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RESEARCH ARTICLE

Antimicrobial and Antifungal Properties of Leaves to Root Extracts and Saponin Fractions of *Chlorophytum Borivilianum*

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Abstract: ***Objective*:** The study was conducted to examine the antimicrobial activity of methanolic crude extract from different parts of the *Chlorophytum borivilianum plant* against pathogenic microorganisms and toassess antimicrobial activity by MIC and structural characterization of purified saponin of *Chlorophytum borivilianum* by using spectrophotometric and NMR analysis.

***Methods*:** The antimicrobial analysis of the extracts of leaves, roots and stems of *C. borivilianum* is based on the agar well diffusion method and minimum inhibitory concentration (MIC). The phytochemical screening and characterization of saponin on the basis of structural and antimicrobial activity present in *C. borivilianum* were analyzed by different spectrophotometric methods such as HPLC, UV-visible, IR, NMR, LC-ESI-MS and pharmacophore modeling.

***Results*:** The results revealed that the methanolic leaf, stem and root extracts have inhibitory potential against the growth of *K. pneumonia, B. subtilis, M. tuberculosis, E coli* and *S aureus* in case of bacteria and *C. albicans*, *A. fumigatus* and *Tricoderma* in case of fungus. The MIC values of leaf, stem and root extracts were found in the range of 1 mg/ml to 0.125 mg/ml. Moreover, the purified saponins indicated MIC in the range of 0.5 mg/ml to 0.0625 mg/ml against the selected microbial pathogens. Saponins act as one of the major phytocomponents present in *C. borivilianum*. The antimicrobial and structural analysis of purified saponins of *C. borivilianum* was also performed using different spectral analysis methods.

***Conclusion*:** The anti-microbial results showed that the extract from the leaf and stems had higher anti-pathogenic activity as compared to the roots. The MIC results showed that the purified saponin also possessed the anti-microbial activity and oleanolic acid content, as detected by spectral analysis the fundamental structure of the extracted saponin.

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1. INTRODUCTION

Due to the climatic change and poor human lifestyle, human beings are more prone to infections and diseases [1]. The prevailing environmental conditions such as high temperature, humidity, *etc*., promote not only the growth of microorganisms but also disturb the symbiotic relationship between host-microbes homeostasis and this leads to an increase in various diseases such as urinary tract infection (UTI), influenza, endocarditis, meningitis, tuberculosis, *etc* [2, 3]. The microorganism resistivity demands a new formulation of antibiotics, which needs more rigorous research and time-consuming process [4, 5]. Because of the increase in toxicity and antimicrobial resistance to synthetic drugs, humans are more attracted towards ethnopharmacology [6]. According to the World Health Organization (WHO) study, more than 80% of the world's population depends on prescription medicines and this is a significant issue of the society with regard to health care [7]. The use of medicinal plants in Asia reflects a long tradition of contact between humans and the diversity of flora found in favorable climate. A large number of phyto-compounds from the medicinal plants have been identified and found to be healthier with little or

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no toxicity [8]. These phyto-compounds are secondary active plant metabolites, which are also known as phytomedicines [9]. The fragments of plant or whole plant or the isolated active metabolites can be used for the preparation of medicines. Such herbal ingredients demonstrate high therapeutic effectiveness to reduce single constituent toxicity and adverse effects [10]. Chlorophytum *borivilianum* (*C. borivilianum*) is commonly referred to as Safed Musli and belongs to the Liliaceae family [11]. It has high rates of medication property. It was assumed in ancient times that the roots are components of numerous common medicines and also belong to an essential class of Ayurvedic herbs, known as Rasayana [12]. Rasayana is made up of herbs and has higher immune-stimulating and adaptogenic properties [13]. In addition, the saponin content (2-17%) of *C. borivilianum* is anticipated to possess various biomedicinal applications [14]. The **e**xtracts of aqueous origin from *C. borivilianum* normalize the levels of serum corticosterone, plasma glucose, cholesterol and triglycerides and also decrease the index of ulcers and adrenal gland weight related to normal subjects [15]. The plant roots have been identified to possess a lot of medicinal properties such as hepato-protective, antimicrobial, anti-inflammatory, hypolipidemic, antipyretic, antioxidant and anti-diabetic [14]. Normally, *C. borivilianum* is used to treat erectile dysfunction, impotence in men, oligozoospermia, antioxidant properties of sperm [16, 17]. Reports have shown that the root extracts of *C. borivilianum* possess anti-pathogenic activities and other medicinal properties. Because of its high medicinal value, the root of the plant has been exploited and reported to be an endangered species [18]. The leaves and stem extracts of *C. borivilianum* also serve as a strong antimicrobial agent against Gram-positive and Gram-negative bacteria [19, 20].

The present work, therefore, provides a comparative analysis of the crude as well as the purified saponin extracts to determine their biological properties, and furthermore, the structural analysis of the saponin has also been performed using spectrophotometric and NMR methods.

2. MATERIALS AND METHODS

2.1. Collection of Plant Sample

The plantlets were obtained from the medicinal garden of Invertis University, Bareilly, Uttar Pradesh, India.

2.2. Extract Preparation

The plant samples were washed with distilled water and then cut into small pieces. The plant parts were dried, crushed and extracted with methanol in a 1:10 ratio. The slurry was filtered and the filtrate was subjected to evaporation under vacuum conditions. The dried extract was weighed and resuspended in dimethyl sulphoxide (DMSO) [21] and stored at -20°C for further use. The percent yield was obtained after the extraction from the leaf (20 mg/g), stem (15 mg/g), root (12mg/g) and purified saponin (10mg/g).

2.3. Microorganisms Tested

The bacterial and fungal strains used in the study included *Bacillus subtilis* (Bs) (MTCC 441), *Klebsiella pneumonia* (Kp) (MTCC 661), *Staphylococcus aureus* (Sa) (MTCC 902), *Escherichia coli* (Ec) (MTCC 1687), *Pseudomonas aeruginosa* (Pa) (MTCC741), *Mycobacterium tuberculosis* (M Tb) (Lohia Hospital, Lucknow), *Aspergillus fumigatus, Tricoderma,* and *Candida albicans* (Ca), which were procured from Lohia Hospital, Lucknow and MRD Life Sciences Pvt. Ltd. Lucknow, Uttar Pradesh, India.

2.4. Antimicrobial Activity Test

This test is based on the agar well diffusion method [22]. The concentration of bacterial and fungal inoculants was 106-108 CFU / mL. Petri plates of nutrient agar and potato dextrose agar were used for bacterial and fungal cultures, respectively. 0.5 mg of plant extracts were loaded in the wells and the plates were incubated at 37°C for 24 hours. Antimicrobial activity in the form of the zone of inhibition (ZOI) was measured in millimeters (mm).

2.5. MIC Test

The MIC test was conducted in 96-well microtitre plates using the broth-micro dilution process [23]. In this experiment, respective microorganisms were inoculated to each well of the plates with 100µl of nutrient broth, and after that, 100µl of antibiotic dilutions were added and the plates were incubated at 37°C for 24 hours for the further experimental procedures. The minimum plant extracts concentrations for optimum inhibition of bacterial and fungal pathogens were evaluated. The study was performed by using 0.5 mg from 5 mg / mL stock solution from a total volume of 100 µl of the methanolic crude extract and purified saponin along with control.

2.6. Antioxidant Activity

With the DPPH photometric assay (2-diphenyl-2-picryl hydrazyl), the free radical scavenging ability of aqueous, alcoholic extract and essential oil of various plants was assessed with the help of a technique defined by Elmastas (2006) [24].

A solution of 0.1 mM DPPH was prepared with the addition of 3 ml to 0.1 ml of water/ alcohol at varying concentrations (viz., 0.25, 0.5, 1.0, and 2.0 mg / mL) as mentioned in the protocol. The absorption should be estimated at 515 nm after 30 minutes. Lower reaction mixture absorbance showed higher free radicals scavenging behaviour. The scavenging potential of DPPH radicals has been determined using the equation below.

$$\text{DPPH scavenging effect (\%)} = \mathbf{A}_{\mathbf{control}} - \mathbf{A}_{\mathbf{sample}} / \mathbf{A}_{\mathbf{control}}$$

Where, $A_{control}$ is the absorption of the trigger reaction, and A_{sample} is the absorption in the presence of water, alcoholic extract and essential oil [25]. B.H.T., ascorbic acid, vitamin E and BHA are used as reference compounds.

2.7. Phytochemical Test

Plant extracts were screened for the characterization of phyto-compounds such as alkaloids, glycosides, saponin glycosides, steroids and tannins [26, 27].

[a] Carbohydrates: 1 ml of 1mg/ml concentration of the extract was taken and 1ml of each Fehling's A and B were added to it. The mixture was boiled, and if red precipitate was formed, the result confirmed the presence of carbohydrate in the extract.

[b] Phenols and tannins: 1mg/ml concentration of 1 ml extract was taken and 5ml of 2% $FeCl_3$ was added to it. Blue or green-black coloration indicated a positive result.

[c] Saponins: 1 ml extract of 1 mg/ml concentration was taken and 3ml of distilled water was added to it. Then it was shaken vigorously. The development of froth indicated the presence of saponin.

[d] Flavonoids: 1ml of 1mg/ml extract has been taken and 2ml of 2% NaOH has been added. With the addition of 2 ml HCl, the yellow colour turned into colorless and this indicated a positive result.

[e] Catecholic tannin: 1ml plant extract (1mg/ml) was added to 2ml distilled water and then a few drops of ferric chloride were added to it. The appearance of green-black colour confirmed the presence.

[f] Alkaloids: 2ml of 1% picric acid was added to 1 ml plant extracts (1mg/ml). Yellow precipitate showed positive results.

[g] Terpenoids: 2ml chloroform was added to the 1mg/ml concentration of plant extract. Then, 2ml of concentrated H_2SO_4 was added slowly to it. A reddish-brown coloured interface was observed, indicating the presence of terpenoids.

[h] Steroids: 1ml of plant extract (1mg/ml) was added to 2ml of chloroform and mixed properly. After that, 2ml of concentrated H_2SO_4 was added to it. The appearance of red colour in the chloroform layer indicated a positive result.

[i] Starch: 1ml of plant extract (1mg/1ml) was added to 2 ml of iodine solution and the development of blue-black colour indicated a positive result.

2.8. Extraction and Purification of Saponin

Dried powder of plant sample was extracted 95% ethanol (1:5 wt/vol ratio) by incubating the mixture at 100 RPM for 12- to 18 hours, which was then filtered. The filtrate was evaporated under reduced pressure at 35 °C to 45°C. Delipidization was performed according to the protocol reported by Sharma *et al.* [28]. Briefly, petroleum ether was added to the concentrated plant extract and after mixing the solution, it was heated for around 30 min to evaporate the solvent. The residue was treated with an equal ratio of ethyl acetate and chloroform mixture and the sample was stirred for 15 min. Chloroform is a deproteinizing agent, hence it is used to remove the proteins from plant samples. Ethyl acetate and chloroform were evaporated by heating the mixture at 45 °C to 55°C. The acetone was added drop by drop to the remaining hot residue and a white-colored precipitate was formed. The precipitate was filtered and dried in an oven to obtain white crystals, known as saponins. The confirmatory test was carried out by dissolving the white residue in 10 ml of distilled water and shaken vigorously for 30 sec to 1 min. The extract was purified using silica gel (SiO_2) with a column chromatographic mode, where silica particles were used as an adsorbent and packed in a cylindrical glass column. Slurry with silica was loaded to the column and saponin was eluted by using solvent system chloroform: methanol: water (60:30:10). The fractions were dried at 45°C to 55 °C and analyzed by the HPLC column.

HPLC system of Shimadzu with C-18 column (4.6X250mm, 5µm), SPD 20 A detector was used for the analysis under isocratic condition. Acetonitrile: water (50:50 v/v) was degassed and used for the analysis of saponin. 20µl sample of 1mg/ml stock solution was injected and the flow rate of 1 ml/min was maintained throughout the run time of 20 min. The saponins were analyzed by the reverse-phase method and scanned at 254 nm.

2.9. Chemical Characterization

2.9.1. UV-Vis Spectrum Analysis

The extracted compound was diluted to a 1:10 ratio with the methanol and the sample was scanned ranging from 200 to 800 nm using the Eppendorf spectrophotometer and the characteristic peaks were detected.

2.10. IR Spectrum Analysis

Infrared analysis of the dried powder of the extracted saponin from *C. borivilianum* was done through FTIR spectrophotometer (Agilent Cary 630) with a scan range of 450 to 4000 cm^{-1}.

2.11. LC-ESI-MS

The purified saponin was dissolved in water and then filtered before use. Mass spectrums were obtained by the Liquid Chromatography Electrospray Mass Spectrometry (Waters UPLC-TQD Mass spectrometer). The column used for the study was Sunfire C18, 250 X 4.6, 5 µm; solvent system acetonitrile: water, with a flow rate of 1.0 ml/min. The mass spectra were scanned in the range of 150 to 1500, with a MS scan run-up to 30 min.

2.12. NMR

The purified saponin was dissolved in D_2O and NMR spectra (^{1}H-NMR and ^{13}C-NMR) were recorded on the Bruker Avance 400 (FT NMR) at 400 MHz.

2.13. Statistical Analysis

All the experiments were performed in triplicate and the data were represented as the percentage mean ± standard deviation. The data were further analyzed by two-tail ANOVA analysis using Graph Pad Prism version 7 software and during the analysis, the level of significance was kept as p-value < 0.05.

3. RESULTS

3.1. Antibacterial Activity Test

The antimicrobial activity of different parts of *C. borivilianum*plant was evaluated against six pathogens (*K. pneu-*

monia (Kp), *B.subtilis (Bs), M. tuberculosis* (M Tb), *E. coli* (Ec), *P. aeruginosa* (Pa) and *S. aureus* (Sa)). The tests were performed by the agar well diffusion and cup plate method, with methanol as negative and tetracycline as a positive control. Whereas, 0.5 mg concentration of both extracts and tetracycline was loaded with 5 mg / mL stock solutions from 100 µl of total volume. Secondary metabolites extracted from all three parts of *C. Borivilianum* were found active against *M. tuberculosis.* Maximum activity was found in the stem extract (18.6 mm) in comparison to leaf extracts (13.3 mm) and root extracts (14.2 mm) (Table **1**). Furthermore, the antimicrobial activity of the mentioned plant extracts was also examined against various Gram-positive and negative bacterial strains and the results are summarized in (Table **1**). From the data, it was observed that against *K. pneumonia*, stem extracts (16.1mm) of *C. borivilianum* possessed higher antimicrobial activity as compared to leaf extracts (13.8 mm) and root extracts (14.7 mm). The leaf extract (19.2 mm) showed good antimicrobial activity against *B. Subtilis* as compared to root extracts (14.3 mm) and stem extracts (14mm). However, root extracts (19.6 mm) of the plant were found to be more effective against *E. Coli* than the leaf (14.9 mm) and stem extracts (16.9 mm).

3.2. Antifungal Activity Test

The antifungal activity of different parts of *C. borivilianum* plant was also examined against three fungal pathogens *C. albicans, A. fumigatus and Tricoderma* (Table **2**) by agar diffusion method using methanol as negative and Fluconazole (0.5 mg) as a positive control. Based on ZOI (Zone of Inhibition), stem extract of the plant was found to be more effective against *C. albicans* (21.7 mm) and *Tricoderma* (21 mm) as compared to leaf extracts and root extracts. However, leaf extract (20.8 mm) was found more effective against *A. fumigates* than root extracts (16 mm) and stem extracts (20.8mm).

3.3. MIC

MIC of methanolic extracts (crude) and purified saponin from *C. borivilianum* was analyzed against *K. pneumoniae* (Kp), *B. subtilis* (Bs), *M. tuberculosis* (MTb), *E. coli* (Ec), *P. aeruginosa* (Pa) and *S. aureus* (Sa), *C. albicans, A. fumigates* and *Tricoderma* (Table **3**). MIC identifies the minimum concentration of the extract, at which it shows the inhibition of microbial growth. MIC of purified saponin is found in the range of 0.500 – 0.0625 mg/ mL, whereas the MIC values of crude extract from stem, root and leaf are found to be higher than the purified one (MIC of stem extract is 1.00 - 0.25 mg / mL; MIC of root and leaf extract is 1.00 - 0.125 mg / mL). MIC experiment was also performed against *M. tuberculosis* and it was found that all the four samples, purified saponin (0.0625 ± 0.06mg / mL), leaf extracts (0.5 ± 0.03mg / mL), root extracts (0.5 ± 0.04mg / mL) and stem extracts (0.25 ± 0.04mg / mL), were found to have higher activity against *M. tuberculosis* as compared to other pathogens. The results of MIC values were verified with the results obtained through the agar well method. It was observed that the purified saponin showed higher activity than the crude ones due to the higher yield of saponin during the purification process. Moreover, the results suggest that the saponin also showed an effective and inhibitory effect against fungal pathogens.

Table 1. Antibacterial analysis of methanolic extract of leaf, root and stem of *C. borivilianum* against *K. pneumonia* (Kp), *B.subtilis (Bs), M. tuberculosis* (M Tb), *E. coli* (Ec), *P.aeruginosa* (Pa) and *S. aureus* (Sa).

S no.	Pathogens	Zone of Inhibition (mm)			
		Leaf Extract (0.5 mg / mL)	Root Extract (0.5 mg / mL)	Stem Extract (0.5 mg / mL)	Tetracycline (0.5 mg / mL)
1	*M. tuberculosis*	13.3 ± 0.6**	14.2 ± 0.1**	18.6 ± 1**	21.3 ± 0.1**
2	*K.pneumoniae*	13.8 ± 0.6**	14.7 ± 1.1***	16.1 ± 1.1***	22.6 ± 1.5***
3	*B. subtilis*	19.2 ± 0.9**	14.3 ± 0.6**	14 ± 0.1***	21.3 ±1.4***
4	*E. coli*	14.9 ± 0.8***	19.6 ± 0.5***	16.9 ± 1.8***	20.6 ± 1**
5	*S. aureus*	17.2 ± 1***	22.5 ± 1.3**	16.9 ± 1.8***	21.1 ± 1.2**
6	*P. aeruginosa*	0	15.33± 1.1**	0	20.9 ± 1.6***

Data presented in the table is the mean value of three independent experiments. *P<0.05 **P<0.001 and ***P<0.005 respectively.

Table 2. Antifungal analysis of a methanolic extract of the leaf, root and stem of *C. Borivilianum* against *C.albicans, A. fumigates* and *Tricoderma.*

S no.	Pathogens	Zone of Inhibition (mm)			
		Leaf Extract (0.5 mg / mL)	Root Extract (0.5 mg / mL)	Stem extract (0.5 mg / mL)	Fluconazole (0.5 mg / mL)
1	*C.albicans*	20.3 ± 1.1***	20 ± 1**	21.7 ± 0.6**	22.7 ± 1.9***
2	*A.fumigatus*	21.1 ± 1.9***	16 ± 1.7***	20.8 ± 1.5***	21.3 ± 1.5***
3	*Tricoderma*	18.9 ± 2.1**	14.1 ± 1***	21 ± 1**	19.1 ± 0.9**

Data presented in the table is the mean value of three independent experiments. *P<0.05 **P<0.001 and ***P<0.005 respectively.

Table 3. MIC test of methanolic extract leaf, root and stem of C. borivilianum against K. pneumonia (Kp), *B. subtilis (Bs),* M. tuberculosis (M Tb), E. coli (Ec), P. aeruginosa (Pa), S. aureus (Sa), Tricoderma, C. albicans and A. fumigatus.

Pathogens	MIC value (mg/mL)					
	Crude Extract			Purified Saponin	Tetracycline	Fluconazole
	Leaf	Root	Stem			
M. tuberculosis	0.50±0.03*	0.50±0.04*	0.25±0.04*	0.06 ±0.06***	0.12 ±0.08***	Nd
B. subtilis	0.50±0.03***	0.25±0.05***	0.50 ±0.05*	0.06 ±0.06***	0.25±0.06***	Nd
E. coli	0.25±0.05*	0.50±0.07*	1.00 ±0.05	0.50 ±0.02*	0.25±0.06***	Nd
P. aeruginosa	0.50±0.05	1.00±0.03*	0.50 ±0.05*	0.50 ±0.06*	0.12 ±0.03***	Nd
K.pneumoniae	1.00±0.04*	0.50±0.03*	0.25±0.08*	0.12 ±0.1***	0.06 ±0.04**	Nd
S. aureus	0.12±0.04*	0.12±0.06***	0.25±0.10*	0.25±0.04***	0.12 ±0.03***	Nd
Tricoderma	0.25±0.04***	0.50±0.03*	0.5±0.05*	0.06 ±0.05**	Nd	0.50 ±0.04*
A.fumigatus	0.12±0.07***	0.12±0.06***	0.25±0.06***	0.125±0.09***	Nd	0.25±0.07***
C.albicans	0.50±0.07*	0.25±0.04*	0.12 ±0.06***	0.25±0.02*	Nd	0.50 ±0.05*

Data presented in the table is the mean value of three independent experiments. *P<0.05 **P<0.001 and ***P<0.005 respectively.

Table 4. DPPH radical scavenging activity of ascorbic acid, BHT, leaf, stem and root extracts of *C. Borivilianum*.

Concentrations (1.0 mg / mL)	0.25 mg / mL	0.5 mg / mL	1 mg / mL	2.0 mg / mL
Root extract	25.4±3.1	35.3±3.5	50.0±3.3	56.2±1.7
Stem extract	23.9±2.6	25.5±3.0	27.3±3.6	36.6±3.4
Leaf extract	28.0±3.2	30.0±2.2	33.0±1.2	37.4±3.1
Ascorbic acid	12.6 ± 0.5	24.5 ± 1.04	24.5 ± 1.0	93.1 ± 0.4
BHT	20.0 ± 1.3	43.5 ± 1.1	43.5 ± 1.1	76.0 ± 0.8

Data presented in the table as (%) is the mean value of three independent experiments.

Table 5. Qualitative phytochemical analysis of the methanolic extracts of fresh roots, stems and leaves of *C. borivilianum*.

S No.	Phytochemicals	Stem Extract	Leaf Extract	Root Extract
1.	Alkaloid	++	+	+
2.	Flavonoid	+	++	+++
3.	Terpenoid	-	-	-
4.	Starch	+	-	-
5.	Steroids	+++	+	++
6.	Carbohydrate	++	+	+
7.	Saponins	+++	+++	++
8.	Phenols and Tannins	+++	++	+
9.	Catecholictanin	+	+	+
10.	Gallic tannin	-	-	-

Data presented in the table indicates -: absence, +: minor presence, ++: moderate presence, and +++: maximum presence.

3.4. Antioxidant Test

The results suggest that with the increasing concentration of the reducing compound, the scavenging activity of the samples also increased. Among the plant samples, root extract showed maximum reducing power (25.4% -56.2%) as compared to stem (23.9% -36.6%) and leaf extracts (28% -37.4%) (Table **4**).

3.5. Phytochemical Test

To check the presence and absence of phyto-compounds in *C. borivilianum,*the phytochemical analysis was performed. Results of the phytochemical analysis of the root stem and leaf extracts are summarized in (Table **5**). On analyzing the results, it has been confirmed that all three parts of the *C. borivilianum* contained 10 phytochemical compounds, as indicated in (Table **5**). Among them, alkaloid, flavonoid, steroid, saponins and phenols were found to be present predominantly in the plant. The higher concentrations of carbohydrates, steroids, alkaloids and starch were present in the stem of the plant, whereas flavonoids, phenols and tannins, were present at higher concentrations in the extract of the roots, and saponins were present at a higher concentration in the leaf extract of the plant (Table **5**).

Flavonoid optical density in the root, stem and leaf extracts was observed to be 1.8, 1.78 and 1.77, while phenols were found to be higher in the stem extract (0.035) than the

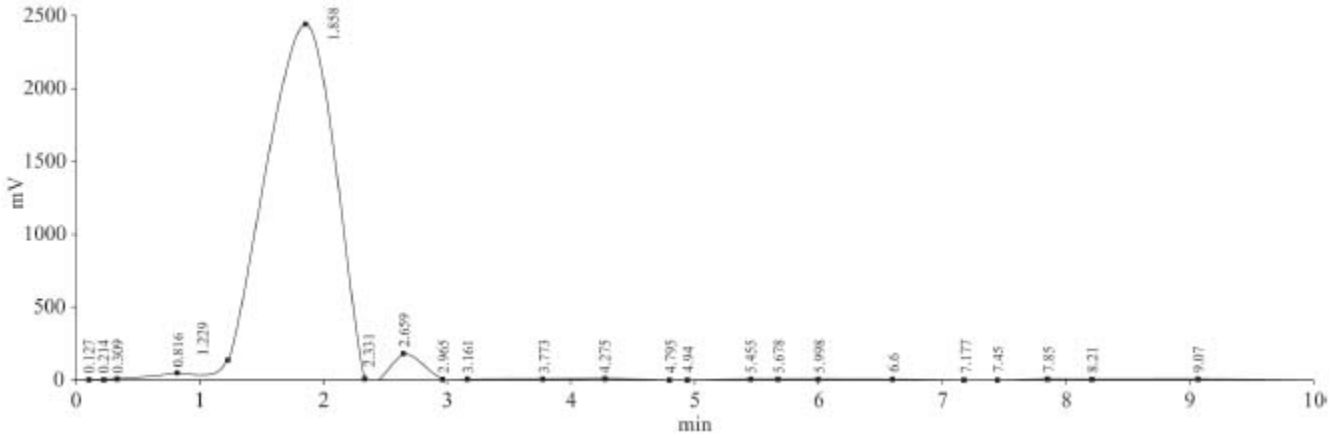

Fig. (1). HPLC chromatogram of extracted saponin from *C. Borivilianum.*

leaf extract (0.024) and lowest in the roots extract (0.011), respectively. It means that in contrast to phenols, the flavonoids are present in a larger quantity.

3.6. HPLC Analysis

The nature of the purified saponin was confirmed by analyzing the HPLC chromatogram. The HPLC profile of purified saponin of *C. borivilianum* was examined at a wavelength of 254 nm (Fig. **1**). The retention time and the percentage area of the biologically active saponin were 1.83 min and 81.775%, respectively.

3.7. Confirmation and Analysis of Saponin Chemical Structure:

The structure of the extracted saponin was confirmed by employing modern spectroscopic techniques such as UV-Vis spectrum, IR spectrum, LC-ESI-MS and ^{1}H, ^{13}C NMR analysis.

UV spectrum of the purified sample in methanol was taken in the wavelength range of 200 to 800 nm. The spectrum exhibited a range of 260 and 450 nm, suggesting the presence of saponin content in the sample (Fig. **2**).

The IR spectrum of the saponin exhibited absorption bands in the range from 3415.82 $cm^{-1}$to 669.06 cm^{-1} (Fig. **3**). The band at 3415.82 cm^{-1} suggests the presence of CONH or OH moiety in the structure. Similarly, the absorption band at 3018.23 and 2934.87 cm^{-1} corresponds to the aromatic and methylene CH stretching, respectively. A sharp band at 1731.01 cm^{-1} and 1222.79 cm^{-1} indicates the presence of C-O and C=O carbonyl moiety in the extracted compound. The peaks at 1376.77 cm^{-1} show methyl deformation vibrations. A sharp band at 1132.66 cm^{-1} reflects the presence of C-O-C stretch, whereas bands at 1047.3 cm^{-1} and 925.2 cm^{-1} suggest the out of plane CH_2- bending vibrations. Likewise, the peak at 795.33 indicates aromatic, out of plane bending.

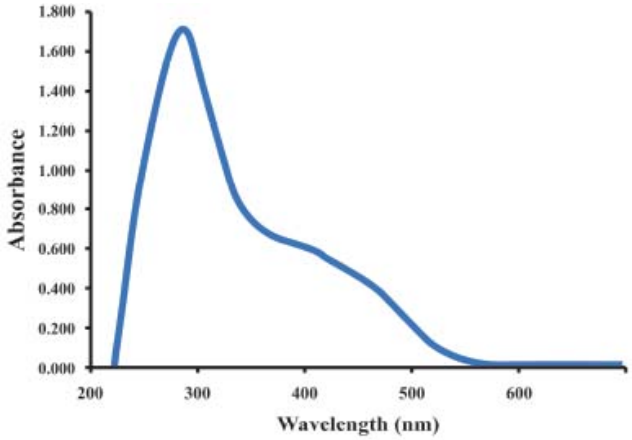

Fig. (2). UV-Visible Spectroscopy Analysis of extracted saponin of *C. borivilianum*. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

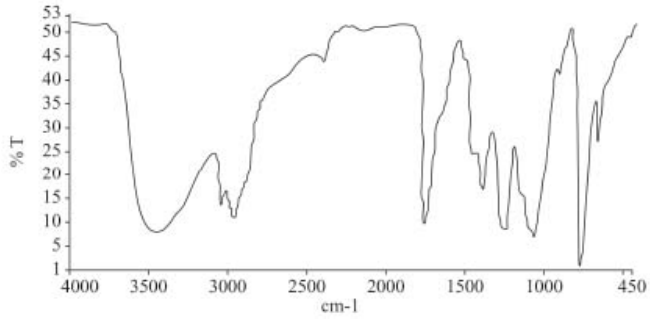

Fig. (3). IR spectrum of the extracted saponin of *C. Borivilianum.*

Purified saponin is a colorless powder; ESI-MS (pos) m/z- containing peaks at 925[$M^{+}H$], 927[$M^{+}2H$] and 958[$M^{+}Na$] suggest the molecular weight of the purified saponin to be around 924 (Fig. **4**).

^{1}H NMR spectrum of the isolated compound revealed one strong solvent (D_2O) peak at 2.132ppm. NMR spectrum

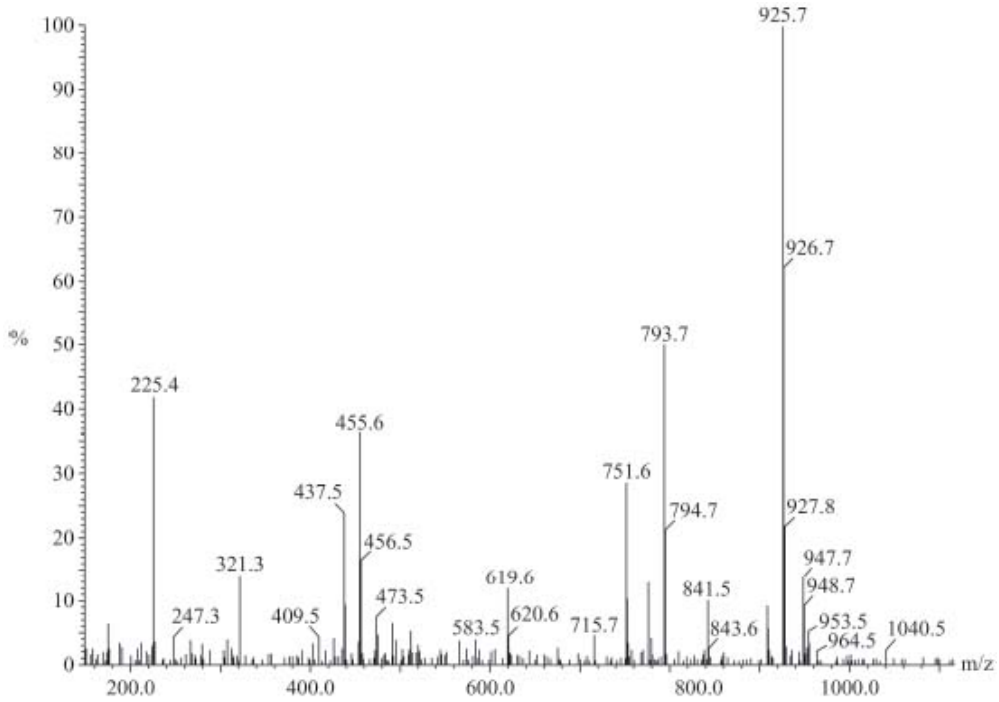

Fig. (4). LC-ESI MS spectrum of the extracted saponin of *C. Borivilianum.*

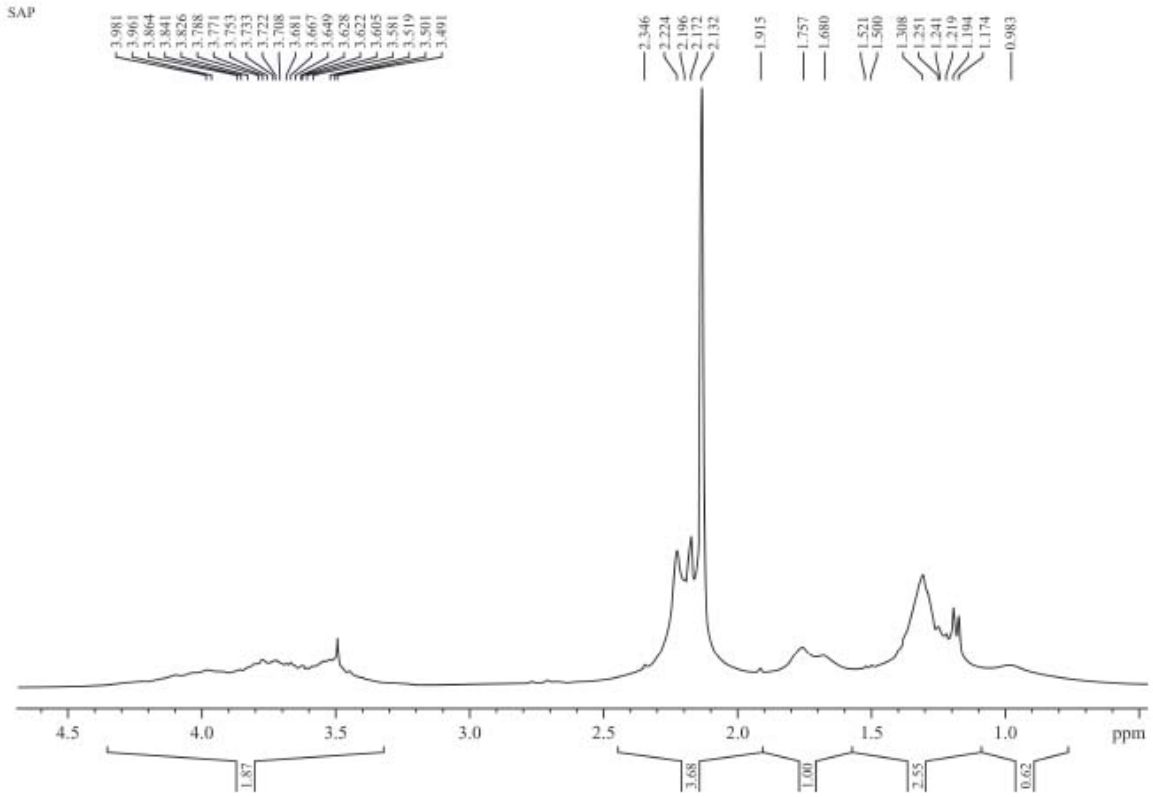

Fig (5). [1]H NMR spectrum of purified saponin of *C. borivilianum*.

of this compound exhibited a peak near around δ 3.65 ppm due to the presence of –OH group and methoxy group, and this result was in agreement with the IR spectral data of this isolated compound (Fig. **5**).

Based on pharmacophore modeling, the following structure of saponin has been proposed, as shown in Fig. (**6**).

The base structure of saponin was found to have the maximum number of plants, such as oleanane, a pentacyclic triterpenoid compound, which exists as a free and frequently as glycoside ester or glycoside bond. The presence of peaks at 454 and 455 nm in the mass spectrum suggests the presence of an oleanolic pharmacophore compound in the given structure (Fig. **6**).

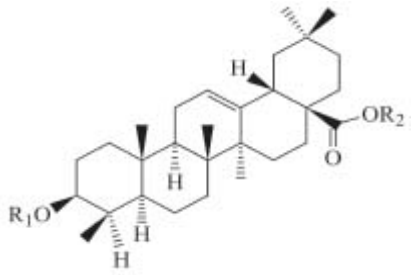

Fig. (6). Structure of purified saponin of *C. borivilianum*.

4. DISCUSSION

The medicinal properties of the roots of *C. Borivilianum* are known for a long time for various therapeutic applications in human society [29]. The comparative examination of roots, leaf and stem extracts has been conducted in this study to investigate the antimicrobial activities and phytochemical compounds found in the plant. The organic metabolites which are present in the various plant parts tend to get dissolved in suitable organic solvents for the extraction of secondary metabolites [30]. Bioactive metabolites from *C. borivilianum* are extracted from methanol, suggesting that it is polar in nature. The plants have various phytochemical compounds or secondary active metabolites that protect the plant from microbial damage [31]. Phytochemical screening confirmed the presence of alkaloids, flavonoids, steroids, carbohydrates, saponins, catechol tannins, phenols and tannins in the extract of *C. borivilianum.* Chakraborthy and Aeri also confirmed the presence of saponin as phytochemical in the root extract of *C. borivilianum* [32]. Sharma *et al.* reported the presence of saponin in the root extract of *C. borivilianum* and also proved it to possess antibacterial activity [28].

Our studies have suggested that the crude methanolic extract of the stem has a higher amount of bioactive secondary metabolites and can act as a potential source of medicinal importance. In the present study, an attempt has been made to purify the saponin content from the whole plant and propose a basic structure of saponin based on spectrophotometric, ESI-MS, NMR and pharmacophore modeling studies. The saponin structure contains five hexagonal rings attached with two side chains of glycosidic linkages. Researchers have identified similar types of structures from different medicinal plant species [33]. Szakiel *et al.* described the structure of oleanolic acid extracted from the saponin of *Calendula officinalis L* [34]. Khathi *et al.* described the structure of oleanolic acid on the basis of ^{1}H and ^{13}C NMR studies and proposed the potential supplements of hyperglycemia. A similar spectrum of ^{1}H NMR of the isolated compound has shown one strong peak at 2.132 ppm and a peak near around δ 3.65 ppm due to the presence of –OH group and methoxy group as compared with the IR spectral data of this isolated compound [35]. The antimicrobial screening of the purified saponin suggests that it has a higher antibacterial, antifungal and anti-tubercular activity as compared to crude methanolic extracts of root, stem and leaf. Sundaram *et al.* also confirmed the antibacterial activity of C. Borivilianum [36].

The results of our antimicrobial screening suggest that the bioactive compound of the *C. borivilianum* is more active towards the Gram-positive bacteria as compared to Gram-negative bacteria. Our findings are also confirmed with the reports of Sundaram *et al.* and Chakraborthy and Aeri, where the antibacterial activity of the root extract of *C. borivilianum* with respect to Gram-positive bacteria has been described [32]. The reason for a higher resistance in Gram-negative bacteria against the extracts is due to the hydrophobic nature of lipopolysaccharides present in the cell wall, which is impermeable to lipophilic compounds as compared to Gram-positive bacteria [37, 38]. The MIC results suggest that the phytochemicals of *C. borivilianum* have the highest activity against *M. tuberculosis*. To the best of our knowledge, this is the first report on the activity against *M. tuberculosis*.

It has been proven in our analysis that the crude extracts of roots, leaf, stems have antioxidant properties in addition to antimicrobial activities. Ashraf *et al.* analyzed the antioxidant properties of crude extracts and total saponin fraction of *C. borivilianum* and found that the crude extract had higher free radical scavenging and bleaching activity, whereas saponin fraction had higher ferrous ion chelating activity [39]. Similarly, Gülçin *et al.* observed that the saponin derivatives were derived from *Hedera helix L.*, which can be used as a natural antioxidant source [40].

The phytochemical screening revealed that both the crude and pure extracts of saponin have antioxidant activity and this antioxidant property may be due to phenolic contents present in the extract. Visavadiya *et al.* [41] have reported a strong association between antioxidant activity and polyphenol and flavonoid contents of the root extract of *C. borivilianum*.

CONCLUSION

In this proposed work, it is proved that the antimicrobial properties of stems and leaves are better than that of the root extract of *C. borivilianum*. Moreover, the MIC analysis reveals that the purified saponin, one of the active components, possesses biomedicinal potential and antimicrobial activity. By using spectral analysis, oleanolic acid has been found to be the core structure of the purified saponin of *C. borivilianum.*

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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