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ORIGINAL ARTICLE

Assessment of Different Crude Extracts of Pashanbheda (*Bergenia ciliata* Sternb. Rhizome) for Phytonutrients Profiling and Polypharmacological Activities Against Respiratory Afflictions Causing MDR- Carbapenem Resistant Organisms

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ABSTRACT
Traditional medications derivative of herbal plants and their formulations were being utilized since long time to treat many human ailments. Many developing countries are dependent on these plants due to its affordability, availability and low cost. In numerous studies it has been observed that active chemical compounds in plants are accountable for their therapeutic activities. In present scenario there is a frightening increase in the antibiotic resistance among many human pathogens which has led to an emergence to find an effective alternative to treat them. Ever since researchers started working on these plants. The antimicrobial compounds produced by such plants have shown incredible activity towards plant and human pathogenic microorganisms. Present study is also centred around one such medicinal plant i.e., Bergenia ciliata. It is used in the form of herb in Himalayas to cure many diseases. This study specially focusing on bioactive analysis of rhizome of Bergenia ciliata against the Multi drug resistant carbapenem resistant organisms (MDR-CRO) pathogens which are triggering many respiratory diseases in human. According to the current study, the antimicrobial and antioxidant competences of rhizome of B.ciliata growing in Joshimath region of Uttarakhand has been reported for the first time. The Agar well diffusion assay was used to evaluate the antimicrobial activity of the plant in which for the concentration of 1mg/100µl, the methanolic extract has revealed maximum antibacterial activity with ZOI of 26.3±1.52mm against P.aeruginosa and lowest ZOI of 21±1mm was shown by ethyl acetate against A.baumannii. For the concentration of 0.5mg per 100µl maximum antimicrobial activity was shown by methanol extract with ZOI of 20.3±1.52mm against P.aeruginosa while minimum activity was observed in aqueous extract with ZOI of 14.3±1.52mm against K.pnuemonia. The scavenging activity was evaluated using DPPH assay method in which ethyl acetate has the extreme inhibition% (81.19%±0.001), followed by methanolic extract (64.46±0.003%) and the least scavenging activity was observed in aqueous (46.63%±0.002). For determining the phytonutrients in all the extracts, GC-MS technique was used.

Keywords: ***Bergenia ciliata****, free radical, DPPH, phytonutrient, antimicrobial, carbapenems, extract, Carbapenem-Resistant Organisms (CRO), Gram-negative bacteria (GNB).*

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INTRODUCTION

Across the range of care, MDR-GNB, eg; CROs, are representing an expanding risk in public health care services such as long -term care facilities, acute care etc. Antibacterial agent consumption, host genetics,

environmental factors, and the complex nature of the interaction among bacteria and antibiotics all play a part in the development of antibiotic resistance (1). GNB are frequently responsible for hospital-acquired infections and community acquired infections, including those of the lower respiratory tract, bloodstream, and urinary tract. They have a broad spectrum of resistance mechanisms. Extended-spectrum -lactamases (ESBLs), ampCs, and carbapenemases are only a few of the various antibiotic resistance mechanisms that these bacteria can produce (2). Antibiotic use and discovery go hand in hand throughout the history of antimicrobial resistance. The majority of beta-lactam antibiotics' effectiveness was harmed by the emergence of extended-spectrum-lactamases (ESBLs) (3). Among all the antimicrobial arsenal for human, carbapenems like imipenem, doripenem, and ertapenem are considered last resort antibiotics which are being used more frequently to treat infections caused by ESBL-producing microbes and for infections that are resistant to more routinely used antibiotics (4). However, cases of disease resistance to carbapenem, particularly those caused by gram-negative bacteria due to misuse of it are creating resistance to this class of antibiotics by putting pressure on these drugs and it has also emerged, raising valid concerns that the development of antibiotics may have come to a stop. Because of this, there are fewer antibiotics that can be utilised to treat these resistant isolates due to the rise of carbapenemases among CRO (5). These extremely aggressive carbapenem-resistant organisms are consequently linked to a high death rate. In a 2017 study, Tamma et al. discovered that 32% of CRO bloodstream infection patients passed away within 14 days (6,7). Due to this increasing drug resistance emergence, there is an immediate need to search for newer alternatives. It is generally recognised that medicinal plant phytochemicals have antibacterial activities, making them potentially crucial for therapeutic applications (8,9). According to Chauhan, V.B.et., al.2021, *Bergenia ciliata* sometimes called elephant's ear has important medicinal potential in treating many human ailments. Its extracts are used to cure a broad range of infection, including liver problems, gastrointestinal issues, malaria, gall bladder stones, kidney stones, wound healing, cough, inflammation, pathogenic microorganisms, oxidative stress, and various ulcer kindslike, and other conditions (10,11,12). Hence, the purpose of the current research work was to examine the antibacterial and free radical scavenging capacity of rhizome of *B.ciliata* extracts against specific MDR-CRO which are responsible for producing various types of respiratory diseases in human. Further study can also be made in -vivo to investigate the remaining aspects of the plant as it still has many hidden properties present in it which need to be explored for human welfare.

MATERIAL AND METHODS

Plant specimen;

Bergenia ciliata, the herb was collected from Himalayan areas. Joshimath was the major site for collection of the sample (Latitude: 30.550552; Longitude: 79.565964). After collection, it was validated by the Botanical Survey of India (BSI), Dehradun with the accession number "375".

Microbial strains;

The microbial strains used for the study were procured from IMTECH Chandigarh. These are Gram-negative MDR-CRO pathogens named *Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter baumannii.* The Culture was prepared first and then maintained on agar slants at 4°C temperature for further investigation.

Chemical requirements;

Many chemicals and reagents were used throughout the working process such as DPPH (1,1-diphenyl-2-picrylhydrazyl), sulfoxide (DMSO), Nutrient agar media, and Nutrient broth media, Muller Hinton Agar media and Broth, Methanol solvent, Ethyl acetate solvent, Hydrochloric acid, Ethanol solvent, Gallic acid, Tris's buffer. All of the chemicals and reagents were of pure grade and bought from Hi-Media, Mumbai, India. Distilled water (D/W) was used in the experiments.

Preparation of Rhizome extracts;

For the extraction three different polarity-based solvents were used i.e., ethyl acetate (4.4) methanol (5.1), and aqueous (9.0). The powdered rhizome was extracted with al the solvents. For each extract 25gm of the sample was placed in the Soxhlet apparatus along with 250ml of solvent. The extraction was performed in series using solvents of increasing polarity. The boiling temperature for each solvent was maintained during the process (100°Celcius for aqueous, 77.1°Celcius for Ethyl acetate, and 64.7°Celcius for Methanol). The rhizome extracts were then evaporated at a temperature between 70-100°C to get in concentrated form. The yield was determined by evaluating all the extracts and then further kept in an airtight tube for storage **(13).**

Susceptibility Testing for the Bacterial strains;

***Agar Well diffusion Assay* (14)**

The well diffusion methodology was used to evaluate the antibacterial activity of each extraction (ethyl acetate solvent, methanol solvent, and aqueous solvent) of *Bergenia ciliata* rhizome. The rhizome extracts were dissolved in DMSO to achieve concentrations of 0.5mg per 100µl and 1mg/100 µl. (dimethylsulphoxide). Cefoperazone + Sulbactam (0.5mg in 100µl and 1mg in 100µl) was applied as a positive control, whereas DMSO as a negative control. MHA plates were inoculated with 20µl bacterial suspension and spread uniformly over the media in this way. The agar plates were made with a 9mm well-cut. A precise quantity of extracts was filled in the well (0.5mg in 100µl and 1.0mg in 100µl) and incubated at 37 °C for a fixed length of time **(15,16).**

Minimum inhibitory concentration Assessment and Minimum Bactericidal Concentration Assessment

The MIC value of all the extracts (methanol rhizome extract, ethyl acetate rhizome extract, and aqueous rhizome extract) was evaluated using the broth dilution method **(14).** First, a two-fold serial dilution of the extract was prepared in well plates using Mueller-Hinton Broth, and at the standard concentration of 5×10^5cfu/ml, 20ml of the bacterial suspension (CRO pathogens) was added to the well and then further the well plates were incubated for 24hrs at 37°C. Every one of the extracts was treated with the same technique, with a positive control (Cefoperazone+Sulbactam) and negative control (DMSO), respectively. MBC is the minimum concentration at which the extract and conventional antibiotics showed no apparent growth (turbidness).

The MBC of the extract was measured using the sub-culturing method after the MIC was obtained. The inoculum was from each microplate well of broth on Mueller-Hinton plates which are incubated at 37°C for 18-24hrs. The number of colonies developing units/ml in the first inoculums and the numeral of colonies forming units per ml on the MHA plate were compared. The MBC was defined as the rhizome extract with the highest bactericidal activity at the lowest concentration (i.e., 99% death of bacterial isolates) **(17).** The MIC value index was used to estimate the bactericidal and bacteriostatic action of an extract, with MIC index values >4 suggesting bacteriostatic activity and MIC index values 4 showing bactericidal activity **(14).**

DPPH Assay for free radical scavenging capacity;

DPPH method was employed to determine the free radical scavenging activity of all the extracts. DPPH (1, 1-diphenyl-2-picrylhydrazyl) was used. The sample was dissolved in 1ml of 1-diphenyl-2-picrylhydrazyl in 450µl of ethanol and 800µl of 0.1M Tris HCL buffer (pH-7.4) with continuous stirring and then stored at room temperature for 2hrs. Now, a certain amount of plant extracts (25.0µl, 50.0µl, 75.0µl, 100.0µl, 125.0µl, 150.0µl, 175.0µl, 200.0µl, 225.0µl, 250.0µl) is further added to this mixture, which is then mixed well and incubated at 37°C for 30min. Spectrophotometer equipment was used to determine the absorbance of the solution after it had been incubated. Below mentioned equation was used to compute the amount of the DPPH free radical:

Effect of DPPH scavenging percentage = (A0- A1) / A0) × 100

where A0 denotes the absorbance under control and A1 denotes the absorbance **(18).** The real reduction in absorption brought on by the test must be contrasted with the outcomes of the positive controls. Plotting the extract concentration vs, the associated scavenging action will allow you to get the IC50 value ie., concentrations that provide 50% inhibition. Using the procedure, we calculated the DPPH inhibitory percentage (I% percent). The standard was gallic acid, and the negative control was dimethyl sulfoxide (DMSO). All results were calculated in triplicate form **(19)**

Qualitative evaluation of phytonutrients using GC-MS technique;

This technique involves the use of a gas chromatograph and a mass spectrometer. To perform GC-MS analysis, HP-5 MS, 30m×250m; 00.25m column at a flow rate of 1ml/min with inlet temperature of 250°C and helium as the carrier gas was used for all the extracts (ethyl acetate solvent, methanol solvent, and aqueous solvent extract). For the ion source 250°C and for the transfer line 280°C temperature with the split ratio 1:10 was employed. The scanning range was 40-500 amu, and the injection volume was 1µl. To identify different components in the extracts, a commercial library (NIST) was used. Temperature programming is as follows shown in Table. 1.

High Performance Liquid Chromatography (HPLC):

The HPLC analysis was carried out using an Agilent 1260 Infinity. A Hi-Olex H 300 X 7.7MM column measuring 8µ was used, and its flow rate was set to 0.7 ml/min. The temperature of the detector was maintained at 55°C and the column at 60°C. A volume of 10 µl of the suitable sample was injected into the column. Gallic acid was used as the standard compound. The UV detector picked up the light at 272 nm.

RESULT

Extraction procedure;

The rhizome *of Bergenia ciliata* was extracted using the Soxhlet method in which three distinct solvents: ethyl acetate, methanol, and water, were used according to increasing polarity. S1 stood for Ethyl acetate extract, S2 for Methanol extract, and S3 for Aqueous extract as the distinct solvent extracts. The complete rhizome extract yield is presented in Table.2.

Antimicrobial Susceptibility Testing;

The Agar well diffusion method was conducted to ascertain the antibacterial activity. The antimicrobial sensitivity test findings have demonstrated the extracts' substantial effectiveness when compared to conventional medicines. *Acinetobacter baumannii (MTCC 9829), Pseudomonas aeruginosa (MTCC 647), Klebsiella pneumonia (MTCC 618)* were the three bacteria that S2 had the most antibacterial activity against, while S1 had the lowest activity when compared to the conventional antibiotics Cefoperazone + Sulbactam which was taken as the positive control (S) in terms of zone of inhibition (mm). The standard medications showed observable inhibition of *Pseudomonas aeruginosa* with ZOI of 23±1mm for 0.5mg per 100µl and 30.3±1.52mm for 1mg/100µl. The effectivity of the drug for *Acinetobacter baumannii* showed ZOI of 22.6±1.52mm for 0.5mg per 100µl and 31.3±1.52mm for 1mg per 100µl and the ZOI for *Klebseilla pneumonia* was 21±1mm for 0.5mg per 100µl and 27.6±1.52mm for 1mg/100µl. The zone of inhibition given by standard drug S was then compared with the ZOI of S1, S2 and S3. The maximum inhibition of bacterial growth was given by S2 in which the ZOI for *Pseudomoanas aeruginosa* is 20.3±1.52mm for 0.5mg per 100µl and 26.3±1.52mm for 1mg/100µl. For *Acinetobacter baumannii* the ZOI was 18.6±1.52mm and 25±2mm for 0.5mg per 100µl and 1mg per 100µl respectively and for *Klebseilla pneumonia* the ZOI was 19±1mm for 0.5mg per 100µl and 24.3±1.52mm for 1mg per 100µl. Following S2, S3 activity was observed by second highest zone of inhibition for all three bacterial strains. The ZOI it formed for *Pseudomonas aeruginosa* was 18.3±1.52mm and 25±2mm for the concentration of 0.5mg per 100µl and 1mg per 100µl respectively. For *Acinetobacter baumannii* the ZOI was 19±2mm for 0.5mg per 100µl and 24±1mm for 1mg per 100µl. The ZOI for *Klebsiella pneumonia* was 14.3±1.52mm for 0.5mg per 100µl and 23.3±1.52mm for 1mg per100µl. The least antibacterial activity was given by S1, in which the ZOI for *Klebsiella pneumonia* is 18.3±1.52mm and 21.6±1.52mm for 0.5mg per100µl and 1mg per 100µl respectively. The ZOI for *Pseudomonas aeruginosa* was 14.6±1.52mm for 0.5mg per 100µl and 24.6±1.15mm for 1mg/100µl. *Acinetobacter baumannii* activity was suppressed with ZOI of 17.3±1.52mm for 00.5mg per 100µl and 21±1mm for 1.0mg/100µl.

The MIC, MBC and MIC Index for S (Control) is as follows for *Pseudomonas aeruginosa* MIC is 0.0208±0.009, MBC is 0.0312±4.24 and the MIC Index value is 1.5. For *Klebsiella pneumonia* MIC is 0.026±0.009, MBC is 0.0208±0.009 and MIC Index value is 0.8 respectively. For *Acinetobacter baumannii* MIC, MBC and MIC index values are 0.0156±2.12, 0.026±0.009 and 1.6 accordingly. The S1 and S2 MIC for *Pseudomonas aeruginosa* was 0.0677±0.05mg/ml and 0.083±0.03mg/ml while 0.0364±0.02mg/ml for S3. For *Acinetobacter baumannii* S2 and S3 has same MIC with 0.0572±0.05mg/ml and 0.1041±0.03mg/ml for S1. But for *Klebsiella pneumonia* the MIC was different for all three solvents i.e., for S1, S2, and S3, it is 0.0364±0.02mg/ml, S2 0.0625±0mg/ml, and 0.1041±0.03mg/ml respectively. The MBC for *Pseudomonas aeruginosa* of S1, S2 and S3 are 0.0364±0.02, 0.0416±0.01 and 0.0364±0.02 along with MIC index value which are 0.5, 0.4, 1 respectively. For *Acinetobacter baumannii* the MBC value of S1 is 0.0625±0, for S2 0.0208±0.009 and for S3 is 0.0312±0.02. The MIC index value for the same is 0.6 for S1, 0.3 for S2 and 0.5 for S3. At last, for *Klebsiella pneumonia,* the MBC value for S1 is 0.0312±0.02, for S2 0.0520±0.01 and for S3 it is 0.0729±0.04. MIC index value for S1 and S2 are same i.e., 0.8, for S3 it is 0.7. All the readings were given in the Table:3,4,5. Pictures for AST, MIC, MBC and MIC value index were shown in figure.1,2,3,4,5.

DPPH Radical Scavenging;

A novel natural source of antioxidant by evaluating the antioxidant qualities of plant extract. Due to its stability in the radical state and ease of the experiment, DPPH radical is a frequently employed substratum for quick assessment of antioxidant capacity **(20).** The method is well established to provide accurate data on the antioxidant capacity of the examined composites **(21)**. The assay's basic idea is change in DPPH colour. Once the antioxidant quenches the radical, the purple colour of the solution changes to yellow **(22).** The colour variations may be quantified using a spectrophotometer absorbance at 517 nm. In this method, ethanol solution is mixed with DPPH and crude extracts following incubation for 20 minutes at room temperature. Then the optical density is measured at 517nm. Observation is given in Table.6 below with graphical representation in Fig. 6.

Plotting the proportion of inhibition versus the concentration of the compounds under research using linear regression allowed for the calculation of the IC50 value, or substrate concentration at which the

DPPH activity reduced to 50%. Table 2 presents the IC50 values for each extract. The ethyl acetate extract had 97.28 µg/ml, the aqueous extract had 281.46 µg/ml, and the methanol extract had 167.38 µg/ml.

Phytonutrients profiling;

Gas Chromatography-Mass Spectroscopy Analysis (GC-MS):

For the evaluation of various phytonutrients present in *Bergenia ciliata* GC-MS technique is primarily used. Plants' internal missile defence system is based on a wide range of tiny molecule antimicrobials. Countless observers have stated that metabolites are really what bring plants significant pharmacological effects. These are organic substances that fall within the classifications of primary and secondary metabolites. Volatile oils, steroids, tannins, terpenoids, flavonoids, alkaloids, saponins, glycosides, as well as other secondary metabolites are common that plants produce. The therapeutic efficiency of plants in treating a broad range of conditions is due to their secondary metabolites, which are referred to as phytochemicals in plants and are pharmacologically active compounds (10**).** Numerous plant secondary metabolites exhibit antibacterial action against bacterial species that are Gram-positive and Gram-negative. To establish the relevance of phytoconstituents in terms of their medication potency, standardization is important, which demands phytochemical research of the drug plant. More than a lakh plant-derived small molecule compounds have been discovered, and many of these have shown antibacterial properties. Therefore, it becomes essential to assess phytochemicals both qualitatively and quantitatively and to define them. **(23).** Several researchers have investigated different phytochemicals in *Bergenia ciliata*. It is an important medicinal herbal plant that contains several key bioactive chemicals. *Bergenia ciliata* rhizome was subjected to phytochemical screening, which revealed that it contained phenol, tannin, fatty acids, glycosides, sterols, terpenoids, and saponins etc **(24).** These chemical compounds are responsible for their antioxidant capacity, with free radical scavenging activity, reducing power, and ability to reduce lipid peroxidation. In all antioxidant studies, the methanolic extract showed better potential **(25)**. Bergenin (0.6%) is a major element in the rhizome. *Bergenia ciliata* rhizome extract in methanolic and aqueous form was discovered to show promising potential for medication development that might be utilized to target tumors for chemotherapy or chemoprevention **(26)**. The rhizome aqueous extract includes phenolic components bergenin, gallic acid, leucocyanidin, catechin, polymeric tannin, methyl gallate **(27)**. The rhizome extracts also confirmed the existence of sitoindoside, -sitosterol, and -sitosterol-d-glucoside as well as (+)-catechin, (+)-catechin-3-gallate, and 11-O-galloyl bergenin **(28).** The evaluation obtained for total number of phytochemicals given by GC-MS are mentioned below in Table.7,8,9 with a graphical representation in Figure.7,8,9.

High Performance Liquid Chromatography (HPLC):

The HPLC chromatogram is show in Figure.10 in which presence of Gallic acid is detected which is the major phytochemical compound in *Bergenia ciliata* rhizome.

DISCUSSION

The different evaluations following the current investigation will help in the proper identification and validation of the drug known as crude *Bergenia ciliata* rhizome extract. Similar investigations using a constructivist approach on many medicinal plants are consistent with the current study and strongly support this work. The practice of plants for therapeutic or medicinal purposes to treat disease and improve human health is termed as phytomedicine. Phytochemicals, or bioactive ingredients, are substances that have medicinal properties and are utilized as medications or treatments. Plants produce secondary metabolites known as phytochemicals (29). Most medicines are based on secondary metabolites and plant chemical structures. Plants' secondary metabolites that have pharmacological effects on humans have been isolated (30). It has been shown that the availability of sterols, glycosides, and other biochemical compounds in the genus Bergenia is what permits those plants to demonstrate antibacterial effects against a broad range of pathogenic pathogens (31). To do this, several crude extracts are prepared using chemicals based on their polarity and tested for their antioxidant and antibacterial properties. Antioxidants, which have the potential to scavenge free radicals, are crucial in treating ailments in animals as well as in human. The DPPH stable free radical method is a subtle technique to assess the antioxidant capacity of plant extracts (32,33). Strong antioxidant characteristics were seen in the *B. ciliata* rhizome's ethyl acetate and aqueous extracts; however, the methanol fraction has the poorest level of antioxidant potential. Phytochemicals from the rhizome of *B. ciliata* were described by Bagul et al. in 2003, however they are in less concentrations than those discovered in the present research. The number of phyto-nutrients in the *B. ciliata* rhizome specifically states that these plant extracts have high medicinal value, including cytotoxic, antioxidant, antifungal and antibacterial activity (34,35). Flavonoids are Benzo-g-pyrone which are naturally occurring metabolites and have a diverse range of chemical and biological traits, such as hepatoprotective, antiviral, anti-inflammatory, and anti-

thrombotic effects (36,37). Most of the aforementioned activities might be attributed to free-radical scavenging and antioxidant activity. There are around 58 phytochemicals in *Bergenia ciliata* in which the rhizome of *Bergenia ciliata* is an excellent source of coumarins, tannins, and alkaloids. Extracts from the rhizomes of *Bergenia ciliata* have also been used as anti-aging agent. Old wounds, gall bladder, kidney stones are also being treated. Septic Rhizome paste is used as an antiseptic Additionally, cough, and colds are also treated by its use. For treating diarrhoea and dysentery, rhizome infusion is taken orally. Rhizome juice is given in cases of severe asthma. Chewing fresh rhizome can treat any form of digestive ailment. Applying crushed rhizome sap to eye conditions. On the newborn baby's head, septic pimples are treated with rhizome paste. Crushed rhizomes are used for several ulcer types. Rhizome paste works well for skin conditions. Fresh rhizome paste is useful for inflammation. Chewing fresh rhizome can treat any form of digestive ailment. Applying crushed rhizome sap to eye conditions. On the newborn baby's head, septic pimples are treated with rhizome paste. Crushed rhizomes are used for several ulcer types. Rhizome paste works well for skin conditions. Fresh rhizome paste is useful for inflammation. Rhizome extract is taken orally and acts as an anti-helminthic and anti-rheumatic. Rhizome extract, both fresh and dried, is used orally for piles. Tonsils are treated with it. Rhizome powder is used for heart issues. Colitis and other interior wounds are treated with rhizome paste. The powder from the ciliate rhizome of Bergenia is administered to enhance spermatozoa. Oral rhizome sap is used to treat various types of urinary issues (38). As science developed, it was able to apply AYUSH to address the fresh problems facing the contemporary healthcare system (29). In the last ten years, interest in examining medicinal herbal plants as a potential source of herbal medications has returned. Research for the design and characterization of unique natural medicines must be advanced via the use of superior vetting procedures derived from plants and other natural sources.

Table.1

SNO	Rate (°C/min)	Value (°C)	Hold Time
1.	-	60	1
2.	20	150	5
3.	10	220	3
4.	8	250	10

Table 2: The physical properties, yield, yield%, polarity index of *Bergenia ciliata* rhizome extracts.

Solvents	Ethyl Acetate (S1)	Methanol (S2)	Aqueous (S3)
Yield (gm/250ml)	8.62gm	5.61gm	**6.62gm**
Yield%	34.48%	22.44%	**26%**
Colour	Light brown	Dark brown	Brown
State	Viscous	Viscous	Viscous
Polarity index	4.4	5.1	9
IC50 Value	97.28% (µg/ml)	167.38% (µg/ml)	281.49%(µg/ml)

Table3: Antimicrobial effectivity of S (Positive control) against selective MDR- CRO (Carbapenem-Resistant Organisms) pathogens.

Positive control (S)	Dose concentration	Zone of Inhibition (mm) for Bacterial culture		
		Pseudomonas aeruginosa	***Klebsiella pneumoniae***	***Acinetobacter baumanii***
	0.5mg	23±1	21±1	22.6±1.5
	1.0mg	30.3±1.52	27.6±1.52	31.3±1.52

The mean ± SD values are in triplicates

Table4: Anti-microbial activity of S1, S2, S3 against selective MDR-CRO pathogens.

S.No	Extracts	Dose concentration	Zone of Inhibition (mm) for Bacterial culture		
			Pseudomonas aeruginosa	***Klebsiella pneumoniae***	***Acinetobacter baumanii***
1.	S1	0.5mg	14.6±1.52	18.3±1.52	17.3±1.52
		1.0mg	24.6±1.52	21.6±1.52	21±1
2.	S2	0.5mg	20.3±1.52	19±1	18.6±1.52
		1.0mg	26.3±1.52	24.3±1.52	25±2
3.	S3	0.5mg	18.3±1.52	14.3±1.52	19±2
		1.0mg	25±2	23.3±1.52	24±1

The mean ± SD values are in triplicates.

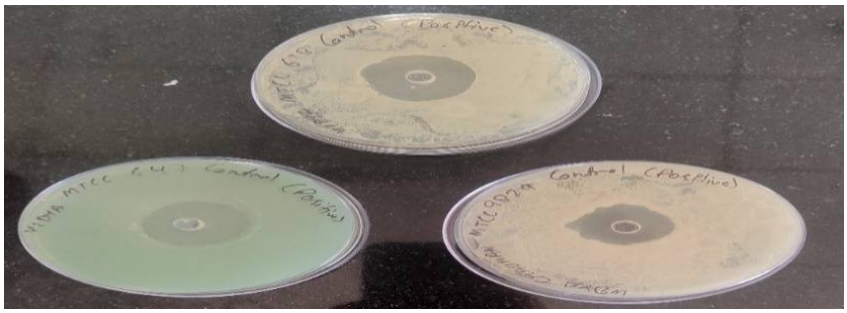


Figure.1. Antibacterial activities of positive control in the form of the zone of inhibition (mm). Key: Positive control (Cefoperazone + Sulbactam), MTCC 647 *(P.aeruginosa),* MTCC 618 *(K.pnuemoniae),* MTCC 9829 *(A.baumannii).*

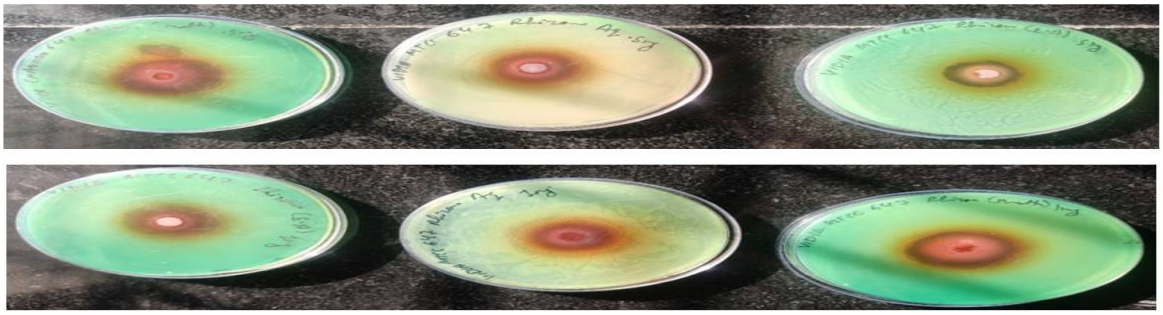


Figure.2. Antibacterial activities of *Bergenia ciliata* rhizome extracts in different solvent in the form of the ZOI (mm) for the concentration of 0.5mg per 100µl and 1mg per 100µl for MTCC 647 *(P.aeruginosa).*

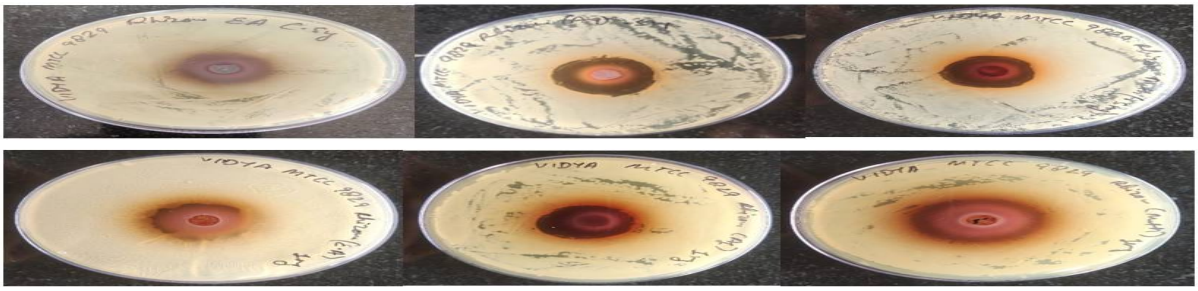


Figure.3. Antibacterial activities of *Bergenia ciliata* rhizome extracts in different solvent in the form of the ZOI (mm) for the concentration of 0.5mg per 100µl and 1mg per 100µl for MTCC 9829 *(A.baumannii).*

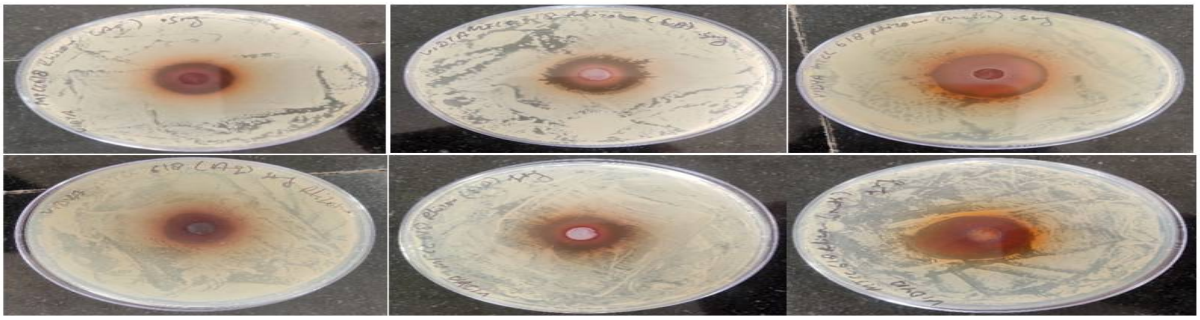


Figure.4. Antibacterial activities of *Bergenia ciliata* rhizome extracts in different solvent in the form of the ZOI (mm) for the concentration of 0.5mg per 100µl and 1mg per 100µl for MTCC 618 *(K.pnuemoniae).*

Table5: The Minimum inhibitory concentration assay and Minimum bactericidal concentration assay values of S, S1, S2, S3 extract against MDR-CRO at concentration of 0.5mg per 100µl.

The MIC, MBC and MIC Index values of *Bergenia ciliata* Rhizome extracts					
S. No	**Extracts**	**Dose concentration (0.5mg/100µl)**	**Bacterial culture**		
			Pseudomonas aeruginosa (MTCC 647)	***Klebsiella pneumonia (MTCC 618)***	***Acinetobacter baumannii (MTCC 9829)***
1		Incubation period	37°C	37°C	37°C
2		Range	0.5-0.0156	0.5-0.0156	0.5-0.0156
3	S (Control)	MIC (S)	0.0208±0.009	0.026±0.009	0.0156±2.12
		MBC (S)	0.0312±4.24	0.0208±0.009	0.026±0.009
		MIC Index (S)	1.5	0.8	1.6
4	S1	MIC (S1)	0.0677±0.05	0.0364±0.02	0.1041±0.03
		MBC (S1)	0.0364±0.02	0.0312±0.02	0.0625±0
		MIC Index (S1)	0.5	0.8	0.6
5	S2	MIC (S2)	0.0833±0.03	0.0625±0	0.0572±0.05
		MBC (S2)	0.0416±0.01	0.0520±0.01	0.0208±0.009
		MIC Index (S2)	0.4	0.8	0.3
6	S3	MIC (S3)	0.0364±0.02	0.1041±0.03	0.0572±0.05
		MBC (S3)	0.0364±0.02	0.0729±0.04	0.0312±0.02
		MIC Index (S3)	1	0.7	0.5

*Incubation period, Range and MIC (Control) are same for all extracts. The mean ± SD values are in triplicates

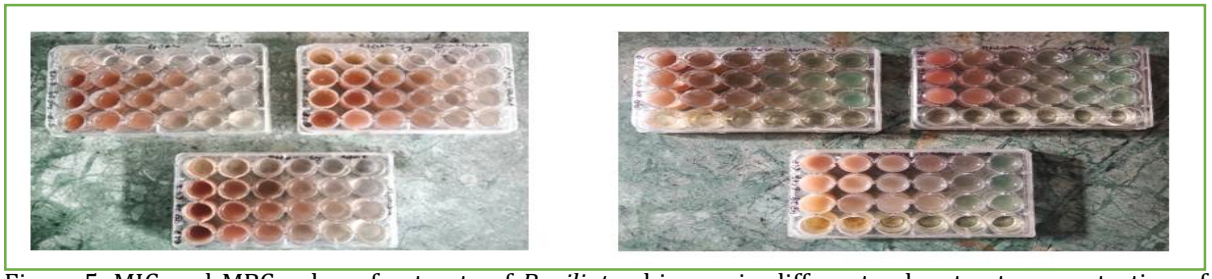


Figure.5. MIC and MBC value of extracts of *B. ciliata* rhizome in different solvents at concentration of 0.5mg per 100µl for MTCC 647 *(P.aeruginosa)*, MTCC 618 *(K.pnuemoniae)*, MTCC 9829 *(A.baumannii)*.

Table6: Reducing Antioxidant power of *Bergenia ciliata* rhizome extract in different solvents along with standard compound (gallic acid)

S.NO	**Concentration (mg/µL**	**Inhibition% ± SD (n=3)**			
		Standard (Gallic acid)	**Ethyl Acetate (S1)**	**Methanol (S2)**	**Aqueous (S3)**
1	25	91.09%±0.001	40.95%±0.002	19.70%±0.002	15.91%±0.002
2	50	91.57%±0.002	41.89%±0.001	21.28%±0.002	18.76%±0.002
3	75	92.41%±0.003	45.82%±0.002	28.52%±0.002	19.70%±0.002
4	100	93.14%±0.003	48.19%±0.003	35.42%±0.005	23.27%±0.001
5	125	93.62%±0.002	51.09%±0.002	43.78%±0.002	29.07%±0.001
6	150	94.82%±0.002	57.51%±0.003	48.41%±0.002	29.88%±0.001
7	175	95.18%±0.002	64.73%±0.003	53.08%±0.001	32.02%±0.001
8	200	95.54%±0.002	69.06%±0.001	57.37%±0.002	41.27%±0.002
9	225	96.02%±0.002	72.61%±0.002	63.00%±0.001	42.30%±0.003
10	250	96.63%±0.003	81.19%±0.001	64.46%±0.003	46.63%±0.002

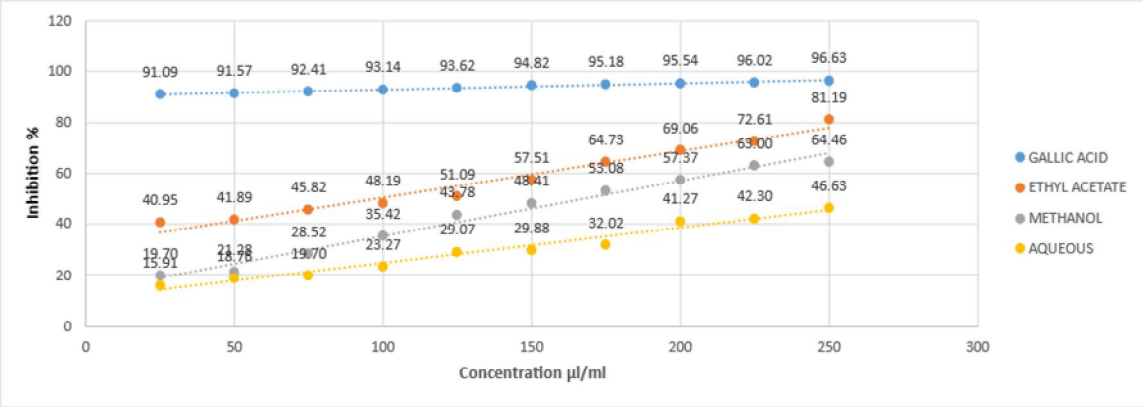


Fig.6: Graphical representation of linear regression for antioxidant capacity of various extracts in different solvents (ethyl acetate, methanal, aqueous) of *Bergenia ciliata* rhizome at increasing concentration along with Gallic acid as reference compound.

Table 7: Phytonutrients in S1 extract using GC-MS technique:

S.NO	RT	Area	Library
1	2.173	0.37	Benzene 1,3-Hexadien-5-yne
2	4.399	0.72	Phenol
3	5.263	4.66	2-Azabicyclo {2,2,1} heptane 2{3H}-Furanone, dihydro-3-methylene 2-Cyclohexene-1-one
4	6.279	0.96	1,2-Benzediol Phenol, 2-{1-methylethoxy}- methylcarbamate 2-Isopropoxyphenol
5	6.457	0.48	Benzofuran, 2,3-dihydro- 1,2,4-Triazolo {4,3-a} pyrazine 1,2,4-Triazolo {4,3-a} pyrimidine
6	6.992	1.16	Hydroquinone Resorcinol 2-Cyclopenten-1-one, 2,3-dimethyl-
7	7.116	0.30	2-Chlorohistidine 2,2-Dimethyl-1-aza-apiro {2,4} heptane 2[1H]-Pyrimidinone, 5-methyl-
8	7.203	0.45	Benzaldehyde, 2-nitroso- 2H-Pyrazole-3-caboxylic acid, 2-methyl Pyrazine, methoxy
9	7.559	5.08	Urea, N, N, -dimethyl- Oxalacetic acid Butanal, 3-methyl
10	7.829	0.63	2,6-Dimethylbanzaldehyde Phenol, 4-{2-propenyl}- 1H-Purine, 2-methyl
11	8.278	7.83	Phenol, 4-propyl- Benzenemethanol, alpha, -2-propenyl Phenol, 3-ethyl
12	8.359	19.58	1,2,3-Benzenetriol
13	9.580	0.29	3-Methyl-2-nitrophenol Pyrazole-5-carboxylic acid, 3-methyl-
14	10.330	0.82	2H-Pyrazole-3-carboxylic acid, 2-methyl 4{1H}-Pyrimidinethione, 2-methyl- 1,3,5-Benzenetriol
15	10.557	0.54	3,4-Dihydro-2-quinoxalinol 3{2H}- Benzofuranone, 6-methyl- 1H-1,5- Benzodiazepine, 2,3,4,5-tetrahydro-
16	10.822	2.98	Benzoic acid, 4-hydroxy-
17	11.616	2.10	Cyclopentane carboxylic acid, butyl ester Cyclopentane carboxylic acid, 2-pentadecyl ester

			Cyclopropane carboxylic acid, propyl ester
18	12.032	0.43	5,6,7,8-Tetrahydrofurazano {4,5-c} azepin-4-one 1,2-Dimethyl cyclopropane 2,4-Hexadiene, 1-chloro-
19	12.443	1.96	Benzene-1,2,3,4-tetraol Piperidin-2-one-5-carboxylic acid, 5,6-didehydro-, methyl {2ster} Cyclobutanecarboxamide, N, N-bis {2-ethylhexyl}-
20	12.605	7.28	1,3,5-Benzenetriol
21	12.886	1.39	Phenol, 2-amino-4-methyl- 4-Pyrimidinamine, 2,6-dimethyl- Levodopa
22	15.727	0.91	Cyclohexane, 1,2,3-trimethyl-, {1, alpha,2, alpha., 3. beta}- Cyclohexane, 1,2,3-trimethyl-
23	16.273	1.10	Benzoic acid, 4-hydroxy-3, 5-dimethoxy-
24	17.947	5.08	n-Hexadecanoic acid Tridecanoic acid
25	20.459	3.65	9,12- Octadecadienoic acid {Z, Z}- 9,12-Octadecien-1-ol, {Z, Z}- 9,12-Octadecadienoic acid, methyl ester
26	20.535	5.15	Trans-13-Octadecenoic acid cis-Vaccenic acid cis-13-Octadecenoic acid
27	20.789	1.11	Pyrene, 1,2,3,6,7,8-hexahydro- Phenanthrene, 2-methoxy- Coumarin, 7,8-dihydro-7-hydroxy-6- Methoxy-8-oxo-
28	20.886	2.62	Octadecanoic acid Tetradecanoic acid
29	25.369	0.43	2-Methyl-5,5-diphenyl-4-{methylthio} imidazole 1H-Indole, 5-methyl-2-phenyl- 3-Amino-7-nitro-1,2,4-benzotriazine 1-oxide
30	25.542	0.61	{9-Oxo-9,10-dihydroacridin-4-yl} acetic acid 3-Amino-7-nitro-1,2,4-benzotriazine 1-oxide Quinoline, 2-chloro-6-methoxy-4-methyl-
31	27.827	1.34	1H-Indole, 1-methyl-2-phenyl- Silane, trimethyl {5-methyl-2-[1-methylethl] phenoxy}- 2-Methyl-7-phenylindole
32	30.118	1.32	2-{Acetoxymethyl}-3- [methoxycarbonyl] biphenylene 1,2-Benzisothiazol-3-amine tbdms 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsily-
33	32.068	3.18	1-Benzazirene-1-carboxylic acid, 2,2, 5a-trimethyl-la-{3-oxo-1-butenyl} perhydro-, methyl ester 2-Ethylacridine Propiophenone, 2-{trimethylsiloxy}-
34	33.553	1.19	Tetrasiloxane, decamethyl- Cyclotrisiloxane, hexamethyl- 1,2-Benzisothiazol-3-amine tbdms
35	38.572	12.28	Stigmast-8{14}-en-3, beta-ol 5-Methyl-2-phenylindolizine Cyclotrisiloxane, hexamethyl-

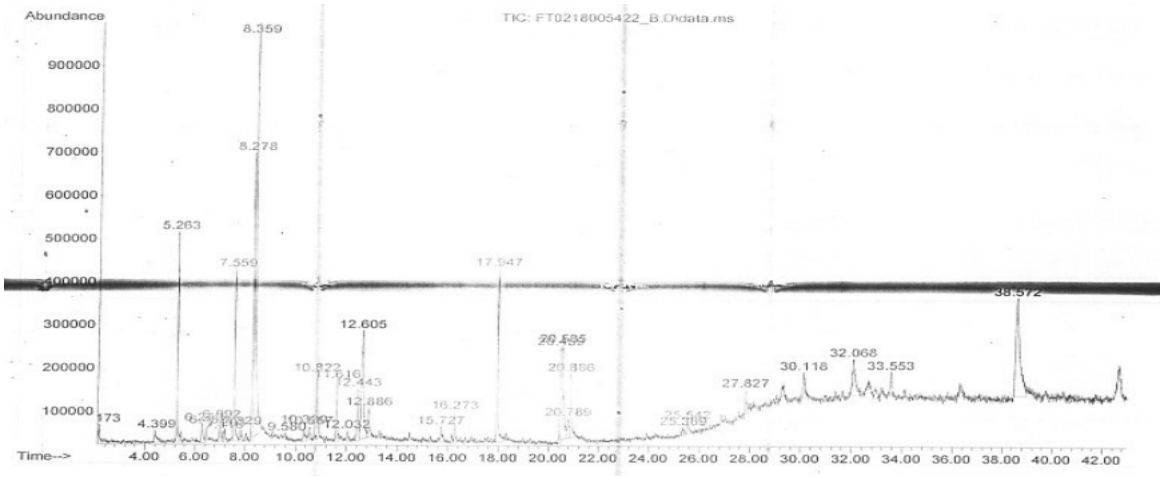


Fig.7: The GC chromatogram of the S1 extract

Table 8: Phytonutrients in S2 extract using GC-MS technique:

S.NO	RT	Area	Library
1	2.692	2.67	Guanidine, methyl- Acetic acid, hydrazide
2	2.832	3.20	Guanidine, methyl Butyric acid hydrazide Carbamic acid, nitrosopropyl-ethyl ester
3	3.097	7.18	Propanal,2,3-dihydro- 1,2-Ethanediol, diformate 1,4-Butanediol
4	3.184	3.59	Furfural Alpha-pyrone-6-carboxylic acid 1H-Imidazole,1,4-dimethyl-
5	3.610	12.53	dl-Glyceraldehyde dimer 1,3-Dihydroxyacetone dimer 2-Propanone, 1,3-dihydroxy-
6	3.934	2.51	2-Cyclopenten-1-one, 2-hydroxy-1-Penten-1-one, 2-methyl- Cucloheptane
7	5.263	33.19	2{3H}-Furanone, dihydro-3-methylene- 2-Azabicyclo {2.2.1} heptane 2H-Pyran-2-one, 5,6-dihydro-
8	5.604	2.54	5-Aminoisoxazole Propanenitrile, 3- {metylamino}- Cyclobutane, methylene
9	5.912	4.11	5-Methoxy- {1,2,3} oxadiazole Trans-1,2-Dimethylsilacyclohexane Cis-1,2-Dimethylsilacyclohexane
10	6.560	3.20	Furazanamine, 4-azido- 1H-Pyrrole-2,5-dione Benzene, {[methylenecyclopropyl]sulfonyl}-
11	7.559	2.64	Butanal, 3-methyl- 3,6-Octadecadiynoic acid, methyl ester Propanenitrile, 3-[methylamino]-
12	8.288	3.03	Cyclohexene, 1-ethyl-6-ethylidene-styramate Pyridine, 4-ethyl
13	10.320	11.02	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2, 5-dioxo-Furazanamine, 4-azido-3Methyl-2-furoic acid
14	17.915	3.35	1,5-Anhydro-4-O-acetyl-2,3,6-tri-O-methyl-D-mannitol n-Hexadecanoic acid Nonanedioic acid, bis{2-methylpropnyl} ester
15	20.756	5.23	1,2-Dimethoxy-4-{1-methoxy-1-propenyl} benzene 5,6,7,8-Tetrahydro-1-phenylnaphthalene 1-Cyclododecanone, 2-ethylidene

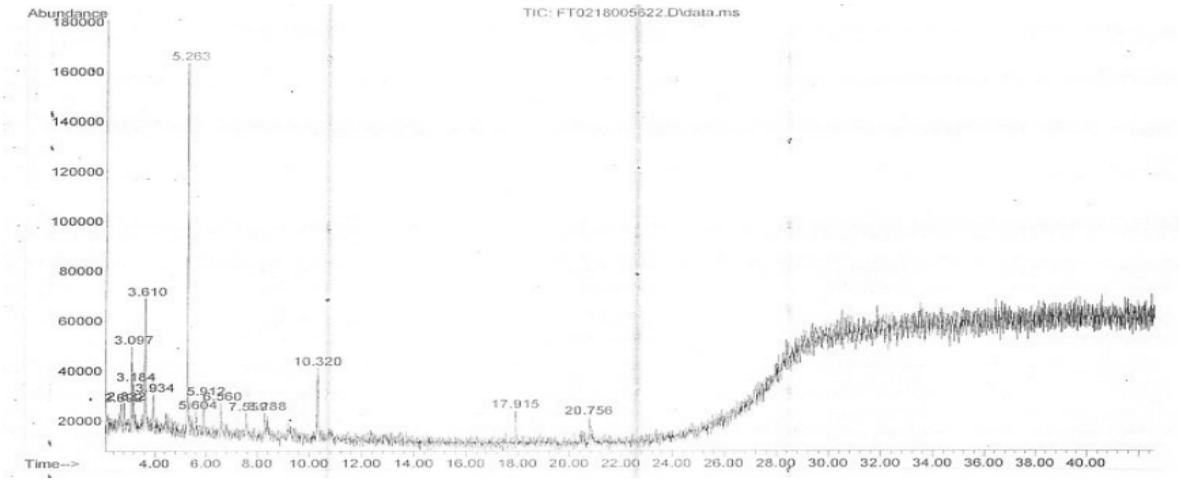


Fig.8: The GC chromatogram of the S2 extract

Table 9: Phytonutrients in S3 extract using GC-MS technique:

S.NO	Retention Time	Area	Library
1.	2.200	1.68	Methylene diamine, N, N-diacetyl-Acetamide, N-methyl 3,6-anhydro
2.	2.611	1.01	1-Heptan-4-ol 3-Butyn-2-ol 4-Heptanol
3.	2.676	0.89	1,2-Epoxy-3-propyl acetate 1,2-Etahnediol, monoacetate Acetic acid, 3-mercapto-3-methybutyl ester
4.	3.102	2.67	dl-Glyceraldehyde Di glycolic acid Methenamine, N-hydroxy-N-methyl
5.	3.102	1.42	Furfural 2-[2,2,2-Trichloro-1-{2-furamido} ethoxy} benzamide
6.	3.329	1.43	2-Furanmethanol
7.	3.508	1.97	Dihydro-2-{3H}-thiophenone Ethanamine, 2-methoxy-N-{2-methoxy ethyl}-N-methyl
8.	3.616	6.86	dl-Glyceraldehyde dimer 2-Propanone, 1,3-dihydroxy-1,3-Dihydroxyacetone dimer
9	3.934	0.97	2-Cyclopenten-1-0ne, 2-hydroxy-Pyrroid-2-one-5-methanol, N-methl 1-acetyl {ester} Benzamide, 3-methyl-N-{2-91-piperidyl} ethyl
10	4.377	1.62	2,4-Dihydro-2, 5-dimethyl-3{2H} furan-3-one Benzene, ethoxy-2,5-Difluoroanisole
11	5.252	11.79	2H-Pyran-2-one, 5,6-dihydro 2-Cyclohexen-1-one
12	5.749	0.88	2-Propanamine, N-methyl-N-nitroso-Ethanamine, N-ethyl-N-nitroso-Acetic acid, hexyl ester
13	5.830	7.40	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl
14	6.268	0.89	1,2-Benzenediol
15	6.441	1.25	Benzaldehyde, 3-methyl Benzaldehyde, 4-methyl 2-Isocyanatopyridine
16	6.554	5.17	2-Furancarboxaldehyde, 5-{hydroxym ethyl}- Thiocynaic acid, 2-propynyl ester
17	6.684	1.60	1,2,3-Propanetriol, monoaetate Hexanoic acid, 3-hydroxy, methyl ester Butaneritrile, 2,3-dioxo, dioxime, o,o-diacetyl
18	6.981	1.44	2,4-Dimethoxypyrimidine Phenol, 2-{methylthiol}-2-Methoxybenzenethiol
19	7.122	2.33	2,4-Dihyroxy-2,5-dimethyl-3(2H)-furan-3-one 5-Undecanol, 2-methyl-3-{Prop-2-enoyloxy} tertradecane
20	7.554	2.12	H-Hydroxycarbamic acid,2-{propoxycarbonylamino} ethyl ester Cycloserine N, N, N, N-Tetramethlsulfonamide
21	7.694	1.35	Propane, 2-{ethenyloxy}- L-serine, dihydrogen phosphate 3-{N-acetyl-N-methylamino} propionic acid
22	7.851	1.02	Semioxamazide Propionic acid, 3-[{diethoxymethyl} ethylphosphinatomethyl}] {ethoxycarbonyl} amino-ethyl ester Isovaleric acid, decyl ester
23	8.034	1.12	Phenol, 2,6-dimethoxy

24	8.380	7.92	1,2,3- Benzenetriol
25	8.942	0.72	3-Piperidinol N-{n-Propyl} acetamide Cyclopropanecarboxamide
26	9.563	9.47	2-Deoxy-D-galactose Hydrazinecarboxamide N1-{4-hydroxybutyl} -N3-methylguanidine acetate
27	10.406	0.72	D-Allose Beta-D-Glucopyranose, 1,6-anhydro-alpha-D-Mannopyranoside, methyl 3,6-anhydro
28	10.816	3.06	Benzoic acid, 4-hydroxy
29	11.594	1.19	1-Octen-4-ol Fumaric acid, dimethyl ester, 2-methoxy 2-t-Butyl-5-methyl-5-propyl- {1,3} dioxolan-4-one
30	12.426	1.37	5-Hydroxymethyl uracil 5-Hydroxy-2-methylthiopyrimidine 5-Hydroxy-2-methylthiopyrimidine
31	12.653	3.71	3-Deoxy-d-mannoic lactone 2R, 3S-9-{1,3,4-Trihydroxy-2-butoxy methyl} guanine N-Methoxymethyl-N-methylacetamide
32	13.031	6.91	Butanoic acid, pentyl ester4(1H)- Pyrimidione, 6-hydroxy-Pentane, 1-propoxy
33	13.237	2.43	N-Hydroxy-N-ethylcarbamic acid, 2-{propoxycarbonylamino} ethyl ester D-Erythro-Pentose, 2-deoxy-3-Pyrrolidicarboxylic acid, 2,4-dioxo-, methyl ester
34	16.267	1.99	Benzoic acid, 4-hydro-3-5-dimethoxy
35	20.783	1.62	2H-1-Benzopyran-2-one, 7,8-dihydroxy-6-methroxy 1,4-Anthracenedione 3,5-Dimethoxy-4-hydrxycinnamaldehyde

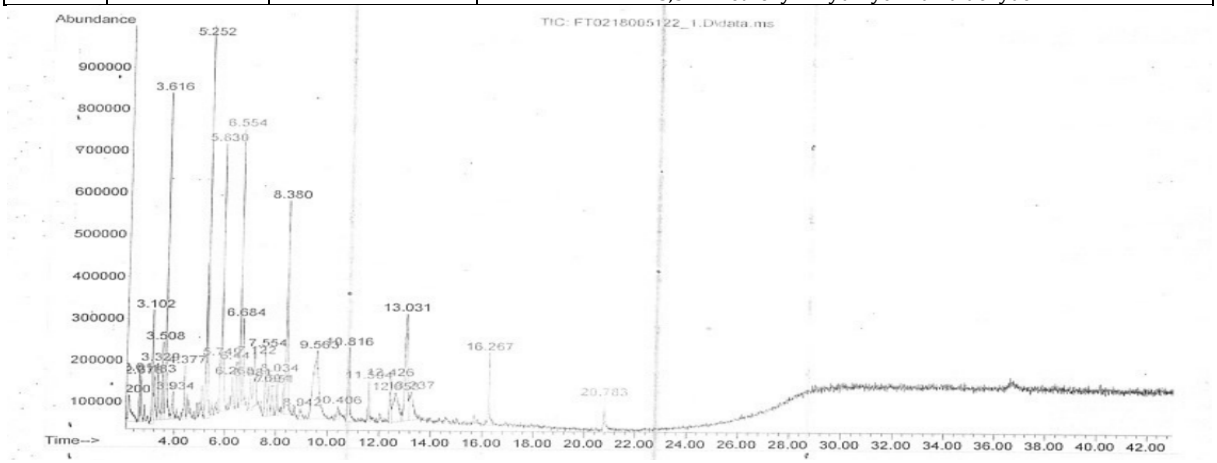
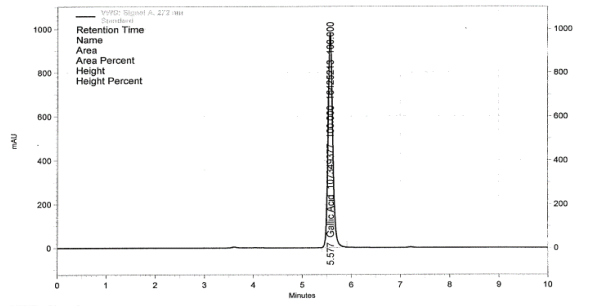


Fig 9: The GC chromatogram of the S3 extract

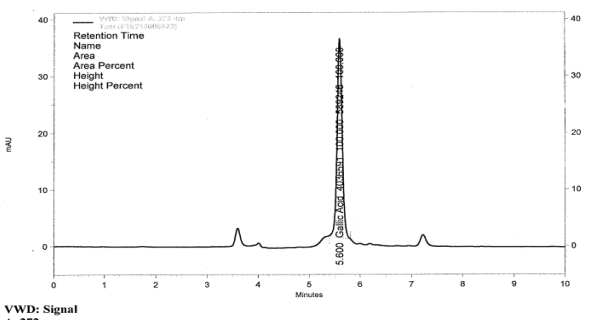
STANDARD



VWD: Signal A, 272 nm Results

Name	Retention Time	Area	Area %	Height	Height %
Gallic Acid	5.577	107349377	100.00	16425213	100.00
Totals		107349377	100.00	16425213	100.00

TEST



VWD: Signal A, 272 nm Results

Name	Retention Time	Area	Area %	Height	Height %
Gallic Acid	5.600	4036591	100.00	589246	100.00
Totals		4036591	100.00	589246	100.00

Fig 10: The HPLC chromatogram of the S2 extract (Test) along with standard as reference.

CONCLUSION

The twenty-first century seems to be coping with a serious challenge in healthcare management due to rising bacterial resistance. The use of old, affordable antibiotics which formerly were enormously successful has been restricted as a result of multiple reports on the development of more recent, multi-drug resistant diseases. The recent fast rise of carbapenem resistance among gram-negative bacteria poses a serious danger to the healthcare system. Therefore, there is an urgent demand for novel CRO-effective antibiotics. The foregoing research indicates that *Bergenia ciliata* rhizome methanol extract has considerable efficacy against all test organisms, perhaps as a result of the plant's secondary metabolites. The study's findings validate the plant's folkloric use and conclude that the majority of its extracts include chemicals with therapeutic activities that might be used as agents in cutting-edge medications to treat infectious disorders brought on by infections. Future studies should concentrate on extracting and concentrating the bioactive compounds associated with Bergenia's antimicrobial activities. It is vital to do continuous research in this field in order to obtain new antimicrobial chemicals, especially from the plant world. Furthermore, research indicates like comprehending whether bacteria build resistance is a key factor that necessitates careful attention if one wants to develop an effective strategy. The antibacterial chemicals that come from plants will be utilized in shaping future methods to combat these complications.

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DISPUTE OF INTEREST

Regarding the research, writing, and/or publication of this work, the authors confirm that there are no conflicts of interest that may have existed.

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