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Chemical Analysis of *Hemigraphis Colorata* Methanol Extract and Its Efficacy Against Human Clinical Pathogens

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KEYWORDS

Hemigraphis colorata, strains, MIC, MBC, GC-MS, FTIR, antioxidant assay.

ABSTRACT:

Introduction: The potential pharmcological values of curing many are high in the traditional herbal plants. *Hemigraphis colorata* is one of the perennial tropical herbs that is used mostly. to achieve aestheticism and new antibacterial treatment.

Objectives: The chemical research analyses composition and biological properties of *Hemighraphis coloratoto* methanol extract. Illustrate its possible use as an antibacterial extract. The methanolic extract was assessed on its possible use as an antibacterial extract. antibacterial action to several bacterial strains. The extract has a maximal inhibitory. zone of *S. mutans* 21mm*, P. aeruginosa* 20 mm*, V. cholerae* 20 mm*, S. aureus* 21mm at a. concentration of 100 ug/ml. Additional MIC values of 60,60, 60, 80,80,100 and100ug/ml and. *S. typhi* has a value of 40,40, 60,60, 80,80, 100,100 ug/ml on MBC. This was determined as *E. coli, S. Paratyphi, Aeruginosa,* and *V. parahaemolyticus* respectively.

Results: The extract showed great antibacterial effect towards some humanoid medical pathogens. There was a presence of bioactive chemicals and therefore, deep research was conducted with the help of such procedures include gas GC-MS, FTIR, and phytochemical screening. It also exhibited high free radical-neutralizing properties which are confirmed from testing antioxidant assay.

Conclusion: These results explicate *H. colorata's* therapeutic potential as a naturally occurring source of bioactive chemicals, underscoring its potential for use in medical research and pharmaceutical development.

1. Introduction

Hemigraphis colorata is a tropical perennial herb that is mostly used for aesthetic purposes, which typically grows to a height of 15 to 30 cm, has oppositely oriented, heart-shaped, toothed leaves. It is a visually attractive addition to any garden, with little white flowers in terminal clusters and growing prostrate [1]. Because of their unique greyish-green hue, which is accentuated by reddish-purple streaks on the top side and a darker purple tinge on the underside, *Hemigraphis colorata* leaves are very attractive. The plant's decorative attractiveness is enhanced by the white, five-lobed, bell-shaped blooms it produces throughout its yearly bloom [2] Since, the beginning of human civilization, natural compounds produced from plants have been used for a variety of medical reasons. The most common method for developing drugs from plants is ethnopharmacology, which examines the use of natural resources in traditional medicine across various cultures [3]. Numerous diseases including as cancer, inflammation, and neurological problems, are linked to free radicals. Furthermore, there is a risk of serious adverse effects from existing antibacterial drugs [4] This study examines the phytochemical characteristics of several *Hemigraphis colorata* (Acanthaceae) extracts using an ethnopharmacological methodology. Building on its historical use for its powerful wound-healing powers, it investigates the plant's antioxidant and

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antibacterial qualities [5]. Traditional healers, particularly in nations with long histories of use such as China and India, have invaluable knowledge of several obscure or little-known wild herbs. These herbs used to cure burns and wounds [6] Human bacterial infections are a major hazard to world health, accounting for a large number of illnesses and fatalities each year [7] These infections are a major burden to the healthcare system across the world and can be mild and fatal. In India, the prevalence of antibiotic-resistant germs is very high, complicating the treatment process and increasing the healthcare costs, which is why the problem is of great concern. To effectively address the increased problem of antibiotic resistance, there is a strong imperative that the improvement of infection control practices, robust antibiotic stewardship programmes and the emergence of new antibacterial therapies be put into place. The current situation of bacterial infections in India illustrates this point [8]. Phytochemical composition of *hemigraphis colorata* was analyzed in different solvents to analyze the methanolic extract of leaves and stems. This extract contains antibacterial activity against clinical pathogens. *H. colorata* has been long indulged in indulgent way of a row of infections such as inflammation, wounds, ulcer, and skin diseases. It has also been used traditionally to treat gallstones and irregular menstruation using its leaves. In Vanuatu, a system of infertility induction and contraceptive action is used with greater effect when the sap of leaf buds prior to sunrise was taken over a 4 day period [9]. These massive pathogens of *E.coli, K. Oxytoca, K. pneumoniae, P. mirabilis, S. aureus*, associated with furuncles and cellulitis, V. cholerae, the cause of cholera, and S. typhi, the cause of typhoid fever. This resistance illustrates that H. colorata can be used in the future to produce antibacterial compounds useful in treating a broad range of illness [10]. The methanolic Leaf extracts, which are available in strippers such as water, acetone, methanol, chloroform, ethanol, and petroleum ether, reveal the contents of proteins, flavonoids, phenols, carbohydrates, steroids, saponins, coumarins, tannins, and carbohydrates [11]. The present studies revealed the leaf extract of *Hemigraphis colorata* as an indigenous provider of characterization using FTIR, GC-MS, antibacterial, and antioxidant in the plant's extract importance in managing infectious diseases and encouraging further investigation into its potential therapeutic applications.

2. Material and methods

Collection of samples

Hemigraphis colorata leaves sample was collected from SIMATS University, Saveetha Nagar, Tamil Nadu 602105, India latitude 13.0283° N, 80.0158° E longitude.

Methanol Extract

After collecting the leaves, they were carefully cleaned using tap water and allowed to air dry in the shade. After drying, the leaves were processed using an electric grinder to a fine powder and kept in airtight jars until required. Fifty grammes of the powdered leaves were steeped for 24 to 48 hours in 150 milliliters of methanol in order to create the methanol extract. Following soaking, the mixture was concentrated using a rotary evaporator set at 40°C under decreased pressure after being filtered with Whatman filter paper. Until further examination, the resultant crude methanol extract was kept in airtight bottles at 4°C [12].

Characterization Techniques

Phytochemical Analysis:

A preliminary phytochemical screening was conducted to ascertain the presence of bioactive substances alkaloids, flavonoid, tannins, saponin, phenolics, and terpenoid in the methanol extract. The analysis has made use of conventional methods, as outlined by Ease and Evans and has followed the usual methodologies [13].

FT-IR Spectrum Analysis

Functional groups in the methanol extract of the plant, Hemigraphis colorata were identified by Fourier-transform infrared (FT -IR) spectroscopy. The IR spectra were indicative of various bioactive compounds that contained phenols, alcohols, alkanes, aldehydes, ketones, esters, alkenes, aromatic compounds, ethers and amines. These results augment the knowledge on the chemical composition of the extract and support the initial phytochemical screening [14].

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GC-MS Analysis

When analyzing the methanol extract of the plant, gas chromatography mass spectrometry (GC-MS) was employed. The technique enabled distinguishing and pinpointing of various bioactive products. The use of GC-MS data showed the existence of alkaloids, phenolic compounds, essential oils and other phyto-transformable products. Such findings are vital in determining the medical potential and use of the extract [15].

Bacterial Strains

The objective of the study was to test the effectiveness of methanol extract of Hemigraphis colorata in relation to diverse clinical pathogens that cause diseases in humans. Pathogens that were selected were: Vibrio parahaemolyticus, Salmonella typhi, Staphylococcus aureus, Vibrio cholerae, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Streptococcus pyogenes, and Escherichia coli [16]

Inoculum Preparation

The nutrient broth was inoculated by passing the test tubes into the nutrient broth which was then autoclaved at 15 pounds pressure during 15 minutes to eliminate bacteria. Each of the ten bacterial strains was then sterilized and inoculated separately and incubated at the temperature of 37 o C in 24 hrs to enable full growth [17].

Antibacterial activity

Agar Well Diffusion method

The agar well diffusion technique was used to determine the antiseptic activity. Bacterial cultures were swabbed onto nutrient agar plates after 24 hours in an aseptic way. Sterile cutters were used to make wells of 5mm in diameter. Methanol leaf extract 10 0 -1. Stock solutions of the leaf extract of methanol were prepared in 10 0 -1 dimethyl sulfoxide (DMSO). Tests were done at the concentration of 25 µg mL -1 and 100 µg mL -1. A standard 10% DMSO solution and a 1mgmL solution tetracycline were used as the standards and placed into the corresponding wells. Plates were incubated at 37 o C after 24 hours in an upright incubator. Antibacterial activity was determined by the diameter of the inhibition zones that surrounded each well in millimeters [18].

Minimum Inhibitory concentration (MIC)

Using the technique outlined byOkekeet al., 2001. the MIC of the methanol leaf extract of Hemigraphis colorata was ascertained [19]. To get focuses ranging from 20 to 100 µg/mL, a standard solution of 1 mg/mL was produced and subsequently diluted serially. 2.0 mL of nutrient broth were combined with 0.5 mL of each dilution for each test, and 0.5 mL of an aged microbial culture was added as an inoculant. Nutrient broth-only control test tubes were also made. For twenty-four hours, all test tubes, including the controls, were incubated at 37°C. The lowest inhibitory concentration was established with the lowest concentration of extract whose growth was not observed during the incubation.

Minimum Bactericidal concentration (MBC)

The approach presented by Jayaprakasan et al. [20] has been used. The lowest concentration of bactericidal (MBC) was determined. Inoculum loopful of the MIC tubes was then transferred to nutrient agar plates, which were then incubated at 37 o C in 24 hours. The minimum concentration that produced no bacterial growth on incubation was considered as the MBC.

Statistical analysis

The statistical analysis was done with SPSS and the impact of different pathogens to various extract concentrations (25, 50, 75, and 100 0.05 ml -1) were evaluated. The plots of the inhibition zones of the methanol extract of *H. colorata* were drawn.

DPPH Radical scavenging activity

To determine the antioxidant capacity of the methanol extract of *Hemigraphis colorata* the DPPH radical scavenging assay was used. The principle behind this method is based on the ability of antioxidants to take away electrons or hydrogen atoms in order to neutralize free radicals. It measures the decrease in the color of a purple solution of methanol that has 2,21 -diphenyl-1-picrylhydrazyl (DPPH), a stable free radical. A 50 2L of the extract or standard compounds at different concentrations were combined with 5ml of a 0.004 2L solution of DPPH methanol 2 in this spectrophotometric assay. The solution was allowed to incubate at room temperature over 30 min and the absorbance was acquired at 517nm in Thermo Spectronic UV

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spectrophotometer. The positive control in these experiments was L -ascorbic acid (21).

3. Results and Discussion

Preparation of *Hemigraphis colorata* was done as follows: Crude extraction with methanol and drying by evaporating. An ethanolic extract of 12g was obtained and dried by evaporation with each 100g of plant powder and Methanolic extract of 15g was obtained and dried by evaporation with each 100g of plant powder. The extract in ethanol produced 10g per 100g of plant powder and the extract in aqueous produced 12g per 100g of plant powder [22].

Phytochemical analysis

The *H. colorata* methanolic leaf extract contains several phytochemicals, and these groups of chemical components serve a specific intent. Some of these groups include alkaloids 0.2%, flavonoids 2.4%, phenolic chemicals 3.5%, terpenoids 0.4%, saponins 0.5%, steroids 0.1% and tannins 1.3% (Fig. 1).

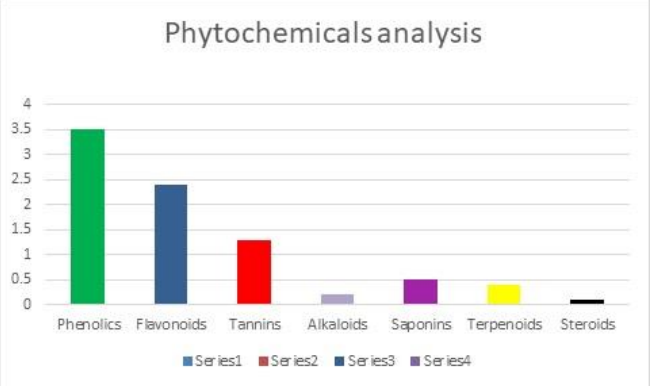

Fig. 1. Phytochemical analysis in methanol extract from *H. colorata*

Likewise, the alkaloids present in the methanolic extract of this plant, which acts as organic insecticide, has a potent pharmacological effect [23]. The pigmentation of plants is attributed to flavonoids, which are well-known for their antioxidant qualities. Because of their potent antioxidant properties, phenolic chemicals are essential to the plant's defence systems. In addition to their fragrant characteristics, terpenoids may possess antibacterial capabilities. Glycosides are defence molecules that protect plants from infections and herbivores. Tannins contribute to the astringency of plants and provide protection against microbial diseases. Finding these substances in leaf extracts from *Hemigraphis colorata* aids in the understanding of the biological functions of the substances and their possible uses in business, agriculture, and medicine.

FT-IR analysis

The methanolic extracts of *Heliographies colorecta* show multiple distinctive absorption peaks in the FT-IR analysis, which suggests the existence of different functional groups (Fig. 2).

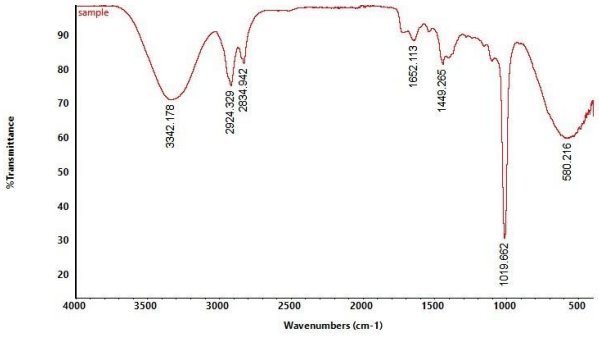

Fig. 2. FT-IR analysis *H. Colorata* from methanol leaves extract

Prominent summits consist of the wide peaks between 3342 and 3000 cm^{-3}, which represent the usual carboxylic acid O-H stretching vibrations. A prominent peak at around 2924,2834 cm^{-3}, corresponding to C-H stretching vibrations that are indicative of alkane groups such carboxylic acids, ketones, or aldehydes, lmines. Peaks close to 1652 and 1600 cm^{-1} are ascribed to aromatic rings' C=N stretching vibrations. Absorption bands centered about 1449–1395 cm^{-3}, which are indicative of carboxylic acids, ethers, or esters and correspond to O-H stretching vibrations. 1019-1000 cm^{-3}, C-O. The range of Peaks. [24]. Absorption alcohols existence of many functional groups is confirmed by these FT-IR spectra, underscoring the complex chemical makeup of the methanolic extract of *Hemigraphis colorata* [25].

GC-MS Analysis

The methanolic extract from *Hemigraphis colorecta* included 15–16 different chemical ingredients, according to the GC-MS analysis. The chemical formula, retention period, and peak area of each drug were given (Table 1 & Fig. 3).

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Table 1. GC–MS analysis of methanol extract

Peak#	R.Time	Area	Area%	Name
1	3.023	2069863	10.68	ETHANE, 1,1-DIETHOXY-
2	9.821	1246421	6.43	Dodecane
3	16.153	1830430	9.44	TETRADECANE
4	22.169	216154	1.11	1-Nonadecene
5	22.407	1023248	5.28	Hexadecane
6	28.181	477365	2.46	Eicosane
7	29.164	2187959	11.29	Neophytadiene
8	29.818	508917	2.62	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
9	30.308	776986	4.01	Phytyl decanoate
10	31.482	671719	3.46	Hexadecanoic acid, methyl ester
11	33.235	1125631	5.81	Hexadecanoic acid, ethyl ester
12	35.658	305588	1.58	9,12-Octadecadienoic acid (Z, Z)-, methyl ester
13	35.795	613533	3.16	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-
14	36.063	4949835	25.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
15	37.161	490410	2.53	Linoleic acid ethyl ester
16	37.29	893409	4.61	ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE
		19387468	100	

This thorough examination demonstrates the plant extract's potential for a range of biological uses by highlighting the different bioactive chemicals it contains. This method involved employing gas chromatography and a Shimadzu mass spectrometer to extract a methanol from *Hemi Graphiscolorecta*plants. Showing the peaks as Present of compound 3,7,11,15-Tetramethyl-2-hexadecen-1-ol R. Time36.063, peakarea (25.53%) high percentage of area in compound (RT) 29.164 peak area(11.29%)- Neophytadiene, R. Time 3.023 area of peak (10.68%) ethane, 1,1-diethoxy-(RT) 16.153 peak area (9.44%) tetradecane R. Time9.821, peak area (6.43%) Dodecane, lower R. Timeand peak area in present of compound R. Time 37.161 peak area (2.53%)Linoleic acid ethyl ester, R. Time 28.181 peak area(2.46%)Eicosane, R. Time 35.658 peak area(1.58%)9,12-Octadecadienoic acid (Z, Z)-, methyl ester R. Time 22.169 peak area(1.11%)1-Nonadecene highly Present of compound 3,7,11,15-Tetramethyl-2-hexadecen-1-ol .the chemical compound high biologicalactivity's so apply to the medical field in antimicrobial test antioxidant, Anti-inflammatory, antimicrobial activity Byanalyzing the GC-MS chromatogram, a qualitative analysis of the methanolic extract of *Chemigraphic colorecta* was conducted Abitha et al., [26] was investigated *H. colorectal* methanolic extract fromEach analyte's transit time through the column and to the mass spectrometer detector is shown. The y-axis, or peak area, shows the quantity of each analytic. Peaks indicate the times at which each component arrived at the detector.

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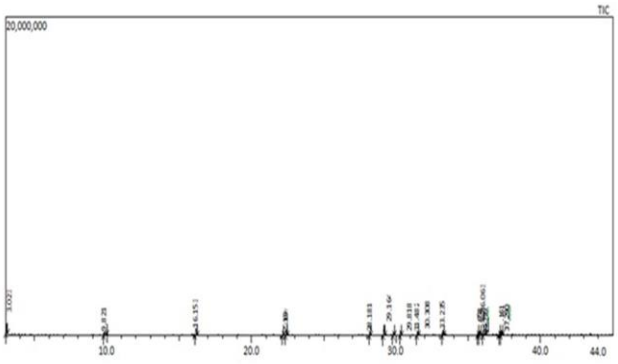

Fig. 3. GC–MS analysis of *H. colorata*

Antibacterial activity

Agar Well Diffusion method

The antibacterial activity of the *H. colorecta* methanolic extract was evaluated against a panel of human clinical pathogens using the well diffusion method (Table 2 and Fig. 4).

Table 2. Antibacterial Activity of the *H. colorecta* Methanolic Extract

NO	Pathogens	ZoneofInhibition					
		25µg/ml	50µg/ml	75µg/ml	100µg/ml	passive	negative
	Vibrio cholerae	16	16	18	21	22	-
	Streptococcus mutans	15	16	19	21	22	-
	Staphylococcus aureus	14	16	18	20	24	-
	Pseudomonas aeruginosa	16	16	18	20	23	-
	Escherichia coli	13	14	14	16	20	-
	Salmonella typhi	8	10	10	12	18	-
	Vibrio parahaemolyticus	8	8	10	12	17	-
	Salmonella Para typhi	-	-	-	-	13	-

The zones of inhibition were measured for various bacterial strains at different extract concentrations (25, 50, 75, and 100 µg/mL) [27]. The methanolic leaf extract exhibited significant antibacterial activity against several pathogens, as observed by the inhibition zones on agar plates [28]. The inhibition zones for the tested bacteria were as follows: *Vibrio cholerae* (20 ± 0.12 mm), *Streptococcus mutans* (21 ± 0.26 mm),

Staphylococcus aureus (21 ± 0.31 mm), *P. aeruginosa* (20 ± 0.11 mm), *Escherichia coli* (16 ± 0.34 mm), *Salmonella typhi* (12 ± 0.45 mm),and*Vibrio parahaemolyticus*(12 ± 0.36 mm) [29].

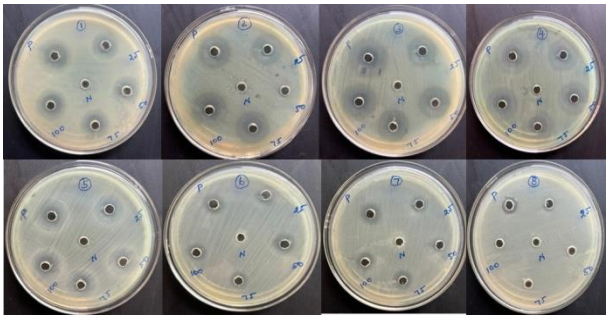

Fig. 4. Zone of Inhibition

The inhibition zones for the tested bacteria were as follows: *Vibrio cholerae* (20 ± 0.12 mm), *Streptococcus mutans* (21 ± 0.26 mm), *Staphylococcus aureus* (21 ± 0.31 mm), *P. aeruginosa* (20 ± 0.11 mm), *Escherichia coli* (16 ± 0.34 mm), *Salmonella typhi* (12 ± 0.45 mm),and*Vibrio parahaemolyticus*(12 ± 0.36 mm) [29]. While some strains exhibited resistance or minimal susceptibility to the methanolic extract, notable inhibitory effects were observed against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with inhibition zones of 24 mm and 23 mm, respectively [30]. In contrast, the lowest inhibition zones were recorded against *Vibrio parahaemolyticus* and *S Para typhi* (16 mm and 12 mm, respectively), indicating variability in the antibacterial potency of the extract.

Minimum Inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of *Hemigraphis colorata* methanolic extract against the range of bacteria cultures (Fig. 5) has been determined [31].

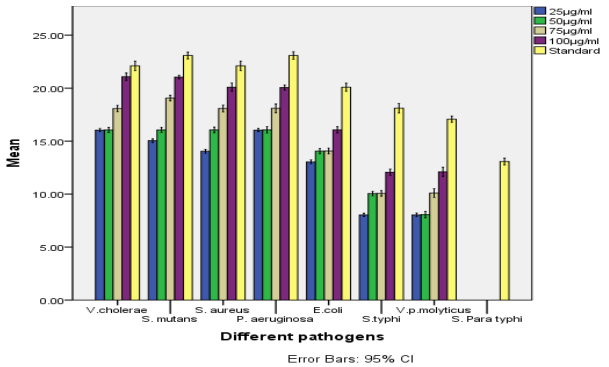

Fig. 5. Statistical analysisAntibacterial Activity (It is a SPSS statistical analysis for different pathogen against different extract concentrations (25, 50, 75, and 100 µg/mL).The zones of inhibition were measured forthe

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methanolic leaf extract exhibited significant antibacterial activity).

It is a crucial indicator of an extract's antimicrobial activity as it shows the lowest concentration of the extract required for stopping bacterialgrowth visibly [32]. The different concentrationof leaf extract ofranging from 20 to100µg/ml was treated individually against each species *V. cholerae, S. aureus, E. coli,P. aeruginosa,S. mutans, P. mirabilis, S. typhi, S. pyogenes,* and *V. parahaemolyticus* respectively. Together, these substances prevent the growth of bacterial diseases.The presence of *H. colorata*plantextracthas arrested growth at MICvalue 60,60,60, 80,80,100,and 100µg/ml(table.2). Jayaprakasanet al., [33] finding the MIC methanolic leaf extracts from *Hemigraphis colorata* extracts. Several chemical componentsincluding alkaloid, flavonoid, phenolic compound, terpenoid, glycoside, and tannin, are thought to be responsible for its efficacy. The methanolic extract establishing minimum inhibitory concentration (MIC) by use of broth dilution technique indicates beneficial effects of treating microbial infection and synchronous antimicrobial therapeutic drugs [34].

Minimum Bactericidal Concentration (MBC)

The MBC values for methanol extracts from*H. colorata* leaf bactericidal efficacy against various bacterial strainsincluding S. *typhi, S. aureus, S. mutans V. cholerae, E. coli, S. paratyphi, P. aeruginosa,* and *V. parahaemolyticus*. Itwas conducted to investigate leaf extract activity of different concentration (20 to 100µg/ml) and revealed impact at 40,40, 60,60, 80,80, 100,100 µg/ml for S. *typhi, S. aureus, S. mutans V. cholerae, E. coli, S. paratyphi, P. aeruginosa,* and *V. parahaemolyticus* respectively (Fig. 6).

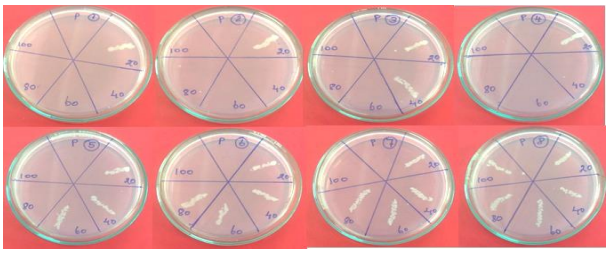

Fig. 6. Minimum Bactericidal Concentration (MBC)

Rajamalar et al., [35] reported the MBC Chemical characterization of *Orchis masculaleaves*. Similarly, Sandrasagaran et al., (2014) found new perspective of *Dendrobium crumenatum* orchid using Minimum Bactericidal Concentration [36]. Another study Recorded by Ruban et al., [37] antibacterial activity against human pathogens to achieve precise data about colony development with the MBCof the ethanolic extract, the methanolic extract's MBC values in this investigation showed that it effectively inhibited *V. cholerae,s mutans* and *p. aer uginosa* at doses of 40,60,80 µg/ml and100 µg/ml, respectively. The results indicate that methanol extracts from H. colorata leaves possess promise as a natural antibacterial agent against several dangerous microorganisms. This illustrates how it might be applied to develop various therapeutic strategies to combat bacterial infections.

Antioxidant activity

A methanolic solvent was used to extract the dried leaves (Fig. 7). The findings assessed *H. colorata's* potent antioxidant activity [38]. A 2014 study by Asha et al., [39] on the analysis of methanolic leaf extracts demonstrated the strong antioxidant properties of concentration-dependent. By scavenging DPPH free radicals and having the potential to the crude extracts demonstrates the outstanding antioxidant activity [40]. Radical scavenging activity of the various concentration suing 50 µg/ml,100 µg/ml, 150 µg/ml, 200µg/ml and 250µg/ml showed raising activity was exhibited by L-ascorbic acid 88%at 250 µg/mL, followed by BHA at all tested doses. The extract showed good natural antioxidant activity with 75% scavenging at 250µg/mL. Methanolic leaves extract. Additionally, it demonstrated the existence of considerable polyphenolic content, including flavonoids, flavanols, and phenolic compounds. The phenolic acids found in the plant, including chlorogenate, cinnamate, coumarate, gallate, and formulate, operated as pro-oxidants and had free radical scavenging assay properties.

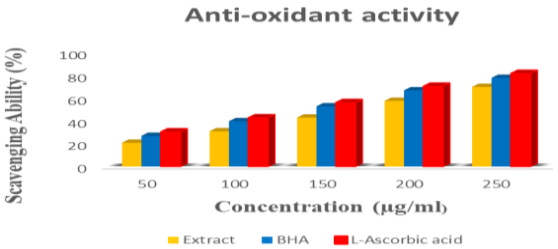

Fig. 7. Anti-oxidant activity of *H. colorata* Methanolic extract solvent

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Conclusion

The *Hemigraphis colorata's* methanol leafextract was examined using FTIR, GC-MS, and phytochemical screening. In human clinical infections, these compounds plays a significant antibacterial effectiveness, backed by excellent MIC and MBC values, indicating their potential as powerful antimicrobial agents. Additionally, the extract exhibited strong antioxidant activity, as demonstrated by its ability to scavenge DPPH radicals, hence mitigating oxidative stress and neutralizing free radicals.As a natural source of bioactive chemicals with strong antibacterial and antioxidant qualities, *Hemigraphis colorata* has medicinal promise, as this study concludes, and it encourages more research into its clinical uses.

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