Phytochemical And Pharmacological Evaluation Of Hydroalcoholic Extract Of Amaranthus Viridis Linn

Manimekalai Pichaivel[1], Devadharshini Dhandayuthapani[1], Omji Porwal[2], Pradeep Kumar Sharma[3*], Divya Manickam[1], Vivek[3], Shekhar Prakash Kushwaha[3], Prabhu Dayal Rajan[3], Durga Devi Udhayakumar[1],Ashish Verma[3],Gowsalya Rathinavel[1], Kavya Raja[1]

[1] Swamy Vivekanandha College of Pharmacy, Elayampalyam, Tiruchengode, Namakkal District, Tamilnadu - 637 205, INDIA.
[2] Department of Pharmacognosy, Faculty of Pharmacy, Tishk International University, Erbil, Iraq Near Filkey Baz (Square), across Qazi, Muhammad, 44001, Erbil, KRG/IRAQ,
[3*]Accurate College of Pharmacy, Knowledge Park-III, Greater Noida, Uttar Pradesh, India 201306

***Corresponding Author:-** Pradeep Kumar Sharma

*Accurate College of Pharmacy, Knowledge Park-III, Greater Noida, Uttar Pradesh, India 201306
E-mail: pradeepbsr17@gmail.com
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Abstract

Infectious disease are the major cause of morbidity and mortality and thus a serious public health problem in developing countries. Despite the arsenal of antibiotics available, the situation is worsening due to emerging drug resistance. Antimicrobial resistance is a major issue these days due to extensive use or misuse of antibiotics. Hence, there is an urgent need to find some new interventions. Amaranthus viridis Linn (Family: Amaranthaceae) is commonly known as“chowlai”is acommonwild vegetable andiswidely grow in gunderawi derange of climatic conditions and has been utilizedasa medicin alherbin traditional ayurvedic medicine for the treatment of inflammation, diabetic, asthma, leprosy, cancer etc. Amaranthus viridis Linnincludes various biologicallyactive constituents such as saponin, tannin, phenols, flavonoids, alkaloids, cardiac glycosides,steroid and triterpenoids. This,medicinalplantsis a sourceformanypharmaceutical drugs and indicated in many infections and diseases.The design of this study was to evaluate the preliminary phytochemical contents and toanalyse thephytocompounds present in the hydroalcoholic leaf extract of Amaranthus viridisLinnby using Liquid chromatography - Mass spectroscopy (LC-MS) and in-vitro evaluationsare need edto exploreagains tpatho genicmicroorganism. Preparation of Amaranthus viridis Linn. Leaves extract using ethanol and water (80:20), followed by preliminary phytochemical screening of the extract, estimation of phenolic content and mass determination with LCMS, and evaluation of in vitro Antibacterial, Antifungal and Anthelmintic activity. Based on the findings of our studies, we conclude that hydro alcoholic extract of Amaranthus viridis Linn has potent anthelmintic, antifungal and antibiotic activity attributed to the presence of an secondary metabolite flavonoids (quercetin).It was confirmed by analyzing the phytocompounds by LC-MS and compared with total phenolic content identified by UV Visible spectroscopy shows that highest amount of flavonoids (quercetin) present in the Hydroalcoholic extract of Amaranthus viridis Linn. However, further studies need to be carried out to investigate the molecular mechanism of the secondary metabolite.

Keywords: Amaranthus viridisLinn, Anti-bacterial, Anti-fungal, and Anti-protozoal

1. INTRODUCTION

Medicinal plants are plants that have phytoconstituents that can be utilized to treat ailments in one or more of their components. Plant-based medicines are well-known for their safety, ease of availability, and low cost. Herbal remedies can be made from the entire plant or largely from the leaves, roots, bark, seed, and flowers. They can be taken orally, breathed, or applied directly to the skin. Alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds, and many more bioactive phytochemical elements are among the most important [1]. According to recent estimates, more than 70% of India's 1.1 billion people still use non allopathic medical systems[2,3].Herbs have been valued for their medicinal, flavouring, and aromatic capabilities for ages, synthetic products of the modern era temporarily overshadowed their relevance[4-97]. In fast-developing countries like Nepal, India, Bhutan, and Kenya, the contribution can be as high as 80%. As a result, medicinal plants are far more valuable to emerging countries like India than to the rest of the globe [98]. The National Council for Alternative Medicine (NCAM) in the United States has accepted the Indian system of medicine, albeit most of the medications that promise to cure various ailments have yet to be scientifically confirmed. Scientific confirmation will not only make these treatments more popular in India but will also make them acceptable to people in other areas of the world in some way [99]. Infectious disease has been a leading source of death and disability since the birth of humanity. Antibiotics are one of the most recent developments in the fight against bacterial illnesses. All main microbial pathogens and antimicrobial medications are involved in drug resistance. Currently, clinically significant bacteria are characterized not only by single drug resistance, but also by multidrug resistance. These are difficult to treat and are the source of a number of infectious diseases [100]. The goal of antimicrobial research is to

find and develop new antimicrobial agents that are effective against a variety of pathogens [101]. Plant-based antimicrobials will aid in the fight against resistance and will be more reliable than synthetic antimicrobials [102,103]. Helminthiasis is a parasitic worm infection in which parasitic worms such as roundworms (Nematodes), tapeworms (Cestodes), or flukes infest a region of the body (Trematodes). Although the worms live in the intestines, they can also burrow into the liver and other organs [104]. These parasites are also transmitted in places with poor sanitation, its geographic distribution overlaps with that of the other soil-transmitted helminthiasis. Some broad-spectrum anthelmintics (e.g., Piperazine citrate, Albendazole) work against parasitic flat worms and nematodes. However, the majority of medicines have a limited action (e.g., Praziquantel) because resistance can develop quickly and toxicity issues can arise [105-106]. As a result, alternative treatment, such as medicinal herbs with broad range anthelmintic activity and low toxicity, is required [107].The medicinal plant chosen for our detailed study is Amaranthus viridis Linn, which has been shown to have a variety of therapeutic properties. Analgesic is one of Amaranthus viridis Linn listed actions. Antipyretic, Anticonvulsant, Antiviral, Antileprotic, Antioxidant, Antiulcer, Fever, pain, asthma, diabetes, dysentery, urinary disorders, liver disorders, eye disorders, and venereal illnesses are all treated using Amaranthus viridis Linn as a traditional medicine[108-110]. The plant is emollient and vermifuge. The entire plant is decocted to treat diarrhoea and inflammations, as well as purify the blood[111]. The root juice is used to alleviate irritation in the urinary tract as well as constipation. Diuretic, febrifuge, and purgative, the leaves are used to treat heart problems, Gonorrhoea, and haemorrhoids. The sap from the leaves is used to cure eye infections [112]. Based on the literary facts current study was planned to evaluate the preliminary phytochemical contents and analyse the phytocompounds present in the hydroalcoholic leaf extract of Amaranthus viridis Linn utilizing Liquid chromatography-Mass spectroscopy (LC-MS) and in-vitro evaluations [113].

2.MATERIAL AND METHODS

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

The whole plant of Amaranths viridis Linn were collected from in and around the region Erode, Tamil Nadu in month of February 2022. The plant materials were identified and authenticated by Botanist. A voucher specimen was submitted and deposited in the department of pharmacology, swamy Vivekanandha college of pharmacy, Tiruchengode Tamil Nadu.

PREPARATION OF PLANT EXTRACT

The plant extract was prepared as per the protocol given in the literature, with slight modifications [114-143]. The Freshly leaves of a Amaranths viridis Linn were collected and cleaned to evacuate the adhered foreign material. The collected leaves were shade dried at room temperature. The dried plant materials were subjected to size reduction to coarse powder by using dry grinder and passed through sieve. No: 40 was used for extraction. Powdered material of Amaranthus viridis Linn was extracted by Soxhlet apparatus. Hydroalcoholic solvent with ratio of (80:20) with water was added to ta round bottom flask, which was attached to a Soxhlet extractor and condenser on a mantle. The powdered leaf material (60gm) was loaded in the muslin cloth, which was placed inside the Soxhlet extractor. The side arm was lagged with 24 glass wool. The solvent was heated using the mantle and began to evaporate, moving through the apparatus to the condenser. The condenser then driped into the reservoir containing the muslin cloth. Once the level of solvent reached the siphon it poured back into the flask and the cycle begins again. The process ran for about 30 hours. The equipment was turned on and off when overnight running was not permitted, and the time split over a number of days. Once the process was finished, the solvent was evaporated using a heating mantle at 5- 10° Cleaving the dark coloured molten extract. The extract was stored in airtight container in refrigerator maintained below 10°C until further use.

PRELIMINARY PHYTOCHEMICAL SCREENING OF AMARANTHUS VIRIDIS HYDROALCOHOLIC EXTRACT

The phytochemical screening was done based on the standard protocol, with slight modifications [128-163]. The following tests were performed to detect the phytochemical constituents in Amaranthus Viridis Hydrochloric Extract:

a. Detection of Alkaloids
b. Detection of Carbohydrates
c. Detection of Anthraquinone Glycosides
d. Detection of Cardiac Glycosides.
e. Detection of Saponin Glycosides
f. Detection of unsaturated Sterols/Triterpenes
g. Detection of Phenols
h. Detection of Flavonoids
i. Detection of Tannins
j. Detection of Proteins and Amini acids
k. Detection of Total phenolic content

LC-MS ANALYSIS OF HYDROALCOHOLIC EXTRACT OF AMARANTHUS VIRIDIS LINN

The samples were analysed by Liquid chromatography and Mass spectroscopy(LC-MS) according to the previously described method (Ilyas khan et al., 2017). For chromatographic separation, an Agilent 1200 series LC system equipped with binary gradient solvent pump, a degasser, an autosampler and column oven was used. Phenolic compounds were separated at 25oC, on Zorbax SB-C18 column (2.1 X 50mm, 1.8µm particle size; Agilent Technologies) using 3µl

injections. MS detections was performed in a 3200 QTRAP Mass Spectrophotometer (AB Sciex) and triple quadruple-ion trap mass analyser that was controlled by the analyst 1.5 software. [16, 18, 32, 105, 164, 165].

ANTHELMINTIC ACTIVITY

Experimental worms

The anthelmintic activity was done based on the standard protocol with slight modifications [104]. It was analysed on Indian adult earthworms (Pheretima posthuma) due to its anatomical resemblance with the intestinal roundworms parasites of human beings. They were collected from moist soil and washed with normal saline to remove all faecal matters.

Administration of Albendazole

The oral suspension of Albendazole was prepared by using normal salineto prepare the stock solution (20 mg/ml).the dilutions were administered as a standard drug in the earthworms.

Administration of Extract

The suspension of hydroalcoholic extract of Amaranthus viridis Linn at different concentration (20, 30, 50 mg/ml) were prepared by using 0.9% $NaCl_2$ as a suspending agent and final volume was made upto 30 ml for respective concentration. Albendazole was used as standard. Groups of approximately equal size (5-6 cm), weight 0.32-0.50 gm worms consisting of five individual earthworms individually in each group were released into in each 30 mL of desired concentration of drug and extracts in the petri-dish.

Experimental Design Group I

Albendazole (20 mg/ml) Group II: HAEAV(10 mg/ml) Group III : HAEAV(20mg/ml) Group IV : HAEAV (50mg/ml) The time taken by each group for causing paralysis and fatality to Pheretima posthuma was observed separately for individual worm. The paralysis was confirmed when there is the loss of movement and contraction in earthworms when it is pressed by a finger, whereas the fatality of the earthworm is represented by the loss of their motility with the fading of their body colour.

ANTIFUNGAL ACTIVITY [144-146]

Test Microorganism

The test organism used for the study were Candida albicans, Aspergillus niger. The morphologically identified microorganisms were then subjected to a biochemical test for identification up to biochemical level. The fungal culture were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young fungal broth cultures were prepared before the screening procedure.

Preparation of potato dextrose agar

39 milligram of potato dextrose agar powder was added into one litre of distilled water in a flat bottomed conical flask. Boiling while mixing was done so as to dissolve the potato dextrose agar powder. The flask was then tightly closed using a cotton wool and further covered with aluminium foil. The mixture was autoclaved for 15 mins at 121◦C after which it was left to cool down to room temperature. 40mg of tetracycline was added to inhibit bacterial growth and the media stirred before dispensing in Petri-dishes. The media was poured in a petri-dishes in a laminar flow to give uniform depth of 3-4 millimetres. The petri-dishes were left to cool and after which they were placed in sterile plastic bags and stored at a temperature of 2-8◦C before use.

Antifungal activity by well- diffusion agar plate method

Antifungal activity was measured using methods of well-diffusion plates on agar to test the antifungal activity, the fraction of different concentration of plant extract were dissolved in 70% ethanol. 20 ml of sabouraud dextrose agar was poured into each 15cm petri-dish. Candidia albicans were grown in sabouraud dextrose broth at 27◦C for 48 hours. Growth was adjusted to OD(600nm) of 0.1 by dilution with sabouraud dextrose broth. Then, Wells were cut and 20µL of the different concentrations of test drug were placed on agar to load 10 and 15µL of each spice sample (1mg/ml). 100 units of fluconazole, obtained from local pharmacy, were used as a positive control. Inhibition zones were determined after incubation at 27oC for 48 hours.

ANTIBACTERIAL ACTIVITY[147-157]

Test microorganism

The test organism used for the study were Staphylococcus aureus, Enterococcus faecalis. The morphologically identified microorganisms were subjected to a biochemical test for identification up to biochemical level. The bacterial culture were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

Preparation of Inoculums

Active cultures of experiment were prepared by a loop full of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hours at 37◦C. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities.

Preparation of Sterile Swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or by dry heat (only for the wooden swabs). It was sterilized by packing the swabs in culture tubes, papers or tins etc.

Sterilization of Forceps

Forceps can be sterilized by dipping in alcohol and burning off the alcohol.

Preparation of Muller-Hinton Agar

38 milligram of Muller-Hinton agar powder was added into one litre of distilled water in a flat bottomed conical flask. The mixture was heated with frequent agitation and boiled for 1 minute to completely dissolve the media. The flask was then tightly closed using a cotton wool and further covered with aluminium foil. The mixture was autoclaved for 15 mins at 121°C after which it was left to cool down to room temperature. The media was poured in a petri dishes in a laminar flow to give uniform depth of 3-4 millimetres. The petri dishes were left to cool and after which they were placed in sterile plastic bags and stored at a temperature of 2-8°C before use [81].

Antibacterial Assay Using Agar Well Diffusion Method

The well diffusion method was used to screen the antibacterial activity. Invitro antibacterial activity was screened by using Muller-Hinton agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 50 ml of molten media into sterile petri plates. The plates put solidify for 5 minutes and 0.1% inoculums suspensions was swabbed uniformly, and allowed to dry for 5 minutes. Wells were cut and 20µL of different concentration of test drug were added. The plates were then incubated 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Chloramphenicol disc was used as a positive control [52].

3. RESULTS

Table 1: Colour, consistency and yields of hydroalcholic extract of Amaranthus viridis Linn

S.no	Extract	Colour	Consistency	%yield (w/w)
1.	Hydroalcoholic extract	Dark Green	Sticky Mass	5.6% W/W

Table 2: Preliminary Phytochemical Evaluation of Amaranthus Viridis Linn Hydroalcoholic Leaf Extract

S.NO	PLANT CONSTITUENTS	HAEAV (80:20)
1.	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Gum and Mucilage	-
5	Saponins	+
6	Unsaturated sterols & triterpenes	+
7	Phenols	+
8	Tannins	+
9	Flavonoids	+
10	Proteins And Amino Acids	+
11	Fixed oil and Fat	-

Presence : + Absence: -

Table 3: Total Phenolic Content of Hydroalcoholic Extract of Amaranthus Viridis Linn

Samples	Peak value	Absorbance value
HAEAV	0.9135	0.6484
Quercetin	0.7568	5.0574

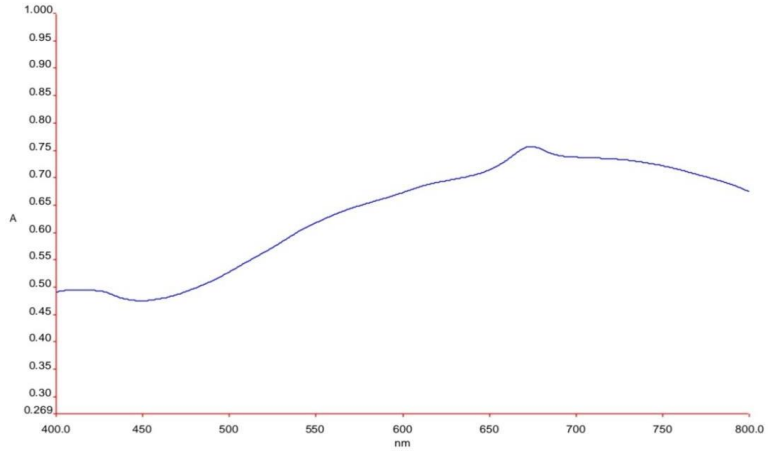


Figure 5: Total Phenolic Content Graph Of Hydro alcoholic extract of Amaranthus viridis Lin

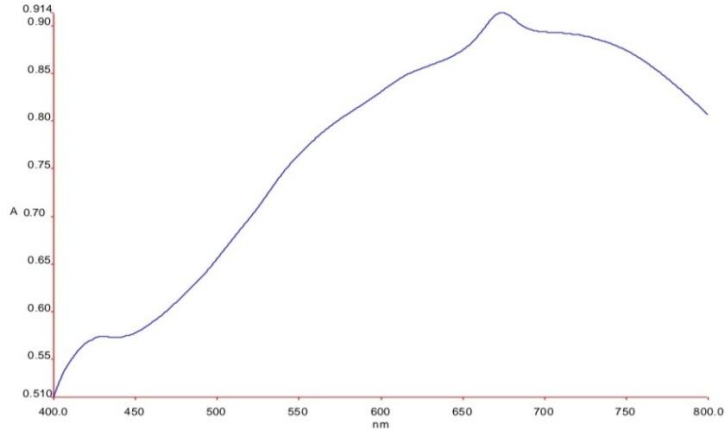


Figure 6: Total Phenolic Content Graph Of Standard (Quercetin)

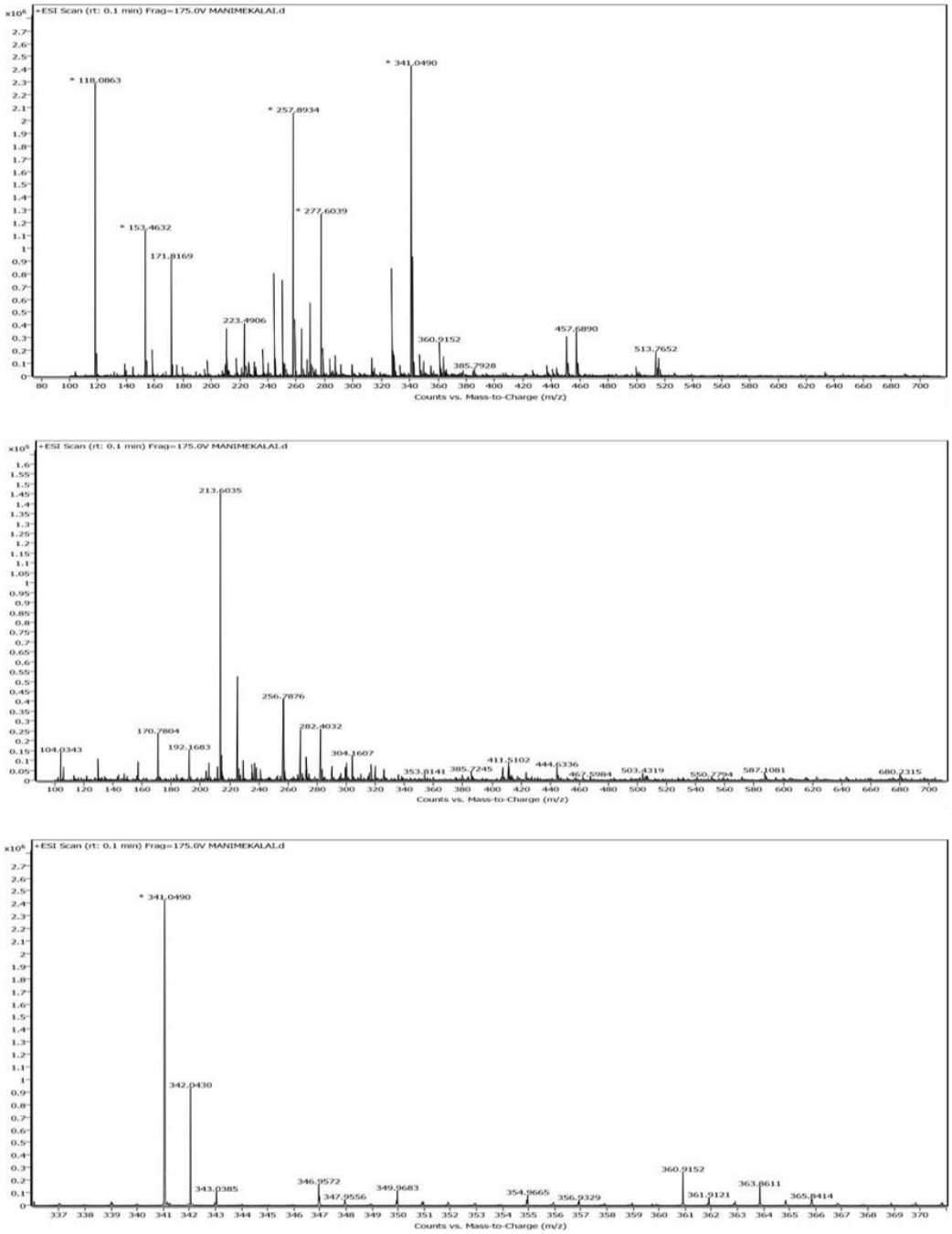


Figure 7: LC-MS Chromatogram of hydroalcoholic extract of Amaranthus viridis Linn Retention time - 0.1

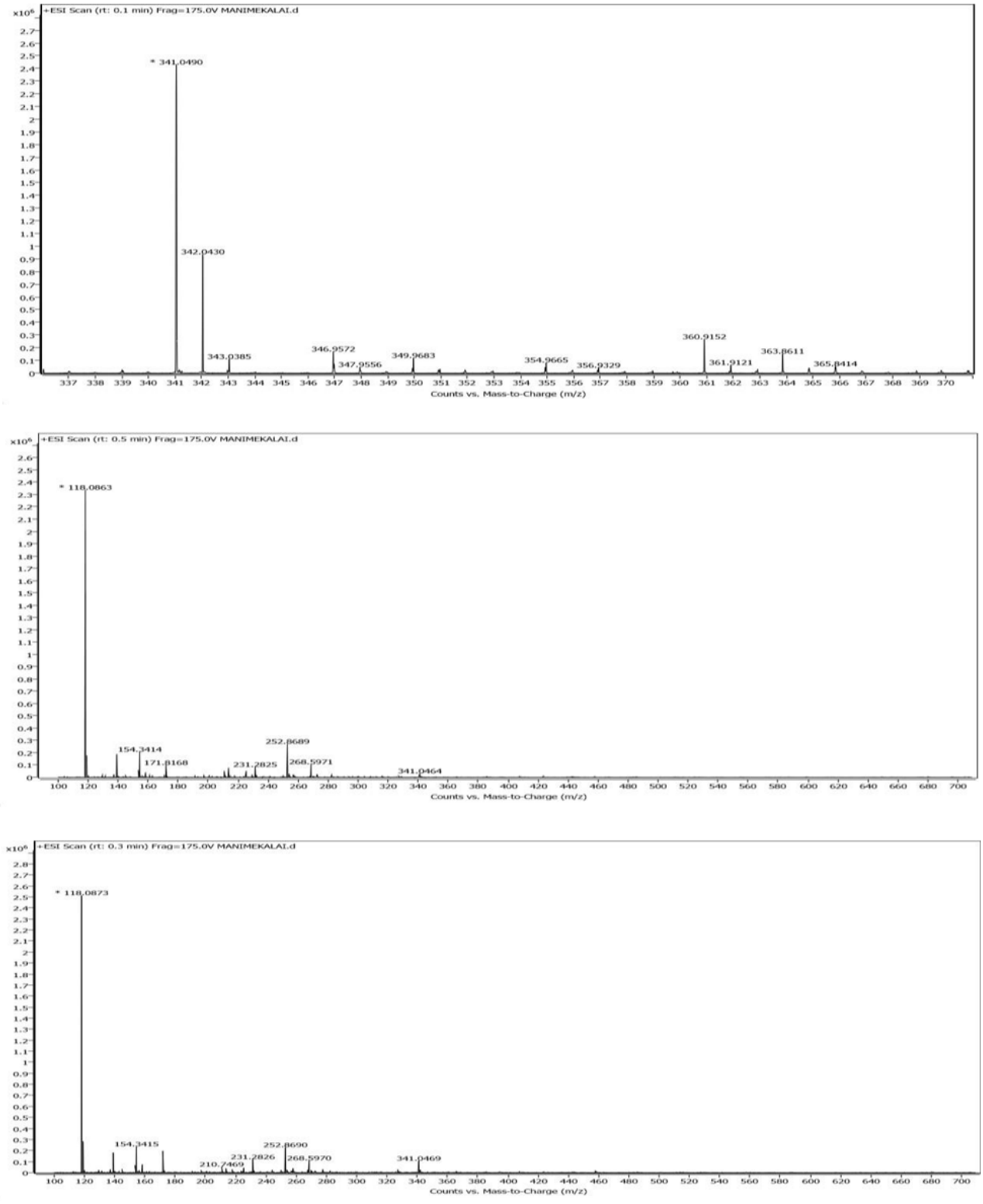


Figure 8: LC-MS Chromatogram of hydroalcoholic extract of Amaranthus viridis Linn Retention time - 0.1, 0.3, 0.5.

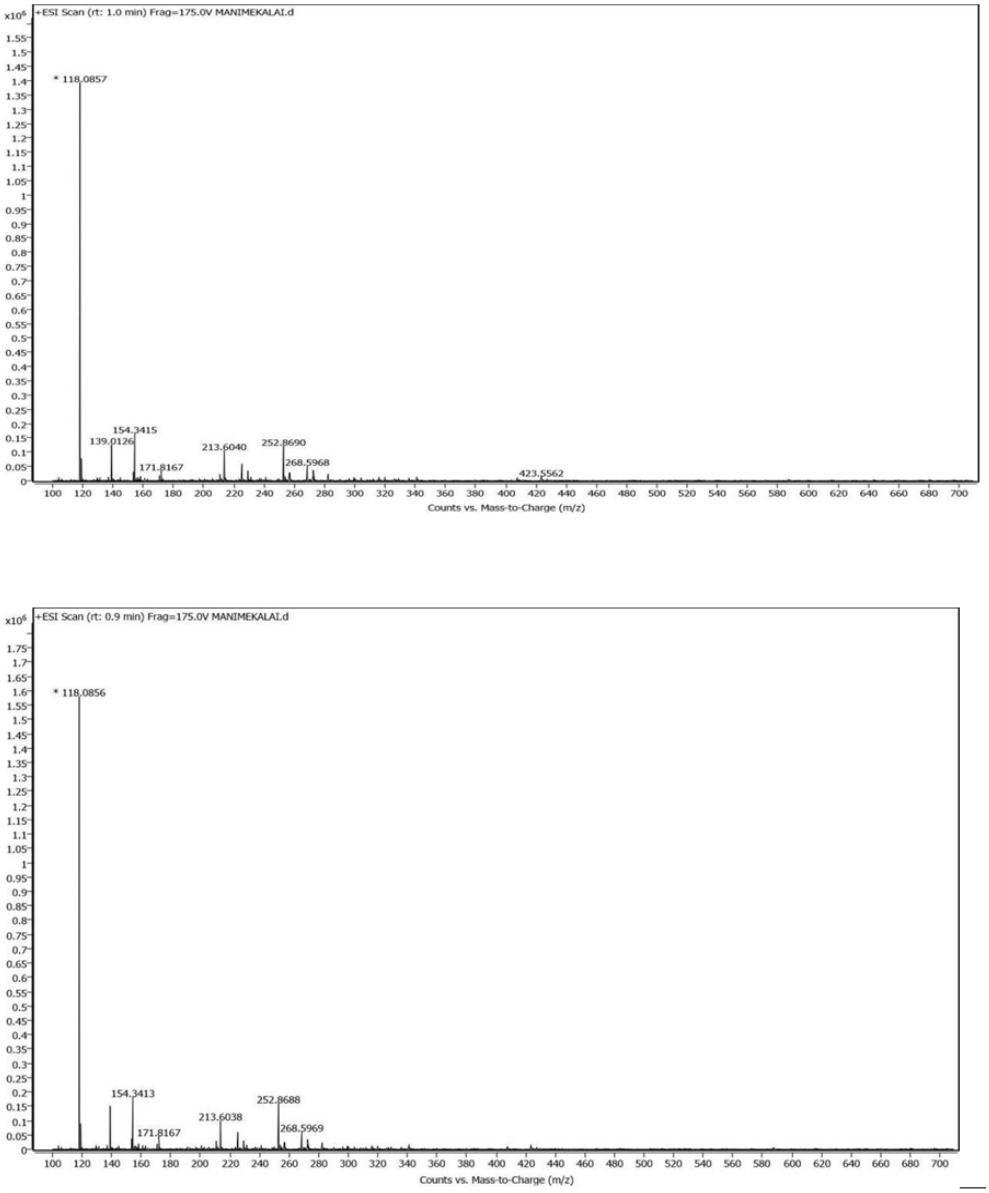


Figure 9: LC-MS Chromatogram of hydroalcoholic extract of Amaranthus viridis Linn Retention time – 0.9, 1.0.

Table 4: Chromatographic and spectral data of the phenolic compounds identified in Amaranthus viridis linn:

S.No	Retentiontime	m/zvalue	Formula	Identifiedcompound
1	0.1	341.04	$C_{16}H_{18}O_8$	Coumaroylquinicacid
2	0.1	118.08	$C_{15}H_{19}O_7$	Quercetin
3	0.1	213.60	$C_{17}H_{20}O_9$	Feruloylquinic acid
4	0.1	277.60	$C_{15}H_{10}O_7$	6-hydroxykaempferol
6	0.1	171.81	$C_{15}H_{16}O_{11}$	Caffeoylglucaricacid
7	0.3	252.869	$C_{27}H_{30}O_{16}$	Quercetin-3-O-neohesperidoside
8	0.1	223.49	$C_{22}H_{30}O_{15}$	Kaempferol-3-O-neohesperidoside
9	0.1	257.89	$C_{22}H_{22}H_{12}$	Isorhamnetin-3-O-glucoside
10	0.3	118.08	$C_{15}H_{19}O_7$	Quercetin
11	0.5	231.28	$C_{15}H_{10}O_6$	Kaempferol
12	0.5	118.08	$C_{15}H_{19}O_7$	Quercetin
13	1.0	268.59	$C_{33}H_{40}O_{20}$	Quercetin-3-O-rutinoside
14	0.9	118.06	$C_{15}H_{19}O_7$	Quercetin
15	1.0	118.08	$C_{15}H_{19}O_7$	Quercetin
16	1.0	423.55	$C_{28}H_{32}O_{16}$	Isorhamnetin-3-O- eohesperidoside
17	0.1	450.7256	$C_{27}H_{30}O_{16}$	Rutin
18	0.5	231.28	$C_{15}H_{10}O_6$	Kaempferol
19	0.1	457.6890	$C_{28}H_{32}O_{16}$	Isorhamnetin-3-O- eohesperidoside

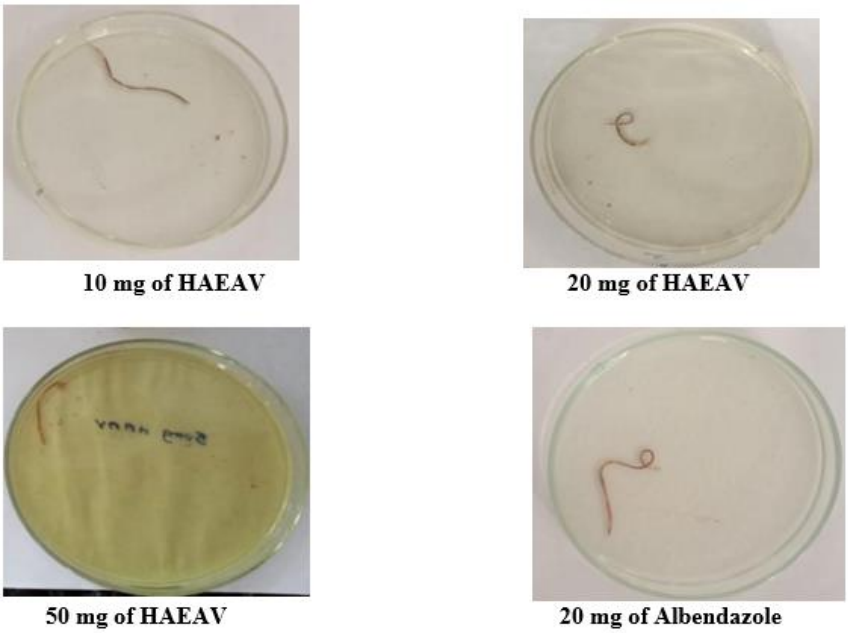


Figure 10: Anthelmintic activity of hydroalcoholic extract of Amaranthus viridis linn at earthworm

Table 5: Results of Anthelmintic activity of hydroalcoholic extract of Amaranthus viridis Linn

Treatment group	Concentration (mg/ml)	Time Taken For Paralysis (inmin)	Time Taken For Death (inmin)
Control (NormalSaline)	-	-	-
Albendazole	20	20.00±0.7071	25.00±0.67
HAEAV	10	20.00±0.7071[a]	25.20±0.8602[a]
	20	14.00±0.7071[a]	19.20±0.8602[a]
	50	6.00±0.7071[a]	11.60±0.9274[a]

Values are expressed as Mean ± SEM, n=5, values were found out by One way ANOVA test. Symbol represents statistical significance: a P<0.0001.

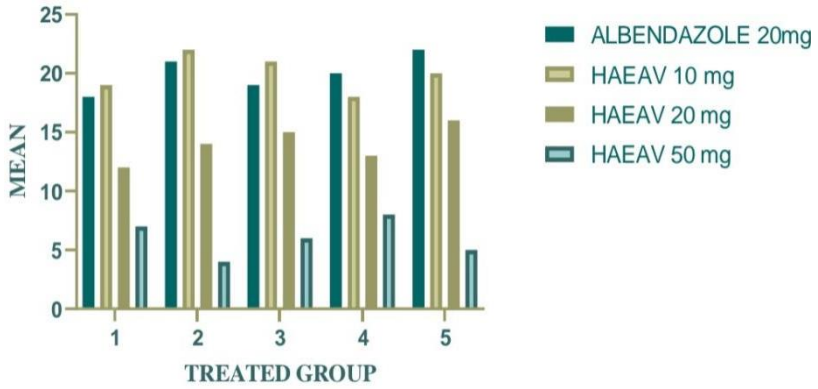


Figure 11: Time Taken For Paralysis (In Min)

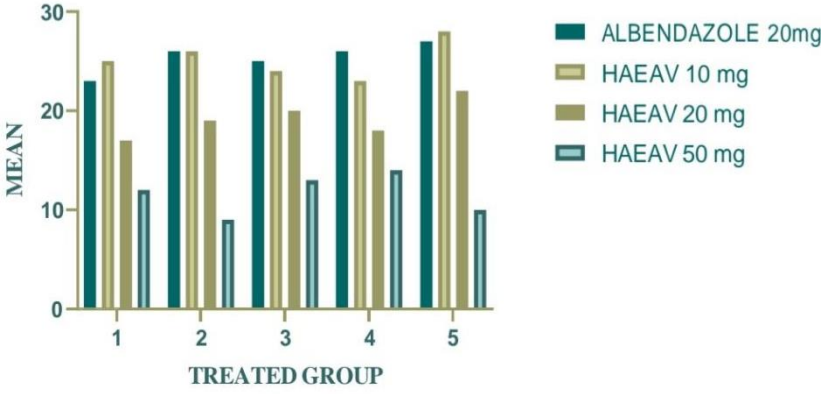
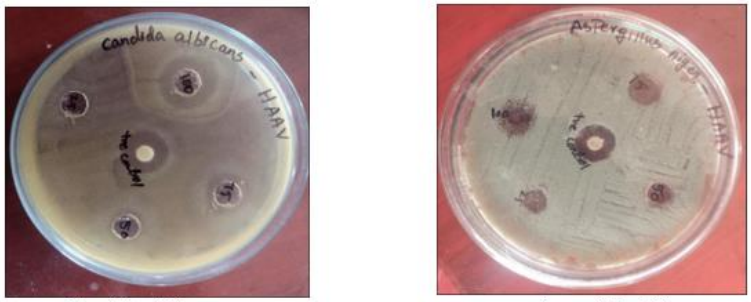


Figure 12: Time Taken For Death (In Min)



Candida albicans Aspergillus Niger

Figure 13: Antifungal activity of hydroalcoholic extract of Amaranthus viridis linn

Table 6: Antifungal Activity of HAEAV In Microorganisms

S. No.	Microorganism	ZoneofInhibition Concentration				PositiveControl
		100µl	75µl	50µl	25µl	
1	*Candida albicans*	17	4	12	10	21
2	*Aspergillusniger*	15	13	10	09	20

Figure 14:Graphical Representation of Antifungal Activity of Hydrochloric Extract of Amaranthus viridis Linn.

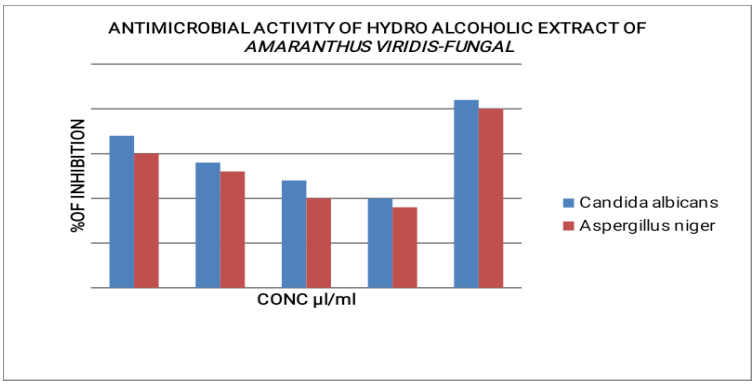
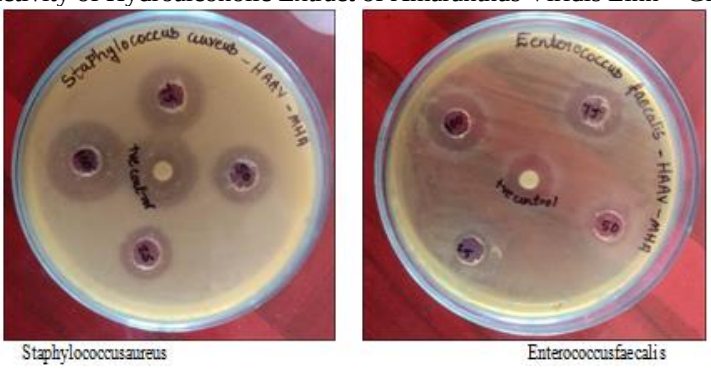


Figure 15: Antibacterial Activity of Hydroalcoholic Extract of Amaranthus Viridis Linn – Gram Positive Bacteria



Staphylococcusaureus Enterococcusfaecalis

Figure 16: Antibacterial activity of hydroalcoholic extract of Amaranthus viridis linn– gram negative bacteria

Escherichiacoli Salmonellatyphi

Table 7: Antibacterial Activity of HAEAV In Microorganisms.

S.no.	Microorganism	Zoneofinhibition				Positive control
Bacteria–Gram Positive						
1	*Staphylococcusaureus*	**CONCENTRATION**				
		100µl	**75µl**	**50µl**	**25µl**	
		20	17	15	12	23
2	*Enterococcusfaecalis*	18	14	13	10	21
Bacteria-GramNegative						
3	*Escherichiacoli*	21	18	17	13	24
4	*Salmonellatyphi*	19	16	14	12	23

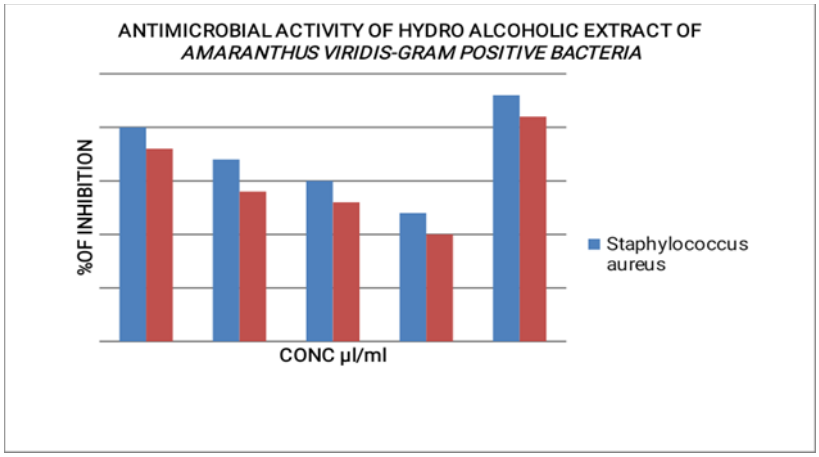


Figure 17: Graphical representation of antibacterial activity of hydroalccoholic extract of Amaranthus viridis linn–gram positive bacteria

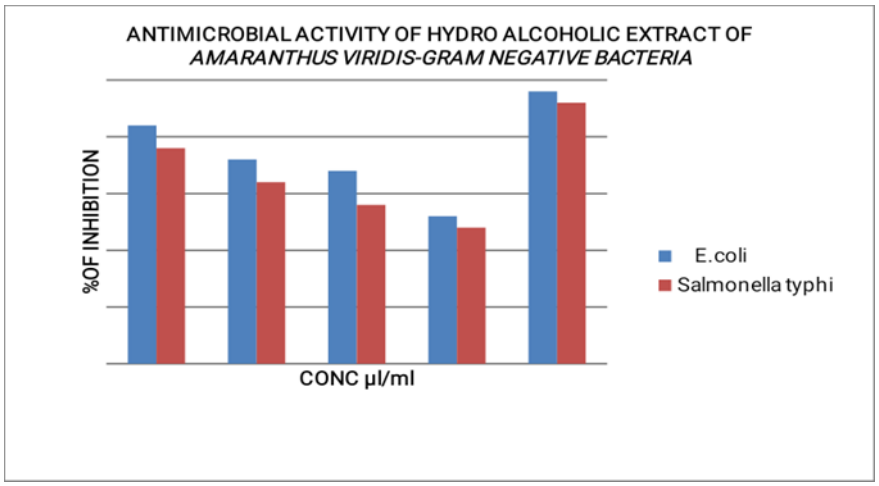


Figure 18: Graphical representation of antibacterial activity of hydroalccoholic extract of Amaranthus viridis linn – gram negative bacteria

4. DISCUSSION

The high cost, development of resistance and associated side effects are the concerns for the researchers [158,160]. The potential of plants, their metabolites, and the plant products always withdraws the researcher's attention [161-204]. Numerous patents have been granted attributed to their biological applications [205-233]. The phytochemical and pharmacological effects of a hydroalcoholic extract of Amaranthus viridis Linn were investigated in this work. The hydroalcoholic extract of Amaranthus viridis Linn has a yield of 5.6 percent W/W. According to the findings of this investigation, Amaranthus viridis Linn leaf extracts contained a variety of pharmacologically active chemicals. Alkaloids, carbohydrates, glycosides, gum and mucilage, saponins, tannins, phenols, unsaturated sterols and triterpenes, flavonoids, proteins and amino acids, fixed oil and lipids were all regularly discovered components in this species [53, 105-109].

The phenolic compounds quantified by estimating the area of peak corresponding to standard. By this comparison we assume that the HAEAV has phenolic contents. The phenolic compounds were measured by calculating the area of the peak that corresponded to the standard [54, 110-116].

The LC-MS technology makes use of an HPLC to separate the different components in a mixture, followed by ionization and separation of the ions based on their mass/charge ratio. In pharmaceutical and food samples, detection of residual

chemical compounds, confirmatory identification of tiny organic molecules, and confirmation and quantification of contaminants and adulterants[55, 116] .

Helminthiasis, the condition resulting from worm infestation is one of the major prevalent diseases in the world. The concentration of the extracts (10,20,50mg/ml) and standard drug Albendazole (20mg/ml) were used in the study. Best action obtained in 10mg/ml (20.00±0.7071) because the HAEAV extract contains desirable amounts of flavonoids (3 hydroxy 2 phenyl chromen- 4-one) as high activity against helminthiasis when compared with the drug Albendazole. The test sample of HAEAV 10mg/ml shows same activity compared to the albendazole 20mg/ml [56].

The greatest antifungal activity against Candida albicans and Aspergillus niger was found in the hydro-alcoholic extract of Amaranthus viridis Linn100l concentration [57]**.** The hydro alcoholic extract of Amaranthus viridis Linn has the highest activity against Candida albicans than Aspergillus niger and serves as the experiment's positive control [58] . Chalcones are naturally occurring flavonoids with potent antifungal properties, and they are the primary inhibitors of efflux pumps (they have noteworthy functional of removing toxic chemicals from fungal body this transported can detoxify the fungal cell through the removal of drug being accumulated the high efflux pump expression can lead to drug resistance) [59].

For the antibacterial activity of Amaranthus viridis Linn hydro alcoholic extract was tested by using both gram positive (Staphylococcus aureus, Enterococcus faecalis) and gram negative (Escherichia coli, Salmonella typhi) [60]. The hydro alcoholic extract of Amaranthus viridis Linn shows highest antibacterial activity against both Gram positive and Gram negative bacteria [61, 117].

5. CONCLUSION

Various microorganisms cause serious infections. Most commonly Bacteria, viruses, fungi, and worms/helminths are typical microbe that causes infection in human beings. An infectious disease will occur if the host immune system is impaired or if the infectious agent overwhelms the immune system. Synthetic medicines are commonly used in clinical settings to treat infectious diseases. Despite their large number, their therapeutic efficacy appears to be impeded by the development of microorganism resistance. When contemporary treatments are ineffectual in treating ailments, however, traditional medicine usage rises. In this study we emphasized the effect of hydro alcoholic extract of Amaranthus viridis Linn on in vitro anthelmintic, antifungal and antibacterial activity. Based on the findings of our studies, we concluded that hydro alcoholic extract of Amaranthus viridis Linn has potent activity against anthelmintic, antifungal and antibiotic due to the presence of an secondary metabolite flavonoids (quercetin) it was confirmed by analysing the phytocompounds by LC-MS and compared with total phenolic content identified by UV Visible spectroscopy shows that highest amount of flavonoids (quercetin) present in the Hydroalcoholic extract of Amaranthus viridis Linn. Further studies need to be carried out to investigate the molecular mechanism of the secondary metabolite.

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