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Comparison of different solvents for Antioxidant and Antibiogram Pattern of *Bergenia ciliata* rhizome Extract from Shimla district of Himachal Pradesh

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Abstract

Bergenia ciliata is a well-known herb commonly known as Paashaanbhed, with various pharmaceutical properties. The scientific exploration of *Bergenia ciliata* is growing in the Western Himalayas for its phytochemical and pharmacological properties. The present study aimed to evaluate the *Bergenia ciliata* rhizome extracts for total phenol content, total flavonoid content, antioxidant and antimicrobial activities. Four different solvents viz., aqueous, chloroform, methanol and ethanol were used for extraction. Among the solvents tested, methanol was found to be the best extractive solvent with the highest total phenolic content of 31.46 mg GAE g^{-1} DE and the lowest IC_{50} values for DPPH and ABTS assays, i.e 80.20 and 73.38 µg mL^{-1}, respectively. Methanol and ethanol extracts exhibited effective antimicrobial activity against *E. coli, S. typhi,* and *S. aureus* (MIC = 6.25 µg mL^{-1}). The current study suggested that the methanol extract of *Bergenia ciliata* has strong antioxidant and antimicrobial potential, which can be used in functional foods and pharmaceutical industries.

Keywords: *Bergenia ciliata*, Antioxidants, Phytochemicals, Antimicrobial

1. Introduction

Nowadays, chief concerns of food and pharmaceutical industries are microbial contamination and side effects of synthetic antioxidants. Increasing tendency for replacing synthetic antioxidant by natural one and growth of microbial resistance to existing antibiotics from the other has stimulated researchers toward considering medicinal plants for dual antioxidant and antimicrobial properties (Savoia, 2012 and Pandey *et al*., 2015). For thousands of years, plants have been used as the source of medicines by mankind. Plant derived natural products or their derivatives play an essential role in both innovation and promotion of new drugs (Mercy *et al*., 2018 and Hazra *et al*., 2010). Exploration of new antimicrobials of plant origin, as reliable source of antibiotics, is receiving attention of the scientific community. According to WHO, about 80 % of the world's population depends on traditional medication from plants. Approximately 25 % of drugs contain phytonutrients extracted from plants (Ebong, 2015) as they are factories of natural phytochemicals (Johnson *et al*., 2015). These plants synthesize bioactive chemicals (phytochemicals) as secondary metabolites (Zulfiqar *et al*., 2017) that are used directly for antibacterial, fungicidal and herbicidal purposes (Agu *et al*., 2017). Many phytochemicals possess antioxidant activity and decrease the risk of diseases (Asgharian *et al*., 2017).

Bergenia ciliata is a perennial herb which belongs to Saxifragaceae family and consists of 30 genera and 580 species (Ruby e*t al*., 2012 and Pokhrel *et al*., 2014). It grows widely in cold and temperate regions of the Himalayas (Islam *et al*., 2002). The plant species flourishes well in rocky areas and on the cliffs. Three species of *Bergenia, i.e. Bergenia ligulata, Bergenia ciliata* and *Bergenia stracheyi* are found in Indian Himalayan region. These are commonly known in Indian system of medicine as *Pashanbheda* (Asolkar *et al*., 1992). The leaves, root, rhizomes and other parts of *Bergenia ciliata* are used as a traditional medicine in Asian countries (Bhattarai, 1994 and Bagul *et al*., 2003) Juice of the rhizome is taken for urinary trouble and hemorrhoids (Manandhar, 1995). *Bergenia ciliata* rhizome is widely used in folk medicines anti-bacterial, antipyretic, analgesic, antioxidant, hypoglycemic, antiviral, anti-inflammatory and antimalarial (Sinha *et al*., 2001 and Walter *et al*., 2013) antidiabetic and anti-tussive properties (Hsieh *et al*., 2001 and Chauhan *et al*., 2012). It is also reported to be helpful for kidney and gall bladder stone, cough, fever, diarrhea and lungs diseases (Farooquee *et al*., 2004; Rai *et al*., 2000 and Pradhan *et al*., 2008). The virtues of plant are attributed to its secondary metabolites such as bergenin, β-sitosterol, arbutin, phytol, gallic acid and catechin, which are therapeutic and account for its use in traditional medicine (Dharmender *et al*., 2010 and Sajad *et al*., 2010).

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The aim of present study was to determine the total phenolic, flavonoid content and their antioxidant and antibacterial activities of different solvent extracts from rhizome of *Bergenia ciliata*.

2. Materials and Methods

2.1. Collection of Plant Material

Rhizomes of *Bergenia ciliata* were collected from the Narkanda region (2700 m amsl) of Shimla district, Himachal Pradesh, India during spring season from April to June, 2018. The plant was identified at CSIR-Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu and the verified sample was submitted in Herbarium at the School of Biological and Environmental Sciences, Shoolini University, Solan, India.

2.2. Morphological Evaluation

In the morphological evaluation various organoleptic characters such as color, odor, shape, fracture, texture, taste and size were determined by Kokate (Kokate, 2005).

2.3. Preparation of Extract

Firstly, the collected rhizomes of *Bergenia ciliata* were washed with water to remove soil and other foreign particles and then dried under shaded place. The dried material was then grinded into a coarse powder using grinder. For the extract preparation, 10 g of powder was soaked separately in 100 mL of different solvents-aqueous, chloroform, methanol and ethanol for 48 h with constant shaking. Extracts were then filtered using Whattman filter paper (No.1). Extracts were dried by evaporation using vacuum evaporator at 40 °C. The filtrate was stored and used for further experiments (Turner, 2006).

2.4. Extraction Yield

The percentage yield of the extraction was calculated using formula: {(V1/V2)/100}.

2.5. Qualitative Phytochemical Analysis

Phytochemical tests were done to identify the presence of chemical constituents in the rhizome extracts of *Berginia ciliata* using standard protocol (Harborne, 2012 and Das *et al*., 2010). These include tests for alkaloids, phenols, carbohydrates, flavonoids, phenols, saponins and proteins.

2.6. Total Phenolic Content (TPC)

TPC was evaluated by a Folin–Ciocalteu assay (Singleton, 1999). Each sample (1 mg/mL; 100 µL) was taken separately in test tubes. 1 mL of Folin–Ciocalteu reagent and 1 mL of sodium carbonate solution (20 %) were added. After 5 min, the mixture was incubated at 25 °C in a water bath for 60 min. Absorbance was determined at 760 nm. The phenolic content values were expressed as mg of gallic acid equivalent per gram of dry extract (mg GAE g DE).

2.7. Total Flavonoid Content (TFC)

TFC was evaluated by the aluminum chloride method (Zhishen *et al*., 1999). Each sample (1 mg/mL; 100 µL) was added to aluminum chloride solution (2 %, 0.5 mL), incubated for 15 min at 37 °C. Absorbance was determined at 510 nm. The total flavonoid content was shown as mg of rutin equivalent per gram of dry extract (mg RE g DE).

2.8. Antioxidant Activity

2.8.1. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging Assay

The effect of extracts on DPPH radical was determined as described (Barros *et al*., 2007). For stock solution, 20 mg DPPH was dissolved in methanol. Then, the DPPH solution was diluted with methanol to reach an absorbance of 0.68–0.76 at 517 nm. To prevent free radicals, the DPPH stock solution was coated with aluminium foil and kept in the dark for 24 h. The extract was placed in a cuvette and mixed with 2 mL of diluted DPPH solution and was kept for 30 min in the dark. Absorbance of the solution mixture was taken at 517 nm using spectrophotometrically.

DPPH scavenging effect (%) = $(A_b\text{-}As/A_b) \times 100$

where A_b= absorbance of blank

As = absorbance of extract/standard.

Ascorbic was used as standard substance. The free radical scavenging activity was expressed as IC_{50} value, which represented the inhibitory concentration of extract/standard required to scavenge 50 % of free radicals.

2.8.2. ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

ABTS radical scavenging assay was performed as described (Re *et al*., 1999). The ABTS radical cation solution was produced by reacting 7 mM ABTS stock solution and 2.45 mM potassium persulfate solution was prepared in 100 mL methanol. These two mixtures were mixed in equal quantities and kept in dark at room temperature for 14–16 h. Then, the ABTS solution was diluted with methanol to reach an absorbance of 0.68–0.76 at 745 nm. 2 mL of solution was mixed extract and incubated for 20 min. Percent inhibition of absorbance at 745 nm was calculated using the formula:

ABTS Scavenged effect (%) = $(A_b\text{-}As/A_b) \times 100$

2.9. Antimicrobial Activitiy:

Antimicrobial activity was tested against three bacterial cultures (Gram negative, viz. *Escherichia coli* (MTCC 82) *and Salmonella typhi* (MTCC734) and Gram positive, viz. *Staphylococcus aureus* (MTCC 96). These strains were obtained from Food Technology Laboratory of Shoolini University, Solan, Himachal Pradesh, India.

2.9.1. Agar Well Diffusion Assay

Agar well diffusion method is widely used for *in vitro* antimicrobial activity (Perez *et al*., 1990). The microorganisms were first inoculated in nutrient broth at 37 °C. The bacterial density reached 0.5 of the McFarland Standard was uniformly spread on the surface of the nutrient agar plates using sterile cotton swabs. The wells were punched aseptically with the cork borer (6 mm). 100 µL of each extract (100 mg/mL) was placed in the wells made in the nutrient agar plate. Then, the petri plates were incubated at 37 °C. Positive control 10 µL of ampicillin (100 mg/mL stock) was used in the experiment; dimethyl sulfoxide (DMSO) was used as a negative control. To determine the effectiveness of extract against each organism the tests were performed in triplicates and results were recorded as mean ± SD.

2.9.2. Minimum Inhibitory Concentration (MIC)

MIC of the different extracts was evaluated by broth dilution method (CLSI, 2012). From stock (100 mg/mL) 100 µL of extract was prepared to check its activity against different bacterial strains in a 96-welled micro titer plate. 10 µL of ampicillin was taken as a positive control; 10 µL of DMSO was taken as a negative control for all the bacterial strains. Plates were incubated under normal conditions at 37 °C for 16-20 h. After 24 h, 10 µL resazurin dye was added in each well, and plates were again incubated for 2 h. The viability of bacterial cells was indicated by color change from purple to pink or colorless (Virk *et al*., 2019).

2.10. Statistical Analysis

All the experiments were done in triplicates for each sample and the results expressed as mean ± SD. IC_{50} values were calculated by linear regression.

3. Results

3.1. Morphological Features

The rhizomes of *Bergenia ciliata* were 4-12.3 cm long, 1.2-2.0 cm in diameter, yellowish brown in color, palmate, barrel shaped, with rough surface, characteristic odour and bitter in taste **(Figure 1 and Table 1).**

Figure 1. *Bergenia ciliata* rhizome.

Table 1. Morphological characteristics of *Bergenia ciliata* rhizomes

Features	Observation
Color	Yellowish brown
Odour	Characteristic and slight
Shape	Barrel shaped
Fracture	Short & fibrous
Texture	Rough
Taste	Bitter
Size	4-12.3 cm long and 1.2-2.0 cm in diameter

3.2. Extraction yield

The yield of extraction from rhizomes of *Bergenia ciliata* was determined by using the solvents of different polarity and was found different for each of the solvent used **(Table 2 and Figure 2).** Among solvents tested, the highest yield was obtained by methanol extract (16.23%), followed by aqueous (13.77%), ethanol (9.54%) and chloroform extract (2.54%).

Table 2. The total yield of different extracts

Extract type	Yield (in %)
Aqueous	13.77
Chloroform	2.54
Methanol	16.23
Ethanol	9.54

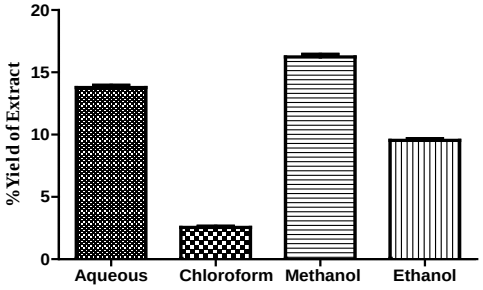

Figure 2. Effect of different solvents on extraction yield.

3.3. Bioactive compounds screening

The present results showed the presence of alkaloids, tannins, flavonoids, phenols, saponins, and carbohydrates **(Table 3).** Large amounts of both primary and secondary metabolites were observed in all the extracts. However, alkaloids were present only in methanol and ethanol extract, whereas proteins were observed only in the chloroform extract.

Table 3. Phytochemicals analysis in different extracts of *Bergenia ciliata* rhizomes

Phytochemical tests	Test Name	Aqueous	Chloroform	Methanol	Ethanol
Alkaloids	Mayer's test	-ve	-ve	+ve	+ve
Carbohydates	Benedict's test	+ve	+ve	+ve	+ve
Flavanoids	Lead acetate test	+ve	+ve	+ve	+ve
Phenol	Ferric chloride test	+ve	+ve	+ve	+ve
Saponins	Froth test	+ve	+ve	+ve	+ve
Proteins	Millon's test	-ve	+ve	-ve	-ve

+ve = present; -ve = not detected

3.4. Total phenolic and flavonoid content

Total phenolic content was calculated from the standard curve of gallic acid using the equation: y= 0.741x+0.1326, while total flavonoid content was calculated using the standard curve of quercetin using the equation: y=0.965x+0.0362 contents.

Total phenolic content obtained was in the range of 11.10 to 31.46 mg GAE g^{-1}. The highest phenolic content was achieved by methanol extract (31.46 mg GAE g^{-1} DE), followed by aqueous (24.66 mg GAE g^{-1} DE) and chloroform extract (12.86 mg GAE g^{-1} DE). The lowest phenolic content was obtained with ethanol extract (11.10 mg GAE g^{-1} DE) (**Figure 3 and Table 4**). Total flavonoid

content was in the range of 10.66 to 19.06 mg RE g^{-1}. The highest flavonoid content was found in aqueous extract (19.06 ± 0.61 mg RE g^{-1} DE), followed by methanol (18.46 mg RE g^{-1} DE), ethanol (14.97 mg RE g^{-1} DE) and chloroform extract (10.66 mg RE g^{-1} DE).

Table 4. Total phenolic and flavonoid content of different extracts of *Bergenia ciliata* rhizomes

Extract type	Total Phenolic content (mg GAE/g DE)	Total Flavonoid content (mg RE/g DE)
Aqueous	24.66 ± 0.69	19.06 ± 0.61
Chloroform	18.46 ± 0.16	10.66 ± 0.93
Methanol	31.46 ± 1.05	18.46 ± 0.16
Ethanol	11.10 ± 0.62	14.97 ± 1.49

Values represent mean ± SD of triplicates; TPC = total phenolic content; TFC = total flavonoid content; mg GAE g^{-1} DE: mg gallic acid equivalents per gram of dry extract of the sample; mg RE g^{-1} DE: mg rutin equivalents per gram of dry extract of the sample.

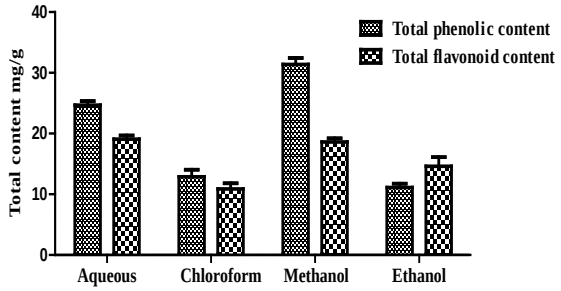

Figure 3. Phenolic and flavonoid content in different extracts of *Bergenia ciliata* rhizomes.

3.5. Antioxidant activity

All the extracts of *Bergenia ciliata* rhizomes exhibited good antioxidant properties, which varied with the type of solvents. The best activity for DPPH assay was observed in methanol extract (IC_{50} = 80.20 µg mL^{-1}), followed by aqueous (IC_{50} = 135.2 µg mL^{-1}) and chloroform extract (IC_{50} = 138.2 µg mL^{-1}), respectively **(Figure 4 and Table 5).**

Table 5. Antioxidant activity of different extracts of *Bergenia ciliata* rhizomes

Extract type	DPPH radical IC_{50} (µg mL^{-1})	ABTS radical IC_{50} (µg mL^{-1})
Aqueous	135.2	94.8
Chloroform	138.2	118.8
Methanol	80.20	73.38
Ethanol	184.6	141.4
Ascorbic acid	22.53	28.3

Each value represents the mean ± SD of triplicates. IC_{50} = half maximal inhibitory concentration; DPPH= 2,2-diphenyl-1-picrylhydrazyl; ABTS= (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))

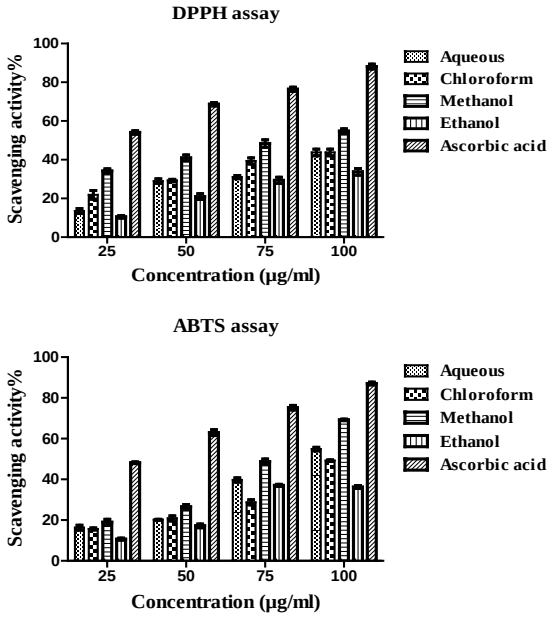

Figure 4. DPPH and ABTS assay of different extracts of *Bergenia ciliata* rhizomes.

In case of ABTS assay, the best activity was observed in methanol extract (IC_{50} = 73.38 µg mL^{-1}), followed by aqueous (IC_{50} = 94.81 µg mL^{-1}) and chloroform extract (IC_{50} = 118.8 µg mL^{-1}). The lowest activity was observed in ethanol extract with IC_{50} value of 184.6 and 141.4 mg mL^{-1} for DPPH and ABTS assay. Ascorbic acid was used as a standard, which exhibited an IC_{50} value of 22.53 and 28.30 µg mL^{-1} for DPPH and ABTS assay, respectively.

3.6. Antibacterial activity and MIC

All the extracts were active against bacterial strains and showed a range between 10.33 ± 0.47 to 17.0 ± 0.81 mm **(Table 6 and Table 7).** The methanol and ethanol extracts of rhizomes appeared to be the most active against all strains. The best activity was obtained by methanol extract of rhizome (IZD = 17 mm; MIC = 6.25 µg/mL^{-1}) against *E. coli* **(Figure 5).** The same extract was active against *S. aureus* and *S. typhi* with (Inhibition zone diameter (IZD) = 15.6 mm and 14.6 mm; MIC = 6.25 µg/mL^{-1}). The chloroform and aqueous extract exhibited the lowest effect against all the tested bacteria (IZD range of 10.3 ± 0.47 to 12.3 ± 0.94 mm, and MIC is 6.25–25 µg/mL^{-1}). However, this antibacterial activity was less than that of ampicillin.

Table 6. Antibacterial activity of different extract of *Bergenia ciliata* rhizomes

	Microorganism		
Extracts	*E. coli*	*S. aureus*	*S. typhi*
Aqueous	12.3 ± 0.94	10.3 ± 0.47	12.6 ± 0.47
Chloroform	11.3 ± 0.47	12.0 ± 0.81	11.6 ± 0.47
Methanol	17.0 ± 0.81	14.6 ± 0.94	15.6 ± 0.94
Ethanol	16.0 ± 0.81	16.3 ± 0.47	14.6 ± 0.47
Amp	27.6 ± 0.47	24.3 ± 0.81	24.3 ± 0.47
DMSO	ND	ND	ND

Zones of inhibition (mm) are presented as mean ± SD. *E. coli* = *Escherichia coli*, *S. typhi* = *Salmonella typhi*, *S. aureus* = *Staphylococcus aureus*; IZD: Inhibition zone diameter (mm); Amp =Ampicillin (positive control); DMSO = dimethyl sulfoxide (negative control); ND (not detected).

Table 7. Minimum Inhibitory Concentration against test organisms (µg/mL^{-1})

Extracts	*E. coli*	*S. aureus*	*S. typhi*
Aqueous	6.25	12.5	12.25
Chloroform	25	25	12.25
Methanol	6.25	6.25	6.25
Ethanol	6.25	6.25	6.25

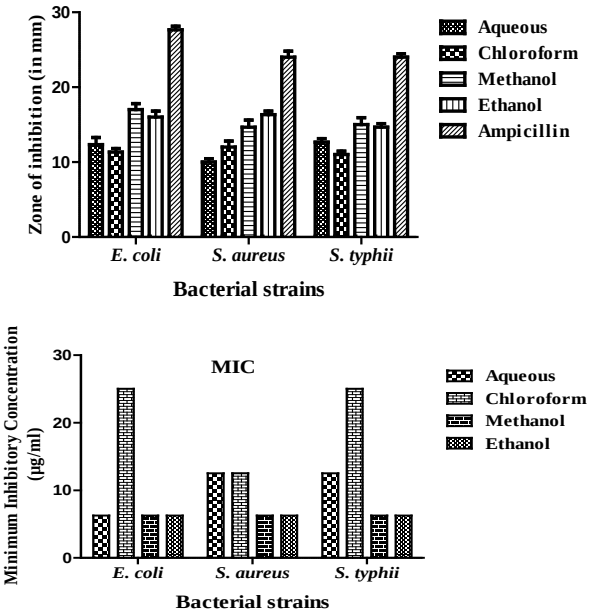

Figure 5. Antibacterial and MIC of different extracts of *B. ciliata* rhizomes.

4. Discussion

Natural products have been traditionally employed since prehistoric times for the maintenance of general health conditions and management of various ailments. They provide clues to investigate and isolate bioactive components in modern era. Results showed that methanol extract yield greater quantities of active compounds as compared to solvent extracts and also possessed good antioxidant and antimicrobial activities. The preliminary phytochemical screening tests might be helpful in the identification of the pharmacologically bioactive components in the plant material and subsequently lead to discovery and development of drugs (Bhandary *et al*., 2012). Results obtained in this study indicated the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins and proteins. The presence of these secondary metabolites in rhizomes extract of *Bergenia ciliata* is in agreement with the previous reports (Uddin *et al*., 2012 and Ahmad *et al*., 2018). These differences could be attributed to the exposure to adverse conditions; parasitic or microbial infection of the plant prior to collection as most phytochemicals are secondary metabolites that are produced in response to such activities (Vila *et al*., 2016). The presence of alkaloids, anthraquinones, flavonoids, and saponins in the leaf and root extracts of *Bergenia ciliata* was similar to other reports (Pokhrel *et al*., 2014). Water also meets the definition of green solvent which has a low environment and health hazard (Li *et al*., 2008 and Welton *et al*., 2015). The extraction yield may be affected by several parameters, including temperature, time, the type of solvent, solvent to sample ratio and the number of extraction cycles (Che *et al*., 2017 and Efthymiopoulos *et al*., 2018). This study showed that total phenolic content of the solvent extracts was in the given order methanol > aqueous > chloroform > ethanol. This is due to the polarity of extracting solvent and the solubility of chemical constituents in the solvent, (Singh *et al*., 2014) which may be due to the influence of dielectric constant, organic solvent structure and chemical properties of phytochemicals (Cheok *et al*., 2012 and Jayaprakasha *et al*., 2003). The total phenolic content reported in the rhizome extract of *Bergenia ciliata* was 442 mg GAE g^{-1} which was much higher than the values obtained for the rhizome extract of *Bergenia ciliata* in this study (Singh *et al*., 2013). These findings are in good agreement with the previous study, which reported methanol as an effective solvent for extraction of antioxidant and phenolic compounds. According to the present study, no difference in the total phenolic content was observed in the rhizome extract of *Bergenia ciliata* collected from Sikkim Himalaya, and the rhizome extract of *Bergenia ciliata* collected from Shimla region. Total flavonoid content was found to be higher in the aqueous extract and the lowest in the chloroform extract. These results are not in agreement with the previous work, which reported that flavonoid content in the extracts depends on the solvent polarity (Singh *et al*., 2017 and Hajji *et al*., 2009).

Antioxidants are important defense mechanisms in preventing the harmful effects of free radicals (Birben *et al*., 2012 and Kedare *et al*., 2011). The methanol extract had high antioxidant activity, when assessed with ABTS and DPPH assay. However, the antioxidant activities were lower as compared to the positive controls. The methanol and aqueous extract of *Bergenia ciliata* rhizome possess strong antioxidant activities as compared to the n-hexane fraction (Uddin *et al.*, 2012).

Another investigation also confirmed that both methanol and aqueous solvents were found to be more active radical scavengers (Rajkumar *et al*., 2010). Our results revealed that there is a significant correlation between phytochemical and antioxidant assays that could be attributed to the different mechanism of the radical antioxidant reaction. Li et al have also reported statistical correlation between the TPC and IC_{50} values which confirmed that phenolic content contributes to the free radical scavenging activity of the plant metabolites (Li *et al*., 2009).

The present study supported strong antimicrobial activity of *Bergenia ciliata* rhizome against important pathogenic bacteria. The methanol and ethanol extract showed highest antibacterial activity and MIC against *E. coli, S. typhi* and *S. aureus.* The earlier study revealed a wide range of antimicrobial activity of different concentrations (200-1000 µg/disc) in methanol extract of *Bergenia ciliata* (Sinha *et al*., 2001). Methanolic extract shows the high antimicrobial activity due to the presence of high phenols and flavonoids content in the plant extract. These results are similar with the earliest study where similar resulted were reported in case of *B. ligulata* leaf extracts (Agnihotri *et al*., 2014).

5. Conclusion

The present study concluded that the methanolic extract of *Bergenia ciliata* rhizome contains the highest Total phenolic content and also exhibited high scavenging activity against DPPH and ABTS radicals. Methanol extract also exhibited strong antimicrobial activity against bacteria. *Bergenia ciliata* is a good source for the isolation of antioxidant and antibacterial compounds that can be a breakthrough for pharmaceutical industry.

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