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PHYTOCHEMICAL SCREENING, INSTRUMENTAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF AMARANTHUS SPINOSUS PLANT.

Rishikesh Sharma, Prabhat Singh Bais

Abstract:

Introduction/Objectives: Amaranthus spinosus ethanolic extract exhibits phenolic compound presence and flavonoid presence, as well as a notable reduction in microbial growth suppression using the agar plate method. The existence of antimicrobial medicines with strong anti-microbial action, such as benzoic acid, O-amino benzohydroxamic acid, 2-aminobenzoyl hydrazide, and 2-hydroxy acetyl acetate, is supported by GC-mass studies. The primary goal of this study is to evaluate Amaranthus spinosus's ability to identify the chemical compounds needed to measure the antibacterial activity using various models.

Methodology:

The chemical molecule for the antibacterial activity was found using the GC-mass spectrometry equipment, phytochemical compounds, and the Agar Plate Method. Several models are utilized for determine the antimicrobial property of the Amaranthus Spinosus.

Results: *In this study data show ethanolic extract of the Amaranthus Spinosus show significant reduction in microbial growth inhibition using agar plate method, phenolic compound presence and flavonoid present. GC-mass study support the presence of antimicrobial drugs like Benzoic acid, O- Amino benzohydroxamic acid, 2-Aminobenzoyl hydrazide, Benzoic acid, 2-hydroxy acetyl acetate which have significant anti-microbial activity.*

Conclusion: *The studies data support significant anti-microbial activity using ethanolic extract of Amaranthus Spinosus leaves for anti-microbial activity. The formation of suitable dosage form by isolate phytochemical compound which responsible for anti-microbial activity and use on the animal models will be increase the chance of better results.*

Keywords: *Amaranthus Spinosus, GC-mass, Isolate Phytochemical Compound, Anti-microbial activity,*

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INTRODUCTION:

Amaranthus Spinosus have around 20 species found throughout the world. In India *Amaranthus Spinosus* cultivated and found in wild regions. Most commonly found species are Amaranthus blitoides, Amaranthus gracilis, Amaranthus hybridus, Amaranthus pulmeri, Amaranthus retroflexues. Amaranthus retrofluxues. *Amaranthus Spinosus* and Amaranthus virdi are species with Alleloptic potential. Some of Amaranthus species are known for their antimicrobial action and use in the antibiotics peperation [1].

The phytochemical substance present in *Amaranthus Spinosus* are reported to inhibit the germination and growth of various species. Amaranthus species is known by the name of Kate wali choli in Hindi and Pigweed in English. This plant is used as vegetable, treatment of the disease and decorative purpose in the houses. This plant cultivated throughout in India, Srilanka, and many tropical countries.

Amaranthus Spinosus are used in the peperation of antibiotics in allopathic medicine but their specific chemical which responsible for their activity is still unknown. In present study we identify the chemical which is responsible for antimicrobial properties of *Amaranthus Spinosus* [2]. For the identification of chemical compound in this study done phytochemical test, U.V. Spectrophotometry, FTIR, GC-mass Agilent 7890A GC with 5975C MS system. After the determination of chemical compounds antimicrobial test is used for determine the activity.

MATERIAL AND METHODOLOGY:

The leaves of *Amaranthus Spinosus* were collected from botanical garden of AKSHAT NURSERY KAROND,BHOPAL. in the month of march 2020, and identified by Dr. Saba Naaz H.O.D Department of Botany, Safia Science college, A voucher specimen (File no. 194/Saif.
/Sci./Collage/Bpl of the authenticated has deposited in the herbarium of the institute. Leaves of *Amaranthus Spinosus* were shade dried and powdered to get coarse granules which were stored in air tight container in the dark.

Preparation of ethanolic extract of the leaves of *Amaranthus Spinosus* leaves

In present study, plant materials were extracted by using cold maceration method; the Wheat grass were collected, washed and rinsed properly. About 3kg of the powder was extracted with different organic solvent ethanol and allow standing for 4-5 days each. The extract was filtered using whattsman no.1 filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated & excessive moisture was removed and extract was collected in air tight container [3].

Drugs and Chemicals

Chloramphenicol and Miconazole were used as standard for the bacterial and fungal strains respectively were purchased from the local market of Bhopal. All remaining chemicals used in the experiment were of the highest grade commercially available.

Apparatus and Instruments

Rotatory vacuum Apparatus, UV Spectrophotometer A1800 Simadzu. GC-Mass Agilent 7890A GCwith Agilent 5975C MS, Heating mantle. Hot Plate, Magnetic stirrer, Commonly used glassware.

Methodology Phytochemical test

The freshly prepared ethanolic leaves extract was subject to qualitative test to identify the phytochemical compound present in it using the standard procedure [4].

Determination of Total Phenolic Content

The total phenolic contents were determined by using Folin-Ciocalteu reagent. Gallic acid wasused as a reference standard for plotting calibration curve. A volume of 0.5 ml of the plant extract (100 µg/ml) was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de- ionized water) and were neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using UV-VIS spectrophotometer. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The total phenolic contents were expressed as mg/g gallic acid equivalent.

Determination of Total Flavonoid Content

The total flavonoid contents were determined using the aluminum chloride assay. An aliquot (0.5 ml) of extracts were taken in different test tubes then 2ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminum trichloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml

of sodium hydroxide (NaOH, 4% w/v) and volume was made up to the 5ml with distilled water.
After 15 min of incubation the mixture turns to pink whose absorbance was measured at 510 nm using a spectrophotometer. Distilled water was used as blank. The total flavonoid content was expressed in mg of rutin equivalents per gram of extract [5-7].

Antimicrobial Activity Models
The antimicrobial activity of extract evaluated using modified agar overlay method the nutrient broth was prepared using (13g/L) in distill water and heating on hot plate assembled magnetic stirrer for making homogenous mixture was obtained. The 50 ml of nutrient broth was transferred into 250 ml stoppered with cotton wool and aluminum foil. The nutrient agarwas prepared using 28g/L agar in distill water in similar manner as nutrient broth. The two nutrient broth and nutrient agar were separately autoclaved for 15 minutes at 121˚ C. The nutrient broth was cooled in bio-hazard while the nutrient agar was kept in oven set 45˚C until ready to use.

Suspension of the 10 ml of the reconstituted pathogens were separately introduced into labelled 250x5 (Flask) containing 100 ml of warm nutrient agar. Using sterile graduated pipette. The pathogens were administered and spread as evenly as possible onto the precoated Silica-gel TLC plate already loaded with different compound using various loading 100, 50, 10,5,1 µg per plate. The nutrient agar containing pathogens administered was allowed to solidify before being incubated for 24 h 37˚C and 28˚C for the bacterial and fungal strain respectively. The zone inhibition in mm after 24 h) were measured using after staining plate with methylthiazolyltetrazolium chloride (MTT-2mg/ml). Chloramphenicol and miconazole were used as standard for the bacterial and fungal strains respectively. The entire microbial assay was conducted under the aseptic condition [8-10].

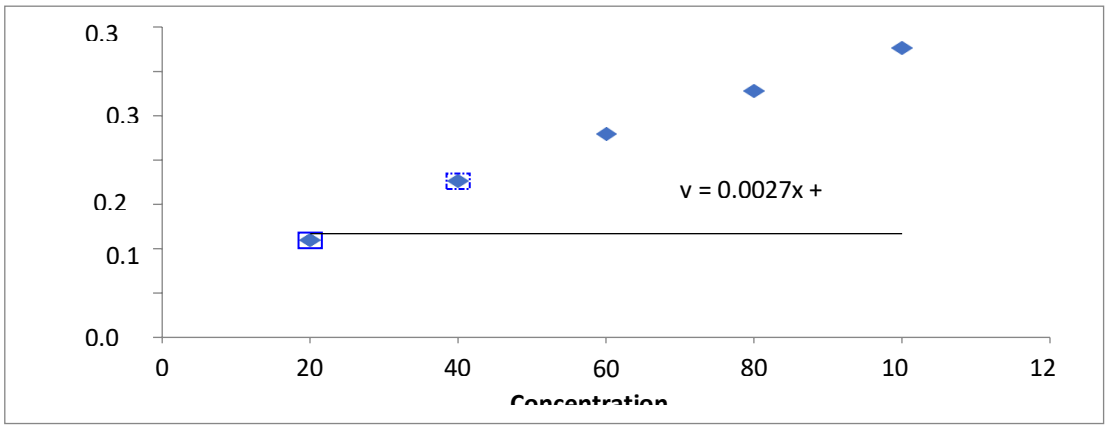
GC-mass analysis
Gc-mass analysis carried out at Central Instrumentation facility of IISER Indian institute of Science Education and Research Bhopal. This technique is very important for the identification of various phytochemical of plants. The equipment used for GC-mass for detection of molecular weight and structure of chemical compound. For GC-mass detection an electron ionization system with ionizing energy of 70 ev was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate 1 ml/min and 1 µl of plant sample was employer (split ratio of 10:1) at the injection temperature 250˚ C ion source temperature of 280˚ C and total running time approx. 60 minutes.

Identification of the GC-mass was conducted using the database of National Institute Standard and Technology having>6200 patterns. The spectrum of the unknown component was composed with the spectrum of known component in the repository of NIST library. The retention of the molecular weight, molecular formula and composition percentage of the sample material were record.

RESULTS:

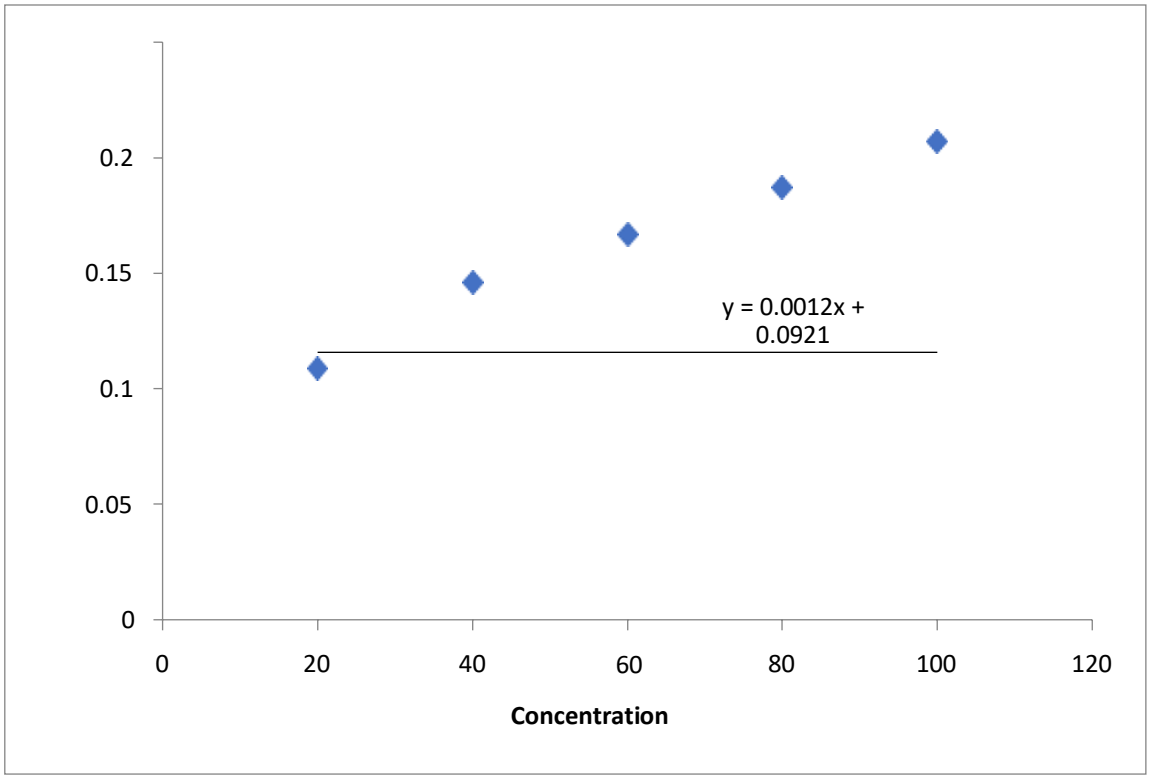
Phytochemical evaluation
Ethanolic extract of *Amaranthus Spinosus* show the presence of Carbohydrate, Protein,Glycosides, Alkaloids, Flavonoids, Triterpenoids, Tannins and Phenol.

Total Phenolic Concentration (TPC):-



Graph-1: Standard curve Gallic acid

TPC Expressed as mg/gm Gallic Acid Equivalent in *Amaranthus spinosus*	
S. No.	
1	0.163
2	0.16
3	0.165
Mean Absorbance	0.163
TPC Value	**50mg/gm equivalent to Gallic acid**



Graph: Standard curve Rutin

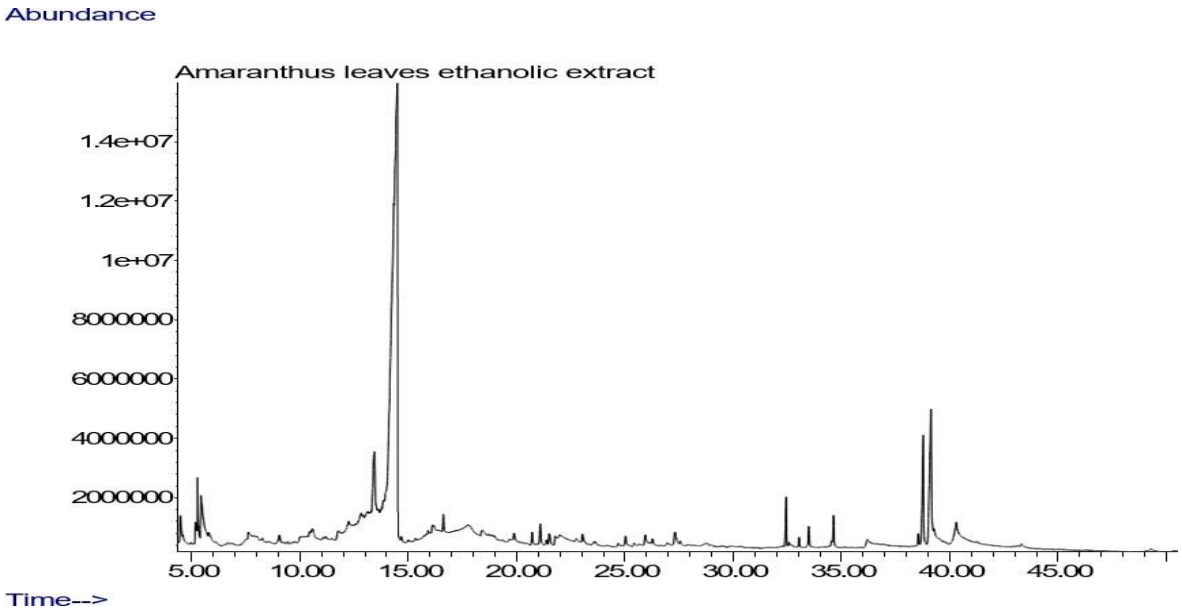
TPC Expressed as mg/gm Rutin Equivalent in *Amaranthus spinosus*	
S. No.	
1	0.151
2	0.156
3	0.138
Mean Absorbance	0.148
TPC Value	**50mg/gm equivalent to Rutin**

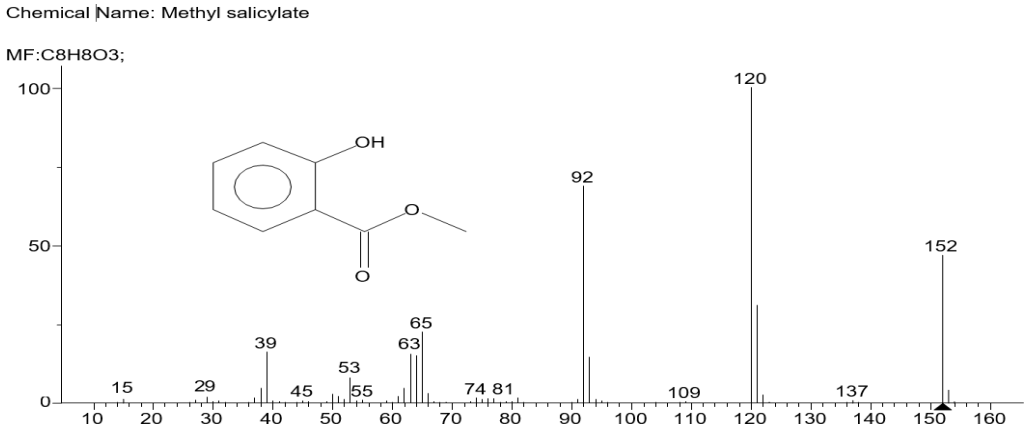
The preliminary anti-microbial assay of extract show different responses to the test organism with best activity observed for both extract ether and ethanolic with MIQ of 1 g. The extract of *Amaranthus Spinosus* ethanolic and ether have significant antimicrobial activity. The preliminary antimicrobial potency of these species is generally categized moderate activity ascompared to the standard drug chloramphenicol and miconazole.

Antimicrobial activity agar plate method:-

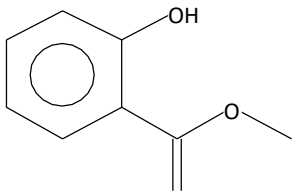
Primarily antimicrobial activity of the different extracts Microbial stain and MIQ in (µg)							
Species	**Parts**	**Extract**	**Gram poistive bacteria**		**Gram negative bacteria**		**Fungus**
			E.Coli	P.aeruginosa	B.Subtilis	S.aureus	C.albicians
Amaranthus Spinosus	leaves	Ethanolic	0.6	15	15	15	0.4
Amaranthus Spinosus	leaves	Ether	0.75	20	20	20	0.35
Chloramphenicol			0.5	10	0.25	0.5	NA
Miconazole			NA	NA	NA	NA	0.25

Amaranthus Spinosus





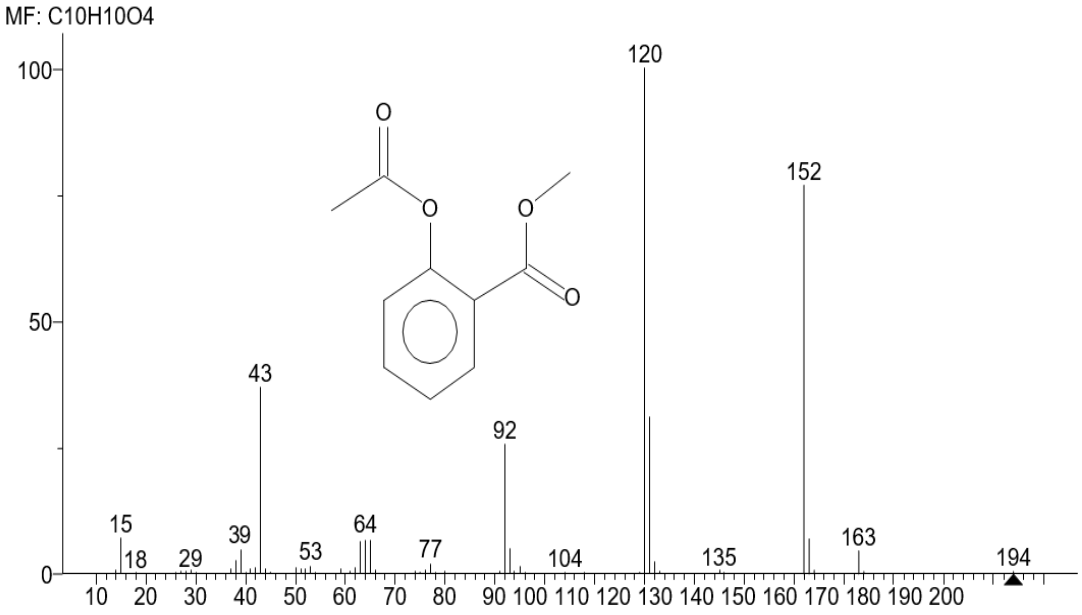
Methyl salicylate: Molecular weight:



10 largest peaks:

120 999	92 688	152 468	121 311	65 225
39 161	63 155	64 149	93 145	53 81

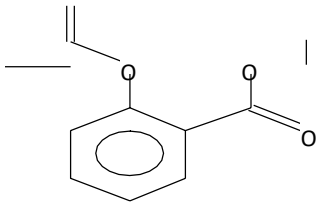
Chemical Name: Benzoic acid, 2-(acetyloxy)-, methyl ester



Benzoic acid, 2-(acetyloxy)-, methyl ester

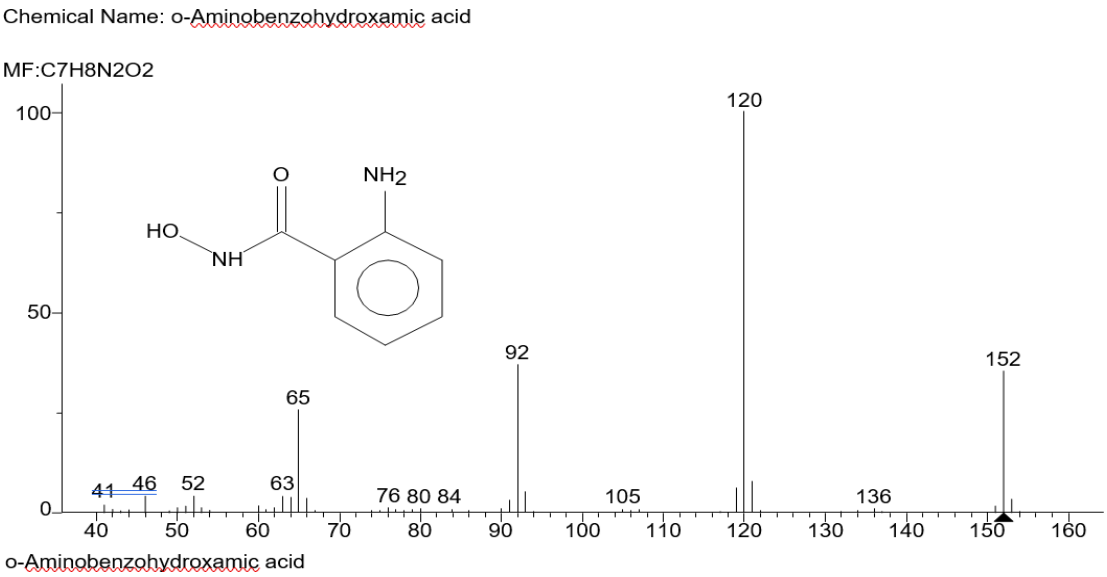
Benzoic acid Molecular Weight: 194

I

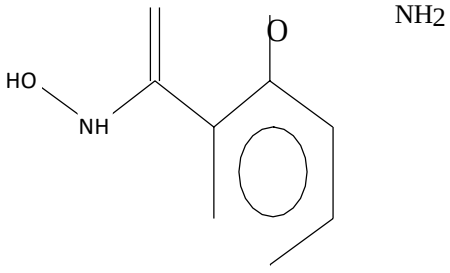


10 largest peaks:

120 999	152 768	43 369	121 309	92 257
15 70	153 69	64 66	65 65	63 62



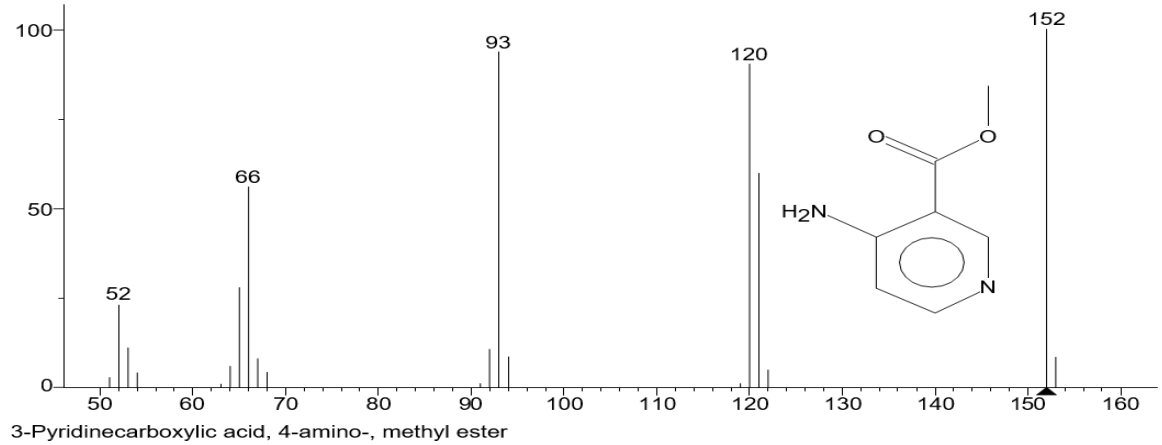
o-Aminobenzohydroxamic acid: Molecular weight :152



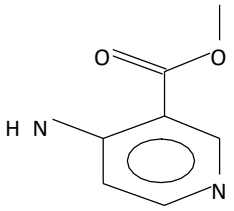
10 largest peaks:

120 999	92 368	152 353	65 257	121 78
119 62	93 53	46 42	52 41	63 41

Chemical Name :3-Pyridinecarboxylic acid, 4-amino-, methyl ester
MF: C7H8N2O2

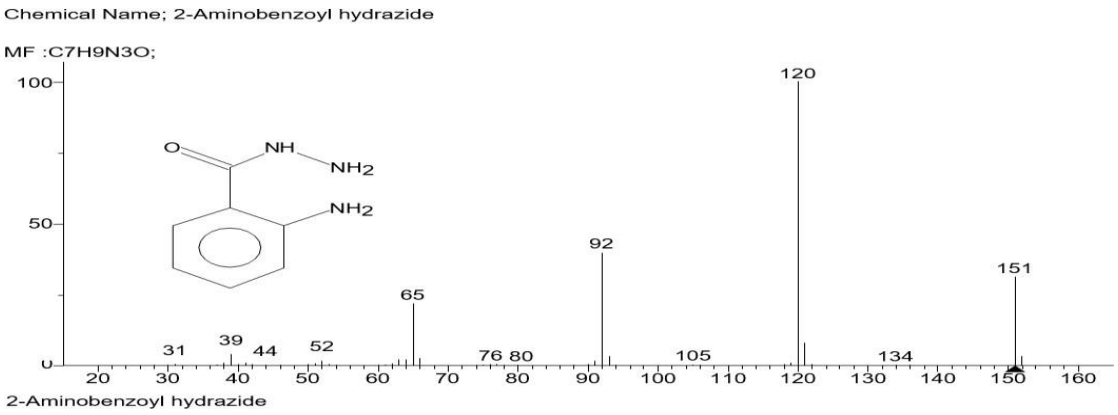


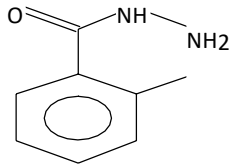
3-Pyridinecarboxylic acid, 4-amino-, methyl ester



3-Pyridinecarboxylic acid, 4-amino-, methyl10 largest peaks:

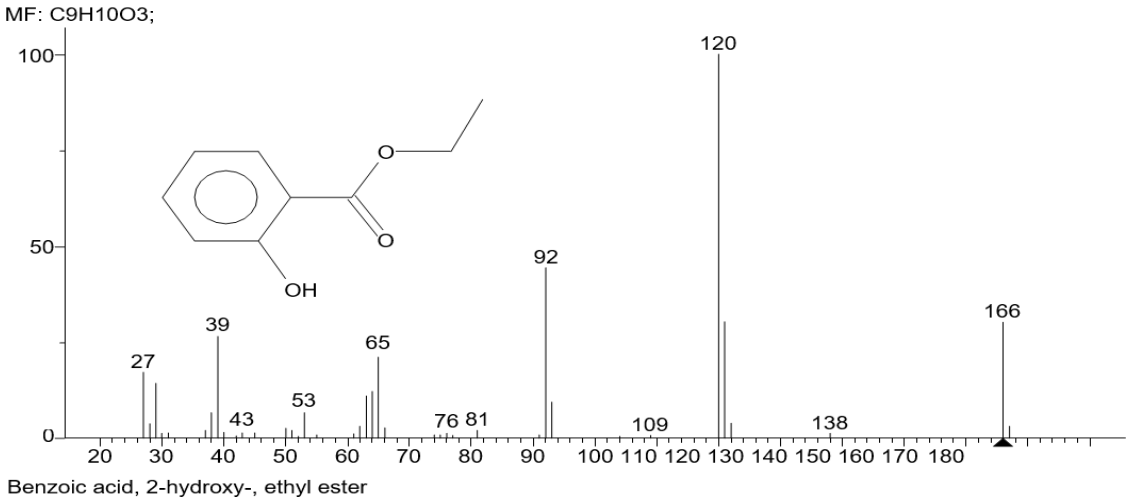
152 999	93 936	120 902	121 597	66 559
65 278	52 229	53 110	92 106	94 85

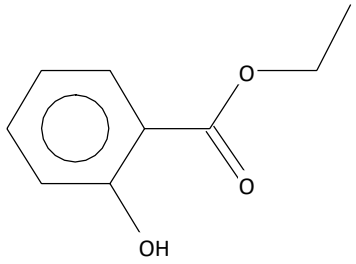




2- Aminobenzoyl hydrazide Mol. Weight:151

120 999				92 397	151 312	65 218	121 80
39 40	152 34	93 33	66 26	64 22		





Benzoic acid, 2-hydroxy-, ethyl ester Molecular Weight:156

120 999 |	92 397	151 312	65 218	121 80
39 40	152 34	93 33	66 26	64 22

Amaranthus Spinosus

S.No	Name of Compound	Molecular formula	M.W.	Pharmacological actions
01.	Methyl Salicylate	$C_8H_8O_3$	152	Analgesic, Anti-inflammatory, Anti-coagulant [11]
02.	Benzoic Acid	$C_{10}H_{10}O_4$	194	Anti-microbial [12]
03.	o-Aminobenzohydroxamic	$C_7H_8N_2O_2$	152	Skin-infection [13]
04	3-Pyridinecarboxylic acid, 4-amino-, methyl ester	$C_7H_8N_2O_2$	152	Skin-infection [14]
05	2-Aminobenzoyl hydrazide	$C_7H_9N_3O$	152	Anti-viral [15]
06	Benzoic acid, 2-hydroxy-, ethyl ester	$C_9H_{10}O_3$	166	Anti-microbial
07	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	Enanthic acid oily liquid with unpleasant odour
08	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	Fatty acid anion and conjugate the base
09	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	278	Enanthic acid oily liquid with unpleasant odour
10	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	306	Enanthic acid oily liquid with unpleasant odour
11	Butyl 9,12,15-octadecatrienoate	$C_{22}H_{38}O_2$	334	Enanthic acid oily liquid with unpleasant odour
12	Methyl 7,10,13-hexadecatrienoate	C17H28O2	264	Enanthic acid oily liquid with unpleasant odour
13	Tetra methyl-2 hexa decan	C20H40O	296	Precursor of Vitamin E, antibacterial and anti-oxidant property
14	Phytol or Isophytol	C20H40O	296	Vitamin E source [16]
15	Oxirane, hexadecyl-	C18H36O	268	Used in the process of sterilization
16	6-Octen-1-ol,3,7-dimethyl (±)-	C10H20O	156	Perfume used in industry
17	1,2-15,16-Diepoxyhexadecane	C16H30O2	254	Oil composed of ester, terpenes and carboxylic acid

GC mass analyzed the results which include the active principles with their molecular formula, molecular weight, and composition of *Amaranthus Spinosus* which are present in thetable No. 2 . The GC -Mass chromatogram responsible for antimicrobial activity shown in the figure no 1 to 06. Manly significant physiological active components were identified from the sample of *Amaranthus Spinosus* by simple GC-mass method are Methyl Salicylate, Benzoic Acid ,O- Amino benzohydrosanilic acid, 2-Aminobenzoyl hydrazide , Benzoic acid, 2-hydroxy-, ethyl ester 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z), 9,12,15-Octadecatrienoic acid, (Z,Z,Z), Methyl 8,11,14-heptadecatrienoate, Ethyl 9,12,15-octadecatrienoate ,Butyl 9,12,15- octadecatrienoate, Methyl 7,10,13-hexadecatrienoate, Tetra methyl-2 hexa decan, Phytol or Isophytol ,Oxirane, hexadecyl-6-Octen-1-ol, 3,7-dimethyl-, (±)- 1,2-15,16- Diepoxyhexadecane.
Antimicrobial activities are shown by various identified compound such as Methyl Salicylate, Benzoic Acid, O-Amino benzohydrosanilic acid, 2-Aminobenzoyl hydrazide, Benzoic acid, 2-hydroxy.

DISCUSSION:

In the present study a total were identified in terms of % amount are main component of the Amaranthus identified compound having among other compound.

The result of GC-MS testing indicated that Amaranthus leaves contained numerous bio-active phytoconstituents belong to various classed such as Carbohydrate, Protein, Glycosides, Alkaloids, Flavonoids, Triterpenoids, Tannins and Phenol.

The leaves quantification by colorimetric method, was found to be rich in phenolic compound (flavonoid} and therefore exhibit very good scavenging activity. It can be concluded that leaves could be used as a natural source of antioxidants and its regular consumption in diet could prove health benefits to humans by protecting against oxidative stress.

Further detailed in Vitro and Vivo in co-relation studies along with isolation of active constituents are needed to novel treatment strategies for free radical indued disease.

CONCLUSION:

The study reveals the presence of bioactive compound of ethanolic extract of *Amaranthus Spinosus*. The present study provides a good concrete base for further research to isolate level bioactive compound in the leaves to develop new anti-microbial agent.

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