IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

GC-MS Profiling and Evaluation of Antioxidant and Antimicrobial Properties of *Rivea hypocrateriformis*

Nayna O. Mishra[1], D. S. Talwankar[2]*

Department of Botany,
G. S. Science, Arts and Commerce College, Khamgaon, District Buldhana Maharashtra, India

Abstract: Wild Vegetables have long been used in traditional healthcare systems and are recognized as valuable sources of bioactive compounds with therapeutic potential. *Rivea hypocrateriformis* (Desr.) Choisy, a member of the family Convolvulaceae, is traditionally used as a vegetable and for the treatment of various ailments such as cough, headache and skin diseases. The present study was undertaken to evaluate the antioxidant potential of the methanolic leaf extract of *Rivea hypocrateriformis* using in vitro assays. Antioxidant activity was assessed through DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. The extract was also analysed for phytoconstituents using GC-MS. The extract exhibited concentration-dependent antioxidant activity, indicating its ability to neutralize free radicals and reduce oxidative stress. The findings support the traditional use of the plant and suggest that it may serve as a valuable natural source of antioxidant compounds with potential applications in pharmaceutical and nutraceutical formulations.

Keywords– Wild Vegetables, GC-MS, *Rivea hypocrateriformis*, Antioxidant, Antimicrobial

Introduction

Traditional drugs has been used in health care programmes specially in developing countries. Ancient literature contains knowledge about the traditionally used medicinal plants and potential benefits of their various parts in medicine and treatment of various ailments. However, the lack of documentation of this information has resulted in declination in use of this plant in ethnomedicine. Therefore, Exploration, proper documentation and standardization is essential to accept this plants for health benefits and as a alternative source of nutrition. This standardization can be done by accurate pharmacognostic studies and biochemical characterization both qualitatively and quantitatively. This will aid in authentication of plant material. (Shweta et al., 2012)

Rivea hypocrateriformis (Desr.) Choisy of family convolvulaceae also known as Phanji is referred in Ayurvedic text Raja Nighantu as vegetables with Grahi, Deepana, Pachana, Ruchya and Tridosha Shamaka properties and known for their traditional use by tribal communities as a wild vegetable in various state. It is reportedly consumed as wild leafy vegetable and known for its ethnomedicinal properties in Cough, Headache, skin disease etc. The antioxidant assay was performed for the leaf extract by scavenging activity of DPPH and FRAP assay to evaluate antioxidant capacity and nutritional evaluation. (Borkar et al., 2015)

Currently, majority of diseases are due to shift in balance of pro-oxidant and antioxidant. Increased free radicals caused due to excessive oxidative stress has lead to pro-oxidant condition. Plants posses phytochemicals such as flavonoids and vitamins that are known to exhibit antioxidant activity that can scavange free radicals from human body. Natural antioxidant exhibit biological functions including protection against oxidative stress, antiviral effects, antibacterial effect, anticancerous activities, antiaging, anti-inflammatory effects, antiallergic and hepatoprotective properties. Therefore, analysis and evaluation of antioxidant activity of this herbs were identified by Ayurveda and utilized till date. Furthermore, there is requirement of identification of their scavenging activity against free radicals. (Nachiar et al., 2020)

Present study evaluated and compared the invitro antioxidant properties of plants which were utilized for medicinal and culinary purpose. The antioxidant activities were calculated through ferric reducing antioxidant power (FRAP), DPPH radical scavenging methods.The data provide information about health benefits and potential use of natural antioxidant in functional foods.(Loganayaki et al., 2010)

Rivea hypocrateriformis (Desr.) choisy belongs to family Convolvulaceae. It is perennial, robust, twining climber reaching upto 2-3m in total height and woody in habit. It has terete, more or less silky, pubescent, glabrous stem with alternately arranged leaves that are 3-10 cm long, broad, orbicular, obtuse, glabrous, pubescent beneath, chordate base, 3-5cm long silky base. Flowers are white 1-3 flowered with short peduncles, stamens 5, linear and oblong. (Khandekar et al., 2015)

Fig.: Morphology of *Rivia hypocrateriformis*

Materials and Method

Methods

1. Collection of Sample: Fresh leaves of *R. hypocrateriformis* were collected from Khamgaon, Dist. Buldhana in month of July 2025. Fresh leaves were washed using tap water and twice by using distilled water and it was dried in shade for 15-20 days. After drying the samples were cut into small pieces and grind in mixer properly. The concentrated extract was collected in sterile bottles and kept in a cool and dark place prior to analysis. This sample were sent for analysis of Antioxidant activity, Antimicrobial activity and GC-MS from Analytical Instrumentation facility(SIF), Department of Botany and RUSA centre for Natural products and alternative medicine, Shivaji University Kolhapur Maharashtra and results were noted.

2. Preparation of Chemicals:

- **DPPH radical scavenging activity-** Chemicals 2,2-Diphenyl-1-picryl-hydrazyl (DPPH•), Ascorbic acid
- **Ferric reducing antioxidant power (FRAP) assay-** 290µl (0.3 M acetate buffer, 5ml 10mM TPTZ, 5ml 20mM FeCl3)

3. Antioxidant activity

- **DPPH radical scavenging activity**

In vitro DPPH radical scavenging activity was measured as the method described by Blois (1958) and Rosidah et. al., (2008) with slight modifications. 10 µl of plant extracts were added to the well and mixed with 290 µl of DPPH. Then the plate was incubated in dark for 20 mins. Absorbance was measured at 517nm. Each fraction's capacity to scavenge free radicals was assessed by comparing its absorbance to that of a control solution (DPPH without sample). Ascorbic acid was used as positive control at concentrations of 20, 40, 60, 80, and 100 µg/ml. The following equation was used to compute the percentage inhibition used to scavenge the DPPH radical. DPPH scavenging activity (%) = [(OD1 – OD2) / OD1] X 100

Where, OD1 = Optical density of control, OD2 = Optical density of test sample

- **Ferric reducing antioxidant power (FRAP) assay**

The ability to reduce ferric ions was analyzed with minor modifications using the FRAP assay method described earlier (Benzie and Strain, 1996). The reaction mixture consists of 290µl (0.3 M acetate buffer, 5ml 10mM TPTZ, 5ml 20mM FeCl3) and 10 µl (1mg / ml) plant extracts. The reaction mixture was incubated at 37°C for 15 minutes while the absorbance at 595 nm was monitored. The antioxidant potential based on the ability to reduce ferric ions was expressed as mmol ascorbic acid equivalent per gm of fresh or dry extracts. Ferric reduction was determined from the linear calibration curve of ascorbic acid.

4. Antibacterial activity

The antimicrobial activity of the plant extract was investigated using the well diffusion method. To set up a homogeneous microbial growth of the plate, bacterial cultures were sub-cultured in Muller Hilton Broth medium and incubated for 24 h at 37 ◦C. Then, the 100 µl cultures were spread out on Muller Hilton Agar plates with 2 Gram-positive *Pseudomonas aeruginosa, Staphylococcus aureus,* and 2 Gram-negative *Escherichia coli, Bacillus subtilis* bacterial strains. To investigate the antibacterial activity, 20 µl different concentrations of extract (20,40,60,80 100 µg/ml), and streptomycin as the positive control, and DMSO was selected as the negative control. The Petri dishes were incubated at 37 ◦C for an entire day. The diameter of the inhibitory zone was measured and allowed researchers to assess the antibacterial efficacy and compare it with the groups in control.

In this assay, four quality control strains, namely Gram-positive *Pseudomonas aeruginosa, Staphylococcus aureus* and 2 Gram-negative *Escherichia coli, Bacillus subtilis* bacterial strains., were used to assess the antibacterial activity of streptomycin (bacteriostatic in action) and extract.

Preparation of bacterial suspension.

The inocula were prepared by culturing the bacteria in 25 ml Liquid Nutrient broth medium and incubating overnight at 37◦ Celsius until the OD at 600 nm reaches 1.00, which determined the growth curve of microorganisms.

Preparation of 96 well-plates.

280 µl of the Nutrient Broth (NB) was dispensed in each well, then 10 µl test sample or standard was added. After these add 10 µl bacterial suspension to each well and incubate 16 Hrs. at 37 ◦C in 96 well sterile plate in Multiskan sky plate reader spectrophotometer. Blank was used as an NB medium and control was used as an NB medium containing bacterial suspension. %Inhibition of bacterial growth = [(A0–A1)/A0] X 100

5. Gas chromatography and mass spectrometry (GC-MS/MS)

GC–MS/MS analyses of extracts of methanol were carried out using the TQ 8050 plus with HS-20 (Shimadzu, Japan) equipped with a SHIMADZU SH-Rxi-5Sil MS column (30 mm in length × 0.25mm ID × 0.25 m df). The carrier gas was pure helium gas (99.99 %) at a consistent rate of 1 mL/min. Fragmentation ranging from 45 to 500 m/z was used for GC–MS/MS spectrum identification. A 10 µl injection volume was utilized, and a constant injector temperature of 250 ˚C was maintained. The temperature of the column oven was initially set at 50 ˚C and then increased to 260 ˚C. This final temperature was kept constant. By

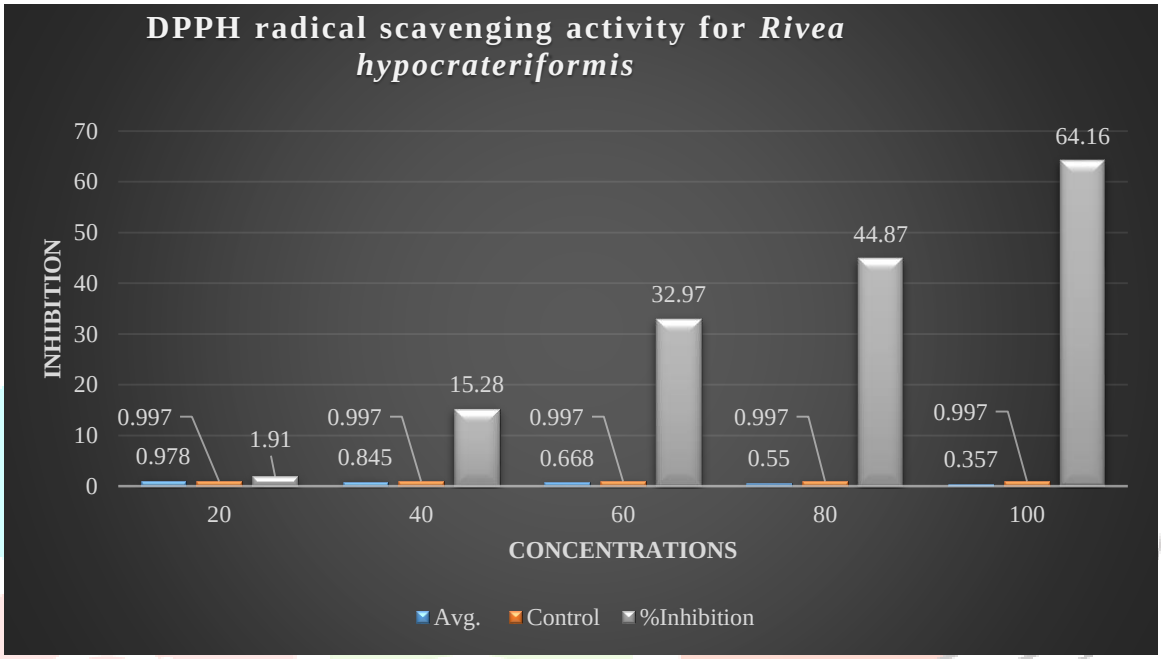
comparing the test samples mass, peak area, peak height and retention time (min) with the authentic spectral compounds data bases kept in the National Institute of Standards and Technology (NIST) library, the phytochemicals present in them were determined.

Result and Discussion

1) Antioxidant activity

a) DPPH radical scavenging activity for *Rivea hypocrateriforms.*

Graph 1: DPPH radical scavenging activity for *Rivea hypocrateriformis*



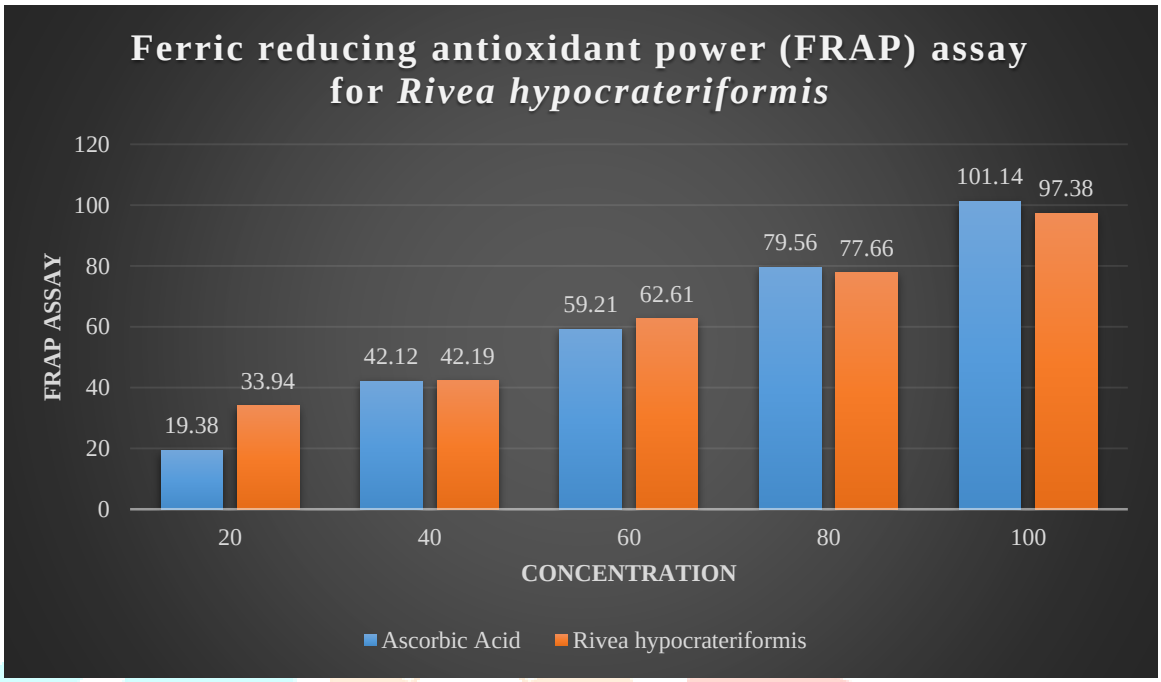
The antioxidant activity of the test sample was evaluated using a free radical scavenging assay by measuring the percentage inhibition at different concentrations ranging from 20 to 100 µg/mL. The results demonstrated a concentration-dependent increase in radical scavenging activity. At a concentration of 20 µg/mL, the sample exhibited only 1.91% inhibition, indicating minimal antioxidant activity. As the concentration increased to 40 µg/mL, the percentage inhibition increased to 15.28%, suggesting enhanced free radical scavenging potential. Further increases in concentration resulted in inhibition values of 32.97% and 44.87% at 60 and 80 µg/mL, respectively. The highest concentration tested, 100 µg/mL, showed 64.16% inhibition, demonstrating significant antioxidant activity.

The IC_{50} value, defined as the concentration required to achieve 50% inhibition of free radicals, was estimated from the inhibition data. Since 50% inhibition lies between the values obtained at 80 µg/mL (44.87%) and 100 µg/mL (64.16%), linear interpolation was used to calculate the IC_{50}. The estimated IC_{50} value was approximately 85.3 µg/mL. This result indicates that a concentration of about 85.3 µg/mL of the sample is required to scavenge 50% of the free radicals present in the assay system.

Overall, the findings demonstrate that the test sample possesses appreciable antioxidant activity and exhibits a clear concentration-dependent free radical scavenging effect. The increase in percentage inhibition and corresponding decrease in absorbance with increasing concentration suggest the presence of bioactive compounds capable of acting as effective antioxidants. The estimated IC_{50} value of 85.3 µg/mL further supports the antioxidant potential of the sample and provides a useful parameter for comparison with standard antioxidants and other test samples.

b) Ferric reducing antioxidant power (FRAP) assay for *Rivea hypocrateriformis*

Graph 2: Ferric reducing antioxidant power (FRAP) assay for *Rivea hypocrateriformis*



The graph no. 2 presents the antioxidant activity of the standard antioxidant **Ascorbic acid** and the extract of *Rivea hypocrateriformis* at different concentrations (20–100 µg/mL). The activity was assessed by measuring absorbance values in triplicate, calculating the average absorbance and determining the equivalent antioxidant concentration.

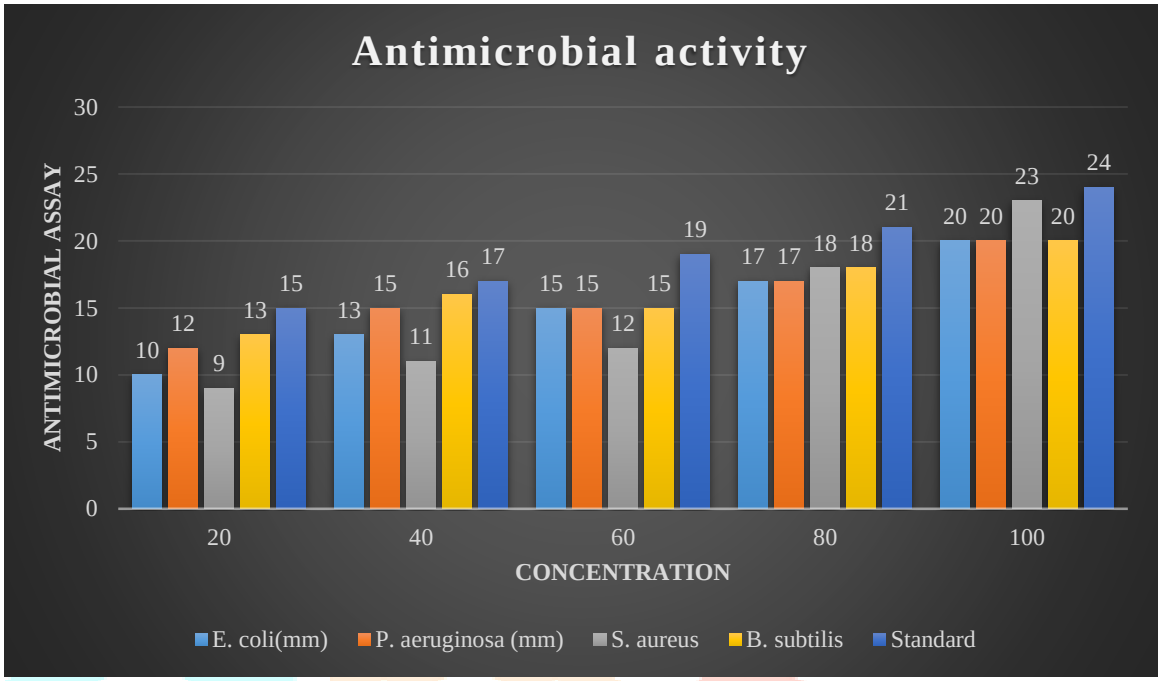
For **Ascorbic acid**, the average absorbance increased steadily from 0.232 at 20 µg/mL to 1.009 at 100 µg/mL. The corresponding antioxidant values ranged from 19.38 to 101.14 µg/mL, indicating a strong concentration-dependent antioxidant response. The highest absorbance of 0.973 indicates the highest antioxidant activity at 100 µg/mL, confirming the effectiveness of ascorbic acid as a standard antioxidant.

Similarly, the extract of ***Rivea hypocrateriformis*** exhibited a gradual increase in absorbance with increasing concentration. The average absorbance increased from 0.371 at 20 µg/mL to 0.973 at 100 µg/mL, while the antioxidant values increased from 33.94 to 97.38 µg/mL. This trend demonstrates that the antioxidant activity of the plant extract is concentration dependent.

These results suggest that the plant extract possesses significant antioxidant potential and may serve as a natural source of antioxidant compounds.

2) Antimicrobial assay of *Rivea hypocrateriformis*

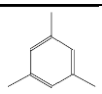
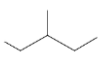
Graph 3: Antimicrobial assay of *Rivea hypocrateriformis*

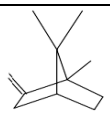
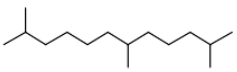
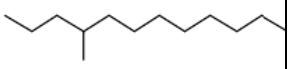
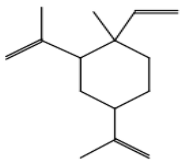
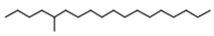
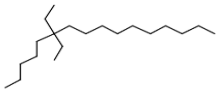
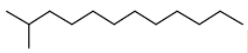
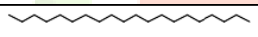
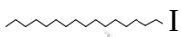
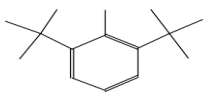
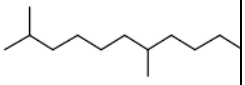
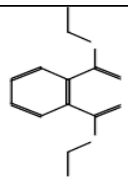
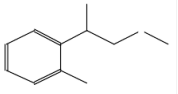


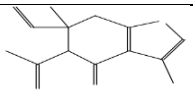
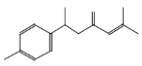
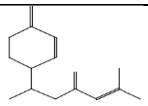
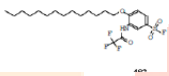
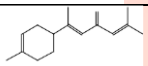
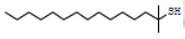
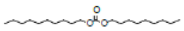
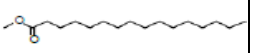
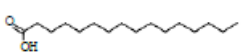
The antimicrobial activity of the plant extract showed a concentration-dependent increase against all tested bacterial strains. At the lowest concentration (20 µL), the extract produced inhibition zones ranging from 9 to 13 mm, indicating moderate antibacterial activity. As the concentration increased from 40 to 100 µL, the diameter of the inhibition zones gradually increased, demonstrating enhanced antimicrobial effectiveness. The highest activity was observed at 100 µL, with inhibition zones of 20 mm against *Escherichia coli*, 20 mm against *Pseudomonas aeruginosa*, 23 mm against *Staphylococcus aureus* and 20 mm against *Bacillus subtilis*. Among the tested organisms, *Staphylococcus aureus* exhibited the greatest susceptibility to the extract, showing the largest inhibition zone (23 mm) at the highest concentration. Although the standard antibiotic displayed stronger antibacterial activity (15–24 mm), the plant extract showed significant inhibitory effects, suggesting the presence of bioactive compounds with potential antimicrobial properties. Overall, the results indicate that the extract possesses broad-spectrum antibacterial activity and its efficacy increases with concentration.

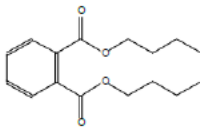
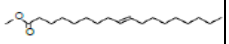
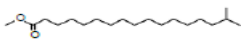
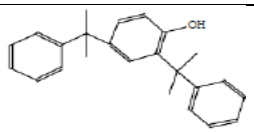
3) Gas chromatography and mass spectrometry (GC-MS/MS)

Table no. 1- Gas chromatography and mass spectrometry (GC-MS/MS)

Sr. No.	Retention Time (min)	Chemical Constituent	Molecular Formula	Structural Formula	Molecular Weight (g/mol)	Peak Area (%)	Reported Pharmacological Activity
1.	6.520	Mesitylene	C_9H_{12}		120	0.59	Antimicrobial, antioxidant
2.	6.664	Glycerin	$C_3H_8O_3$		92	14.63	Antimicrobial
3.	8.528	Benzene, 1,2,3,4-tetramethyl-	$C_{10}H_{14}$		134	0.41	Antioxidant, antimicrobial

4.	8.977	Camphor	$C_{10}H_{16}O$		152	0.46	Antimicrobial
5.	10.722	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$		212	0.64	Antimicrobial
6.	11.361	Dodecane, 4-methyl-	$C_{13}H_{28}$		184	0.45	Antimicrobial
7.	12.435	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	$C_{15}H_{24}$		204	0.36	Antimicrobial, antioxidant
8.	12.634	Octadecane, 5-methyl-	$C_{19}H_{40}$		268	0.32	Antimicrobial
9.	13.147	6,6-Diethylhexadecane	$C_{20}H_{42}$		282	1.19	Antimicrobial
10.	13.230	Dodecane, 2-methyl-	$C_{13}H_{28}$		184	0.40	Antimicrobial
11.	13.580	Eicosane	$C_{20}H_{42}$		282	4.70	Antibacterial
12.	13.698	Hexadecane, 1-iodo-	$C_{16}H_{33}I$		352	1.31	Antimicrobial
13.	13.951	Phenol, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$		206	6.58	Antioxidant
14.	14.260	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$		212	2.19	Antimicrobial
15.	15.090	Diethyl Phthalate	$C_{12}H_{14}O_4$		222	3.18	Antimicrobial
16.	15.248	1-(2-Methoxy-1-methylethyl)-2-	$C_{11}H_{16}O$		164	2.32	Antimicrobial

		methylbenzene					
17.	15.310	Epicurzerenone	$C_{15}H_{18}O_2$		230	1.93	Antioxidant
18.	15.429	Tetracosane	$C_{24}H_{50}$		338	0.82	Antimicrobial
19.	16.216	ar-Turmerone	$C_{15}H_{20}O$		216	10.17	Antimicrobial
20.	16.864	Curlone	$C_{15}H_{20}O$		216	11.09	Antioxidant
21.	17.226	1-Nonadecene	$C_{19}H_{38}$		266	0.54	Antimicrobial
22.	17.875	Benzenesulfonyl fluoride, 4-(tetradecyloxy)-3-	$C_{20}H_{33}FO_3S$		372	0.71	Antimicrobial
23.	18.166	(E)-Atlantone	$C_{15}H_{22}O$		218	1.27	Antimicrobial
24.	18.322	tert-Hexadecanethiol	$C_{16}H_{34}S$		258	0.62	Antimicrobial
25.	20.414	Carbonic acid, decyl nonyl ester	$C_{20}H_{40}O_3$		328	0.35	Antimicrobial
26.	20.550	Tetrapentacontane	$C_{54}H_{110}$		759	3.11	Antimicrobial
27.	20.650	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		270	3.76	Antioxidant
28.	21.415	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		256	2.14	Antioxidant, antimicrobial

29.	21.530	Dibutyl phthalate	$C_{16}H_{22}O_4$		278	1.06	Antimicrobial
30.	23.855	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$		296	1.23	antioxidant
31.	24.292	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$		298	1.55	Antimicrobial, antioxidant
32.	31.390	Phenol, 2,4-bis(1-methyl-1-phenylethyl)-	$C_{22}H_{26}O$		306	5.09	Antioxidant, antimicrobial

The GC–MS analysis of the extract revealed the presence of **32 phytochemical constituents** with diverse biological activities, indicating a complex mixture of bioactive compounds. The identified compounds belonged to different chemical classes, including terpenoids, phenolic compounds, fatty acids, hydrocarbons, esters and aromatic compounds. The retention times of the detected compounds ranged from **6.520 to 31.390 min**, demonstrating the presence of both volatile and semi-volatile constituents.

Among the identified compounds, **Glycerin (14.63%)** showed the highest peak area, followed by **Curlone (11.09%)**, **ar-Turmerone (10.17%)**, **Phenol, 2,6-bis(1,1-dimethylethyl)- (6.58%)**, **Phenol, 2,4-bis(1-methyl-1-phenylethyl)- (5.09%)**, and **Eicosane (4.70%)**. The high peak areas of these compounds suggest that they are the major constituents of the extract and may contribute significantly to its biological activities.

Several compounds identified in the extract, such as **ar-Turmerone**, **Curlone**, **Epicurzerenone**, **Camphor**, and various phenolic derivatives, have been reported to possess **antioxidant, antimicrobial and anticancer properties**. Additionally, fatty acids and their esters, including **n-Hexadecanoic acid**, **Hexadecanoic acid methyl ester** and **9-Octadecenoic acid methyl ester**, are known for their antioxidant and anti-inflammatory activities. (Godipurge et al., 2015)

The presence of numerous antimicrobial compounds, including **Mesitylene**, **Camphor**, **Eicosane**, **Dibutyl phthalate** and several hydrocarbon derivatives, suggests that the extract may possess broad-spectrum antimicrobial potential. Furthermore, the occurrence of antioxidant compounds such as phenolic derivatives and terpenoids supports the antioxidant activity observed in the biological assays.

Overall, the GC–MS profile demonstrates that the extract is rich in bioactive phytochemicals with potential **antioxidant, antimicrobial and therapeutic properties**, which may be responsible for the pharmacological activities exhibited by the plant extract.

Conclusion

The results of the antioxidant assays and GC–MS analysis demonstrate that the extract of *Rivea hypocrateriformis* possesses significant antioxidant potential. The DPPH assay showed concentration-dependent free radical scavenging activity with an IC_{50} value of approximately 85.3 µg/mL, while the FRAP assay confirmed strong ferric reducing power comparable to the standard ascorbic acid at higher concentrations. GC–MS analysis identified 32 bioactive phytochemicals, including phenolic compounds,

terpenoids and fatty acids known for their antioxidant and antimicrobial properties. These findings suggest that *Rivea hypocrateriformis* is a promising natural source of bioactive compounds with potential pharmaceutical and therapeutic applications.

Reference

1. Borkar, S., Naik, R., Shukla, V., & Acharya, R. (2015). Evaluation of phytochemical content, nutritional value and antioxidant activity of Phanji - Rivea hypocrateriformis (Desr.) Choisy leaf. *AYU (An International Quarterly Journal of Research in Ayurveda)*, *36*(3), 298. https://doi.org/10.4103/0974-8520.182755
2. Dhruvi Kasvala*, Priyanshi Monpara, P. P. P. and D. U. U. (2020). World Journal of Pharmaceutical Sciences. *World Journal of Pharmaceutical and Life Sciences*, *6*(4), 72–80.
3. Godipurge, S. S., Rahber, S., Biradar, J. S., & Mahurkar, N. (2015). Evaluation of pharmacological activities of rivea hypocrateriformis in experimental animal models. *International Journal of Toxicological and Pharmacological Research*, *7*(2), 65–73.
4. Khandekar, U., Bobade, A., Bansod, S., Ghongade, R., & Jolhe, S. (2015). Studies on pharmacological and chemical composition of crude plant extract of Rivea hypocrateriformis. *American Journal of PharmTech Research*, *5*(3), 297–306.
5. Loganayaki, N., Rajendrakumaran, D., & Manian, S. (2010). Antioxidant capacity and phenolic content of different solvent extracts from banana (Musa paradisiaca) and mustai (Rivea hypocrateriformis). *Food Science and Biotechnology*, *19*(5), 1251–1258. https://doi.org/10.1007/s10068-010-0179-7
6. Shweta, S., Ganesh, T., & Somshekhar, K. (2012). Morpho-anatomy, physicochemical and phytochemical standardization with HPTLC fingerprinting of aerial parts of Rivea hypocrateriformis. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2 SUPPL.), S689–S694. https://doi.org/10.1016/S2221-1691(12)60297-4