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Comparative Analysis of the Phytochemical Profile of Amaranth Leaf Varieties Using LC-MS Analysis

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Abstract

Amaranth leaves are a highly nutritious vegetable with high protein, vitamin, and mineral content. This study aimed to compare the chemical composition of leaves from different amaranth varieties. Leaves from three amaranth varieties were analysed for their bioactive compounds, mineral content (calcium, phosphorus, iron, and zinc), and vitamin content. Significant variations were observed among the varieties for all the parameters studied. The results indicate that amaranth leaves are a rich source of essential nutrients and that the nutritional value can vary depending on the variety. Further research is needed to identify the most nutrient-rich varieties and to promote their consumption for improved public health.

Keywords: Amaranth, Nutrients, Phytochemical profile.

Introduction-

Amaranthus is a worldwide genus that may be found in temperate, tropical, and subtropical, climates around the world. Grain amaranths are grown in Asia and America in a variety of species. *Amaranthus* species exhibit a wide range of morphological variability (Anjali *et al*; 2013). It is cultivated as a minor crop in Argentina, Bolivia, Ecuador, Mexico, Nigeria, and Kenya. The greatest species diversity occurs in warm, temperate, subtropical, and tropical regions of the world (Mosyakin and Robertson, 1996). Central and South East Asia are the world's major diversity hubs, with secondary variety hotspots in West and East Asia.

According to Maiyo *et al.*, (2010) *A. hybridus*, *A. spinosus* and *A. caudatus* leaves contain various pharmacologically active compounds. According to Nathan *et al.*, (2015) Protein, vitamin C, and mineral components are abundant in the leaves and grains of Amaranthus species. Flavonoids compounds were found in the roots, leaf, inflorescences and seeds *of Amaranthus* species. (Mitra,2015). According to Simpson (2010), the Amaranthaceae family has a global distribution, with members found



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in deserts, estuary or alkaline environments, tropical areas, and temperate zones. Amaranth plants have been framed by Mesoamerican civilizations in central and South America for more than 8000 years, according to the family Amaranthaceae (Safia, 2004). Members of the Amaranthaceae family have a wide and international range, but are most common in the tropics, subtropics, and warm-temperate zones, with alpine and arctic regions clearly lacking. (Eshete *et al.*, 2016). The Amaranth family has been identified as a future food source and a medicinal plant with great promise. The amaranth family is strong in nutritional content and provides the colorant betalain. (Fatinah *et al.*, 2012)

Material Method -

Phytochemical Studies

- 1. Plant Material: Leaves from several *Amaranthus tricolor, Amaranthus caturus,* and *Amaranthus tenuifolius* species were collected from different localities, washed, air dried at room temperature for 4 weeks, and finely pulverized. Each sample's leaves were extracted differently.
- 2. Bioactive Compound Analysis of *Amaranthus* leaves by HR-LCMS.

A TOF/Q-TOE mass spectrometer was used to perform LC-MS analysis of an alcoholic extract of *Amaranthus* species (G6550A). A Hip Sampler (GA226A), a Binary gradient solvent pump (GA220B), and a Quadruple Time of Flight Mass Spectrometer (MS-QTOF) with twin Agilent set stream electro spray (AJS ESI) ion sources constitute the liquid chromatographic system. With needle wash, a 3.00 L sample was injected. The solvent systems used were 0.1% formic acid in water (Solvent A) and Acetonitrile (90 % acetone and 0.1 percent formic acid in water) (Solvent B). MS detection was accomplished in MS-Q-TOF at a flow rate of 0.300 ml/min. The mass spectra and distinctive mass fragmentation patterns of compounds were used to identify them. Phytochemical components were identified using Pub Chem. and Chem. Spider tools.

Results-

Bioactive compound analysis by LC/MS:

The various bioactive compounds detected in leaf extract of selected *Amaranthus* species by LC-MS/MS analysis mainly exhibits Glycophospholipids, Fatty Acids, Alkaloids, Steroids, Carboxylic Acid, Amino Acids, Terpens, Proteins, Quinolines, Flavonoides, Glycosides, Carbohydrate, Sulfonylurea, Phenols, Lipids, Phenothiazines, and Sulfanilamide

a. Amaranthus tricolor L.

Table.1 to 3. LC-MS Profiling of Bioactive Compounds From Ethanolic extract of Amaranthus
tricolor leaves.

sr.No.	Clain	Identified Compound	Molecular Formula	Mass	RT (Min.)
1.		3alpha,7alpha- Dihydroxy-5beta- cholestan-26-oic acid	C8 H9 N 03	7.058	4.094
	Carboxylic Acid	Isocitrate	C6 H8 O7	192.0273	1.037
		Fluorocitric acid	C6 H7 F O7	210.0195	3.521
		Alangimarckine	C29 H37 N3 O3	475.2789	12,103
		Isoproterenol	C11 H17 N O3	211.1175	3.254
		Oxidized dinoflagellate luciferin	C33 H38 N4 O7	602.2699	4,094 1.037 3.521 12.103
	Amino Acids	Endomorphin-2	C32 H37 N5 O5	571.2828	17.427
2.		Acetyl-L-tyrosine	C11 H13 N O4	223.0852	3.483
		Myxochelin A	C20 H24 N2 O7	404.1539	6.453
		N5-Dinitrophenyl-L-ornithine methyl este	C18 H20 N4 O6	388.1389	5.217
		N2-Maltulosylarginine	C18 H34 N4 O12	498.2173	7.075
		Jervine	C27 H39 N O3	425.2873	13.125
3.	Steroides	Hydroxyprogesterone caproate	C27 H40 O4	428.2916	13,469
		7a,12a-Dihydroxy-5b-cholestan- 3-one	C27 H46 O3	418.3416	15.622
		1-Acetyl-3,14,20- trihydroxywitha-5,24-dienolide 3-glucoside	C36 H54 O12	678.3656	



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Sr.No.	Class	Identified Compound	Molecular Formula	Mass	RT (Min.)
		Solanocardinol	C27 H45 N O3	431.3314	15.352
4.	Fatty Acid	3-Methylbutyl 2- methylpropanoate	C9 H18 O2	158.130	9.364
		9,10-Dihydroxy-12,13- epoxyoctadecanoate	C18 H34 O5	330.242	9.216
5	Glycophospholipids	LysoPE(18:2(9Z,12Z)/0:0	C23 H44 N O7 P	477.2938	13.206
б.	Alkaloids	Lyngbyatoxin	C27 H39 N3 O2	437.3011	15.09
		2- Propionylpyrrole	C7 H9 N O	123.069	2.746
		Debromohymenialdisine	C11 H11 N5 O2	245.089	3.518
		Irinotecan	C33 H38 N4 O6	586.2752	17.138
		Pyridoxamine	C8 H12 N2 O2	168.0899	5.073
		Somniferine	C36 H36 N2 O7	608.2568	17.485
7.	Terpens	2,9-Bis(3-methyl-2E- pentenoyl)-2b,9a-dihydroxy- 4Z,10(14)-oplopadien-3-one	C27 H38 O5	442.2678	11.434
		Absintholide	C30 H38 O8	526.2559	16,476
_		Ophiobolin F	C25 H42 O	385.328	7.11
		Ganoderic acid F	C32 H42 O9	570.28	18.045
		Gibberellin A39	C20 H26 O8	426.1518	8.322
		Ganosporelactone A	C30 H40 O7	512.2755	17.986
		Dukunolide D	C26 H28 O8	466.158	5.069
		12-epi-Scalaradial	C27 H40 O4	428.2971	14.43
		Azafrin	C27 H38 O4	426.2797	14.325

Sr.No.	Class	Identified Compound	Molecular Formula	Mass	RT (Min.)
8.	Bernstein	Biliverdin-IX-a	C33 H34 N4 O6	582.2411	14.628
ð.	Proteins	1-Methylhistidine	C7 H11 N3 O2	169.0856	3.75
9.	Ostantian	Cepharanthine	C37 H38 N2 O6	606.2762	18.817
- 36	Quinolines	Fabianine	C14 H21 N O	219.1595	5.748
10,	Flavonoids	Catechin	C15 H14 O6	290.0807	3.833
10.	Flavonoids	Lilaline	C20 H17 N O7	383.1032	2.478
		Deoxycytosine	C4 H7 N3	97.0648	4,557
11.	Glycosides	Niazimin A	C18 H25 N O8	383.1546	10.498
		Perilloside E	C17 H22 O9	370.1324	9.177
12.	Carbohydrates	4-Methoxybenzenepropanol 1-(2-sulfoglucoside)	C16 H24 O10 S	408.1124	4.398
12	Sulfonylurea	Sulfometuron methyl	C15 H16 N4 O5 S	364.0851	5.517
13.		Sulfometuron	C14 H14 N4 O5 S	350.0683	4,403
		Dihydrocapsaicin	C18 H29 N O3	307.2106	7.505
14.	Phenols	Neogrifolin	C22 H32 O2	328.2336	8.775
		Lancerin	C19 H18 O10	406.0948	7.277
15.	Lipids	Sulfoglycolithocholate	C26H43 N O7 S	513.2735	6.949
16.	Phenothiazines	Triflupromazine	C18 H19 F3 N2 S	352.1236	5.585
		Sulfadiazine	C10 H10 N4 O2S	250.0526	5.425
17.	Sulfanilamide	Citalopram-N-Oxide	C20 H21 F N2 O2	340.1558	5,493



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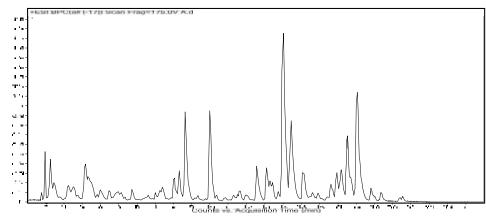


Plate 1: (HR)-LCMS Spectrogram of Leaves of (a) Amaranthus tricolor L.

The ethanol extract of *Amaranthus tricolor* was discovered to contain 54 chemicals of around 17 classes using high resolution liquid chromatography-mass spectrometry analysis (HR)-LCMS. The structure of unknown chemicals eluted at different periods is examined using a mass spectrometer and summarized in Table 1-3,Glycophospholipids, Fatty Acids, Alkaloids, Steroids, Carboxylic Acid, Amino Acids, Terpens, Proteins, Quinolines, Flavonoides, Glycosides, Carbohydrate, Sulfonylurea, Phenols, Lipids, Phenothiazines, and Sulfanilamide were among the important 17 classes of Phytochemical confirmed by (HR)-LCMS analysis. The spectrum profile of ethanol extract of *Amaranthus tricolor* (HR)-LCMS High-Resolution Liquid Chromatography Mass Spectrometer study (Plate-1) indicates 41 components, of which 19 main compounds were verified based on their retention time, mass, and molecular formula.

b. Amaranthus caturus Heyne ex Hooker
Table.4 to 7. LC-MS Profiling of Bioactive Compounds From Ethanolic extract of Amaranthus
caturus leaves.

Sr.No.	Class	Identified Compound	Molecular Formula	Mass	RT (Min.)
		cis-Aconitic acid	C6 H6 O6	74.0172	1.179
0.00		Syringic acid	C9 H10 O5	198.0535	3.394
1.	Carboxylic Acid	Nonate	C9 H16 O4	188.1056	6.571
		9,10-Dihydroxy-12,13- epoxyoctadecanoate	C18 H34 O5	330.2437	9.185
		Ureidoglycine	C3 H7 N3 O	133.0505	0.931
		Oxidized dinoflagellate luciferin	C33 H38 N4 O7	602.27	1.179 3.394 6.571 9.185
	Amino Acids	Gabapentin	C9 H17 N O2	171.126	11.552
2.		LysoPE(0:0/20:1(11Z))	C25 H50 N O7 P	507.3287	9.746
		DL-Omithino-L-alanine	C8 H17 N3 O4	219.123	4.907
		(2S)-2-[[1-(R)-Carboxyethyl] amino]pentanoate	C8 H15 N O4	189.0996	2.949
3.	Steroids	l alpha,3beta,22R- Trihydroxyergosta- 5,24E-dien-26-oic acid 3-O-b-D-glucoside 26- O-b-D-glucosyl ester	C40 H64 O15	784.4198	9.877
		Jervine	C27 H39 N O3	425.2873	13.125
		Withaperuvin H	C30 H42 O9 S	578,2548	16.866



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Sr.No.	Class	Identified Compound	Molecular Formula	Mass	RT (Min.)
		2 -Oxo-10- methylthiodecanoicacid	C11 H20 O3 S	232.1151	7.175
4.	Fatty Acid	Corchorifatty acid F	C18 H32 O5	328.2254	7.986
79	Faily Seid	Colnelenic acid	C18 H28 O3	292.2058	9.911
		0-Oxo-11-octadecen-olide	C18 H30 O3	294.2221	12.923
		2-Hexylbenzothiazole	C13 H17 N S	219.1077	2.498
		2-Propionylpyrrole	C7 H9 N O	123.0687	3.483
	Alkaloids	Fabianine	C14 H21 N O	219.1596	5.752
5.		Icaceine	C22 H33 N O4	375.2363	7.922
		Somniferine	C36 H36 N2 O7	608.2566	17.697
		Irinotecan	C33 H38 N4 O6	586.2751	16.657
		Epivoacorine	C43 H52 N4 O6	720.3786	10.696
		Ganosporelactone A	C30 H40 O7	512.2752	17.918
		Ganoderic acid F	C32 H42 O9	570.2794	18.009
12		Lucidenic acid E2	C29 H40 O8	516.2695	16.71
6.	Terpens	Absintholide	C30 H38 O8	526.255	17.79
		Limonoate	C26 H34 O10	506.2148	13.083
		Glucosyl (2E,6E,10x)-10,11- dihydroxy-2,6-farnesadienoate	C21 H36 O9	432.238	7.069

Sr.No.	Class	Identified Compound	Molecular Formula	Mass	RT (Min.)
		Lamioside	C18 H28 O11	420.164	11.513
		2,8- Dehydroastaxanthianthin	C40 H50 O4	594.3725	12.803
		8-Epideoxyloganin tetraacetate	C25 H34 O13	542.1993	13.092
		delta-Maslinic acid	C30 H48 O4	472.3588	13.226
		Liquiritic acid	C30 H46 O4	470.3428	14.307
	Quinolines	Cepharanthine	C37 H38 N2 O6	606.2764	18.806
7.		Calafatimine	C38 H40 N2 O7	636.2751	11.401
		Sparfloxacin	C19 H22 F2 N4 O3	392.1674	10.2
8.	Flavonoides	Lilaline	C20 H17 N O7	383.1028	2.838
9.	Glycosides	Phenylethyl primeveroside	C19 H28 O10	416.1706	5.084
1825		Canavalioside	C26 H42 O12	546.2676	17.489
10.	Sulfonylurea	Sulfometuron methyl	C15 H16 N4 O5 S	364.0858	5.498
11.	Lipids	9(S)-HpOTrE	C18 H30 O4	310.2167	9.908
12.	Sulfanilamide	Sulfadiazine	C10 H10 N4 O2 S	250.0517	5.354
13.	Diacylglycerols	Glycerol 1,2-diacetate	C7 H12 O5	176.0673	3.803
14.	Umbelliferones	7,8-Dihydroxycoumarin	C9 H6 O4	178.0265	4.348



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15.	Lactone	alpha-Carboxy-delta- decalactone	C11 H18 O4	214.121	4.952
16.	Naphthofuran	(3b,6b,8a,12a)-8,12-Epoxy- 7(11)-eremophilene-6,8,12- trimethoxy-3-ol	C18 H30 O5	326.210	9.826
17.	Gamma-keto acids	Diplodiatoxin	C18 H28 O4	308.201	10.197
		Amaranthussaponin IV	C47 H70 O20	954.455	10.271
	Saponins	Tragopogonsaponin B	C50 H70 O16	926.461	10.625
18.		Spinacoside C	C46 H70 O19	926.458	11.025
18.		Elaterinide	C38 H54 O13	718.360	12.318
		Oleanolic acid 3-O-beta- D-glucosiduronic acid	C36 H56 O9	632.397	12.683
		Elaterinide	C38 H54 O13	718.362	14.096
19.	Guanidine	Hordatine A	C28 H38 N8 O4	550.294	13.469
20.	Porphyrin	Harderoporphyrin	C35 H36 N4 O6	608.2659	17.14
21.	Sesquilignan	Lappaol D	C31 H36 O10	568.2254	17.479

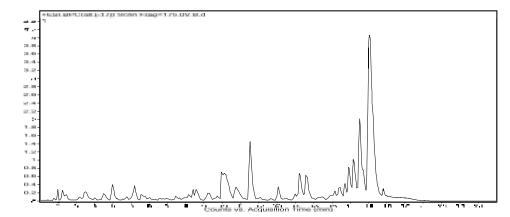


Plate 2: (HR)-LCMS Spectrogram of Leaves of (b) A.caturus Heyne ex Hooker

The ethanol extract of *A. caturus* was found to contain 54 components of around 17 classes using high resolution liquid chromatography-mass spectrometry analysis (HR)-LCMS. The structure of unknown chemicals eluted at different periods is examined using a mass spectrometer and summarized in Table no. 4-7.

Carboxylic Acid, Amino Acids, Steroids, Fatty Acids, Alkaloids, Terpens, Quinolines, Flavonoides, Glycosides, Sulfonylurea, Lipids, Phenothiazines, Sulfanilamide, Diacylglycerols, Umbelliferones, Lactone, Naphthofuran, Gamma-keto acids, Saponins and Guanidine were among the important 17 classes of Phytochemical confirmed by (HR)-LCMS analysis. The spectrum profile of an ethanol extract of *Amaranthus tricolor* (HR)-LCMS research (Plate-2) reveals 54 compounds, of which 12 main compounds were verified based on their retention time, mass, and molecular formula.



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1. Amaranthus tenuifolius Wild.

Table. 8 to 10. LC-MS Profiling of Bioactive Compounds From Ethanolic extract of Amaranthus tenuifolius leaves.

Sr.No.	Class	Identified Compound	Molecular Formula	Mass	RT (Min.)
		2,4-Dichloro-3- oxoadipate	C6 H6 Cl2 O5	227.9615	0.85
1.	Carboxylic Acid	Malic acid	C4 H6 O5	134.0222	1.053
		Benexate	C23 H27 N3 O4	409.1923	5.902
		Alangimarckine	C29 H37 N3 O3	475.2789	12.103
2.	Amino Acids	Carvedilol	C24 H26 N2 O4	406.1876	6.727 7.168
1992	Annua Actus	Octopine	C9 H18 N4 O4	246.1325	7.168
		Argiotoxin 659	C31 H53 N11 O5	659.4197	9,61
	Steroids	23-Acetoxysoladulcidine	C29 H47 N O4	473.3443	15,637
3.		Halcinonide	C24 H32 C1 F O5	454.1908	5.902
4.	Thia Fatty Acid	(±)-2-Hydroxy-4- (methylthio)butanoic acid	C5 H10 O3 S	150.0354	1.018
		Jubanine A	C40 H49 N5 O6	695.3805	10.857
		Septentriodine	C37 H52 N2 O11	£2 O11 700.3608	9.644
5.	Alkaloids	Retronecine	C8 H13 N O2	155.0925	1.18
		Irinotecan	C33 H38 N4 O6	586.2752	17.138
		Convolamine	C17 H23 N O4	305.1607	6.934
6.	Terpens	Ganosporelactone A	C30 H40 O7	512.2752	17.918

Sr.No.	Class	Identified Compound	Molecular Formula	Mass	RT (Min.)
_		Ganoderic acid F	C32 H42 O9	570.2794	18.009
		Euphornin	C33 H44 O9	584.2958	18.752
		Lilaline	C20 H17 N O7	383.1028	2.738
	Planardia	Gallocatechin-4beta-ol	C15 H14 O8	322.071	5.767
7.	Flavonoides	Allivicin	C27 H30 O16	610.1604	5.619
		Sophoranone	C30 H36 O4	460.2613	8.359
8.	Glycosides	Caffeic acid 3-O- glucuronide	C15 H16 O10	56.0764	2.315
1750		Alangiside	C29 H47 N O7	505.1888	6.144
9,	Lactone	Mycinamicin VII	C29 H47 N O7	521.3416	16.417
10.	Sulfones	2,3,5,7,9- Pentathiadecane 2,2-dioxide	C5 H12 O2 S5	263.9404	0.84
11.	Carbohydrates	D-Glucarate	C6 H10 O8	210.038	1.517
		O-Feruloylgalactarate	C16 H18 O11	386.0875	3.239
		beta-D-Glucopyranosyl- 11-hydroxyjasmonic acid	C18 H28 O9	24,1808	4.664
12.	Pteridines	Lumazine	C6 H4 N4 O2	164.0333	1.919
13.	Vitamin	Folic acid	C19 H19 N7 O6	441.1317	2.728



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Sr.No.	Class	Identified Compound	Molecular Formula	Mass	RT (Min.)
14.	Pyrrolizines	Crotanecine	C8 H13 N O3	171.0902	3.381
15.	Antioxidant	Esculetin	C9 H6 O4	178.028	4.249
16.	10 A	2-Caffeoylisocitrate	C15 H14 O10	54.0624	4.735
10,	Tricaboxylic Acid	cis-Aconitic acid	C6 H6 O6	174.0172	4.773
17.	Arene	Dibenzo [h,rst]pentaphene	C28 H16	352.1241	5.512
18,	Triazole fungicide	Fluotrimazole	C22 H16 F3 N3	379.132	5.587
		N-trans-Feruloyloctopamine	C18 H19 N O5	329.1274	5.866
19.	Phenols	Vanillin acetate	C10 H10 O4	194.0591	6.765
		Gossypol	C30 H30 O8	518.1935	5.007
20.	Imidazoles	Imidapril	C20 H27 N3 O6	405.195	4.533
21.	Coumaric Acids	Grossamide	C36 H36 N2 O8	622.231	7.963
22.	Antibiotic	Lymecycline	C29 H38 N4 O10	602.2583	8.872

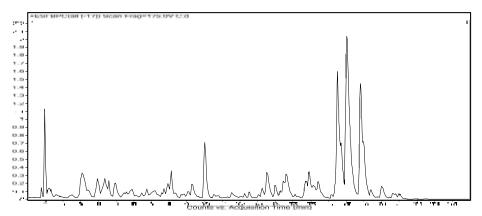


Plate 3: (HR)-LCMS Spectrogram of Leaves of (c) A.tenuifolius Wild.

The ethanol extract of *A.tenuifolius* was discovered to contain 43 Phytochemical of around 21 classes using (HR)-LCMS. The structure of unknown chemicals eluted at different periods is examined using a mass spectrometer and summarized in Table no.8 to 10.

Carboxylic Acid, Amino Acids, Steroids, Thia Fatty Acid, Alkaloids, Terpens, Flavonoides, Glycosides, Lactone, Sulfones, Carbohydrates, Vitamin, Pyrrolizines, Antioxidant, Tricaboxylic Acid, Arene, Triazole fungicide, Phenols, Imidazoles, Coumaric Acids, and Antibiotics were among the important 21 classes of phytochemicals. The spectrum profile of an ethanol extract of *Amaranthus tenuifolius* (HR)-LCMS research (Plate-3) reveals 54 compounds, of which 16 main compounds were verified based on their retention time, mass, and molecular formula.

Conclusion

This study aimed to investigate the Phytochemical profile of different amaranth leaf varieties using LC-MS analysis. The results revealed the presence of various bioactive compounds, including flavonoids, Phenolic acids, and other Phytochemicals, across all varieties. However, significant variations were observed in the abundance and composition of these compounds among the different varieties. These findings highlight the potential of amaranth leaves as a rich source of natural antioxidants and other beneficial compounds. Further studies are needed to elucidate the specific health benefits associated with the unique phytochemical profile of each variety.



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