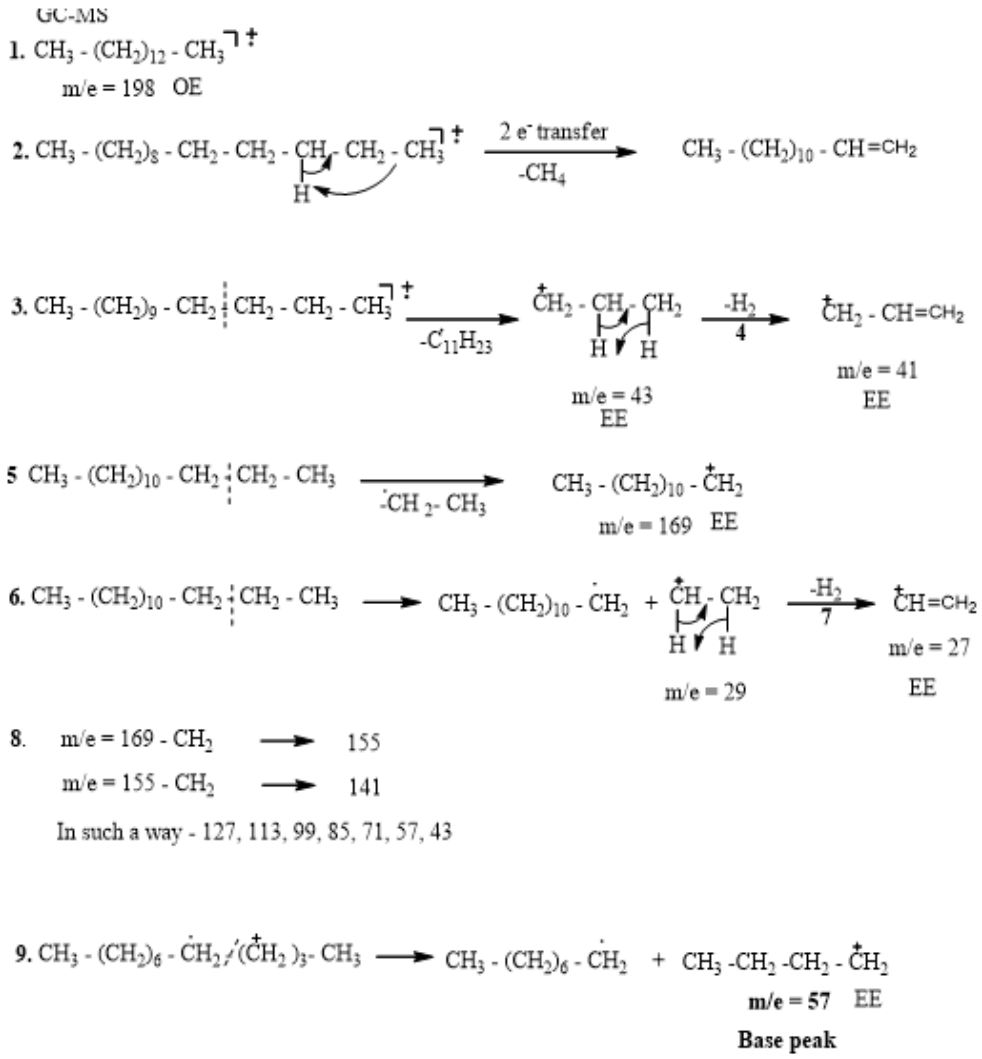


Figure 4. Fragmentation Pattern of Some Identified Compounds.

Based on published research, pharmacological characteristics and occurrence of the identified phytochemicals, in other natural sources were further studied. According to the results, each of them may be used therapeutically in one way or another.

Tetradecene

Tetradecane is an alkane hydrocarbon that is mostly utilized in chemical synthesis, air conditioning and refrigeration as a phase change material, solvent, animal feed and detergent. In nature, tetradecane occurs in *Dryopteris carthusiana* and *Vanilla planifolia* and functions as a plant metabolite in kiwi fruit blossom, nectarine and chickpea seed. Tetradecane is present in dog hair and acts as a biomarker for canine visceral leishmaniasis [20].

Heptadecane

It is a part of essential oils made from plants such as *Annona squamosa* and *Opuntia littoralis*. It functions as both a volatile oil component and a plant metabolite [20].

2-Methyltetracosane

2-Methyltetracosane is a natural product and it has been identified as a free radical scavenger. *Cannabis sativa* and *Plantago ovata* synthesize and accumulate 1-methyltetracosane as part of their metabolic processes. is found to be a Free radical Scavenger [21].

Heptadecanoic acid/ Palmitic acid

Palmitic acid is saturated fatty acid commonly found in palm oil, olive oil, fats, beeswax, body lipids, milk and meat. It is also found as a metabolite in *Daphnia magna*, a common water flea and algae. It inhibits prostaglandin-E2 9-reductase enzyme, which is involved in the metabolism of prostaglandins. It is widely used in a variety of applications, including personal care products, cosmetics, food additive, cosmetics and surfactants [22].

3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)

Phytol is a diterpene alcohol and found in various species, including *Cryptomeria japonica, Parasenecio auriculatus, Excoecaria acerifolia* and *Artemisia carvifolia*. It is primarily used as a fragrance ingredient in cosmetics, toiletries and household products [23].

Phthalic acid butyl undecyl ester (Butyl undecyl phthalate)

Numerous plants belonging to the Lamiaceae, Rosaceae and Solanaceae families as well as several algae have been found to contain butyl undecyl phthalate. Water, soil and air are examples of environmental samples that include phthalates, including butyl undecyl phthalate. Both industrial sources such as the creation of plastic and natural production by plants and other organisms can produce these [24].

Pentadecanoic acid

Pentadecanoic acid is naturally found in milk and cheese like dairy products, ruminant meat and some plants and fish. Researchers highlight its possible function in metabolic health such as immunological, hepatic and cardiometabolic. Additionally, it serves as a biomarker for the consumption of dairy fat and for its possible anticancer and radiotracer therapeutic uses [25].

Nonadecane

Nonadecane is a straight-chain hydrocarbon, been found as a component of essential oils extracted from *Artemisia armeniaca* and *Rosa damascene*. It functions as a component of volatile oils and as a plant metabolite. Nonadecane may acts as a potential biomarker for the watermelons, yellow bell peppers, pome and papaya like food [26].

Z, E-3,13-octadecadien-1-ol

(Z, Z)-3,13-octadecadien-1-ol is a natural compound found in the sex pheromones of various insects, including the redwood resin borer (Synanthedon sequoiae) and the Western Poplar Clearwing moth. Additionally, it is also found in the plant *Dendrophthoe falcata* and in essential oil of *Boswellia sacra.* It can be used in pest control strategies, as a part of synthetic straight-chain lepidopteran pheromones, primarily against the Western Poplar Clearwing moth [27].

cis, cis, cis – 7, 10, 13 – Hexadecatrienal

(Z, Z, Z)-7,10,13-hexadecatrienal is an unsaturated hydrocarbon possessing a seaweed-like odour. It shows antioxidant activity and has potential application in perfumery and cosmetic industry [28].

Butyl octyl phthalate

Butyl octyl phthalate (BOP) is a natural product found in *Amaranthus caudatus L., Saussurea heteromall, Launaea arboresens* and *Acanthopanax sessiliflorus*. The main application for BOP is as a plasticisers, adhesives, coatings, insecticide carriers, medical equipments and cosmetics [29].

Heneicosane

Heneicosane is a naturally occurring waxy substance, present in a variety of food, like pepper, orange, yellow bell pepper and lemon balm. It functions as a volatile oil component, plant metabolite and pheromone. It is a pheromone in the *Reticulitermes flavipes* termite species. Heneicosane lures *Aedes mosquitoes* and can be used in mosquito baits. It is waxy hydrocarbon, isolated from *Origanum vulgare, Periploca laevigata, Carthamus tinctorius* and *Vanilla madagascariensis* plants [30].

n-Eicosane

n-Eicosane is a naturally occurring straight-chain alkane, found in various plants and microorganisms, including *Agave attenuata*, *Vanilla madagascariensis* and *Gymnodinium nagasakiense*. It functions as a as a plant metabolite and was isolated from the *Agave attenuate* leaves. Its applications include use in paraffin waxes for candles, lubricants, plasticizers and as a phase change material (PCM) for thermal energy storage [31].

Oleic acid, 3-(octadecyloxy)propyl ester

Oleic acid, 3-(octadecyloxy)propyl ester is a natural ester, found in *Lepidagathis cristata* plant and sea-weed algae. It may be used as a potential antifungal agent [32].

The chemical compounds identified in the leaves of both plants are found to possess significant therapeutic potential and natural occurrence. GC-MS screening of both hexane extracts showed the presence of several long chain saturated and unsaturated hydrocarbons, aldehyde, carboxylic acid, esters and alcohols. While some of these chemicals are important for industry, the majority of these compounds have a wide range of pharmacological actions, including being plant metabolites, biomarkers, essential oils, antioxidants, enzyme inhibitors, pheromones, pesticide carriers, anticancer, antifungal and radiotracers.

CONCLUSION

The extracts of *A. tricolor* and *A. viridis* leaves in non-polar hexane solvent were used for the analysis of phytochemical constituents. In the current investigation, a total of nineteen important bioactive compounds have been identified from the hexane extracts of both leafy vegetables by means of GC-MS technique. The present investigation describes occurrence of many long chain saturated and unsaturated hydrocarbons, esters and alcohols. Looking carefully at the fragmentation pattern of mass spectral data, it reveals the presence of long chain saturated/ unsaturated compounds. The purpose of this study was to identify and quantify health promoting phytochemicals (non-polar to semi-polar phytochemicals or organic substances) in *A. tricolor* and *A. viridis* leaves. According to analysis, the distinct pharmacological and therapeutic qualities of these leafy vegetables may be a consequence of the chemical compounds that have been identified. However, further investigation is required to ascertain their bioactivity and development of potential drugs.

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Conflict of Interest

The authors declare no conflict of interest.

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