Chemical composition, antioxidant and antibacterial activities of Drymaria cordata (Linn.) Willd. against chloramphenicol-resistant Bacillus subtilis and β-lactams-resistant Pseudomonas aeruginosa

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Article

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Abstract

The current study was done to evaluate the chemical composition, antioxidant and antibacterial activities of *Drymaria cordata* extract in three solvents i.e. methanol (DCM), hexane (DCH) and water (DCW) against chloramphenicol-resistant *Bacillus subtilis* and β-lactams-resistant *Pseudomonas aeruginosa*. Phytochemical screening and Fourier transform-infrared spectroscopy (FTIR) showed the presence of phenols, amines, hydroxy group, and amino-related components. Gas chromatography-mass spectrometry (GCMS) depicts the presence of antibacterial compounds such as hexanedioic acid, hexadecanoic acid, nonadecadiene, hexadecen-1-ol, octadecadienoic acid and neophytadiene. DPPH free radicals scavenging assay exhibit maximum percentage inhibition in DCM (71.57 %) at the concentration of 1000µg/ml. The total antioxidant through phosphomolybdate assay was observed in DCM with values 834.44 ± 4.01 mg AAE/g of extract. For antibacterial activities, DCM shows best zone of inhibition (ZOI) (8 mm at 100 µg/ml to 15.17 mm at 1000 µg/ml) and minimum inhibitory concentration (MIC) (75 µg/ml) as compared to other extracts against *Bacillus subtilis*. Also, DCM shows zone of inhibition (ZOI) (6.83 mm to 13.17 mm) and minimum inhibitory concentration (MIC) (70 µg/ml) as compared to other extracts against *Pseudomonas aeruginosa*. These results indicate that the *Drymaria cordata* methanol extract possesses good antibacterial and antioxidant properties which justify its use against pathogenic bacteria.

Introduction

Bacteria are ubiquitous and have a large impact on human health. Gram-positive and negative bacteria cause fatal diseases such as brucellosis, salmonellosis, anthrax, botulism, tetanus, cholera, gonorrhea, tuberculosis, pneumonia, whooping cough, syphilis, typhus, shigella, yersinia, vaginosis, vibrio and sepsis. The antibiotic resistance against gram-positive and gram-negative bacteria is dependent on the overuse and misuse of antibiotics. The recent COVID 19 pandemic increased our interest in the search for antimicrobial compounds from medicinal plants. We have observed that antimicrobial and immunity booster compounds are quite popular during the global crisis of COVID 19. Globally, it has been observed that over 70% of patients on COVID 19 treatment are receiving antimicrobials. Only 10% of patients suffered from antibacterial infections which further contributed to the antibiotic resistance crisis [1]. New chloramphenicol resistance locus was found in *Bacillus subtilis* [2]. *Pseudomonas aeruginosa* displays resistance to a variety of antibiotics, including aminoglycosides, quinolones and β-lactams [3]. So we need alternative treatment strategies to deal with the growing threat of drug-resistant bacteria. Bacterial infectious diseases are more in number as compared to the viruses and parasites infection [4]. Medicinal plants contain high levels of antibacterial and antioxidant compounds. Antioxidant compounds such as polyphenols can delay or inhibit the production of free radicals. Antioxidants are known as free radical scavengers. Free radicals are generally produced during respiration in the cell. In small amounts, free radicals have a beneficial role to play. But they become harmful if produced in excessive amounts in response to external stresses such as x- rays, gamma rays, UV irradiation, pollutants and any other pathological condition that leads to oxidative stress. Oxidative stress leads to the pathogenesis of both micro and macrovascular diseases such as cancer, asthma, diabetes, cardiovascular disease, stroke, infertility, neurodegenerative diseases, and urolithiasis [5]. Under stress, human bodies produce more oxygen and nitrogen species (ROS and RNS) such as superoxide anion radicals, hydrogen peroxide and hydroxyl radicals than enzymatic antioxidants (superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx)) and non-enzymatic antioxidants (tocopherol (vitamin E), ascorbic acid (vitamin C), carotenoids, glutathione, and flavonoids). DPPH free radical scavenging assay is one of the popular and oldest antioxidant evaluation methods which is described by Marsden Blois using cysteine as a model for antioxidant activity [6]. *Drymaria cordata* (Linn.) Willd belongs to family Caryophyllaceae. It is an herbaceous plant widely utilized in In tropical Africa, *Drymaria cordata* preparations are used for the treatment of diverse ailments including cold, coryza, bronchitis, headaches, aching, inflamed, tumours, painful parts, leprosy, as a fumigant for eye troubles, cerebral stimulant and antifebrile agent. *Drymaria cordata* have been reported to have anti-inflammatory, antitussive, and anxiolytic activities [7]. In Nigerian folk medicine, *Drymaria cordata* is used to treat children sleeping disorders, convulsions, and febrile conditions [8]. This study aim is to investigate the antibacterial, antioxidant and chemical composition of the whole plant extract of *Drymaria cordata* using the different pharmacological assays. To the best of our knowledge, the biochemical composition, antioxidant and antibacterial potential of alcoholic and hexane extracts of *Drymaria cordata* has not yet been analysed. It is very important to study crude drugs to identify possible alternatives to overcome resistance, but the combined studies also provide valuable information for use in the clinical setting.

Material And Methods

Chemicals

All chemicals (Methanol, Ethanol, Luria-Bertani (LB) agar, Gentamicin, DPPH, sodium phosphate, sulfuric acid, ammonium molybdate, ascorbic acid, NaCl) were of good analytical grade.

Collection and extraction

A wild-growing fresh *Drymaria cordata* herb was manually collected from Machi Tehsil (24.530417, 94.213295) located in Chandel District of Manipur State, India. We followed the guideline on good field collection practices for Indian medicinal plants which are issued by National Medicinal Plant Board (NMPB), Department of AYUSH, Ministry of Health and Family Welfare, Government of India. Various plant bioactivities were evaluated according to the biosafety procedures, rules and guidelines. The whole plants were air-dried in a shaded place (25 ± 2 °C) for 8-10 days. The dried plants were powdered using an electric blender/mixer. The plant powder was stored in glass bottles to preserve it from light, dust, and moisture. Then samples were subjected to a selective extraction using three solvents i.e. methanol, hexane and water in a specific ratio (1:6) through the soxhlet extractor respectively. A rotary vacuum evaporator was used to obtain viscous semisolid masses. The crude extract was stored in dark containers at 4 °C.

Plant extraction yield

The Percentage extraction yield of the whole plant extracts was calculated using the formula

Extraction Yield (%) = (W1×100)/ W2

Where W1= weight (g) of the dry extract after extraction

W2= weight (g) of the dry plant powder before extraction.

Secondary metabolite screening

Different extract i.e. methanol, hexane and aqueous were investigated to verify the presence and absence of phytochemicals such as alkaloids, cardio-glucosides, Cardiac glycosides, flavonoids, phenolic compounds, Saponin, Tannin, Terpenoids, Anthroquinone and Phlobatannin [9 10 11 12 13].

Bacterial culture

Two bacterial cultures i.e. chloramphenicol-resistant *Bacillus subtillis*-MTCC-441 (gram-positive) and β-lactams-resistant *Pseudomonas aeruginosa*-MTCC-1688 gram-negative bacteria were procured from Microbial Type Culture Collection (MTCC), Chandigarh-Punjab-India.

Chemical composition

The GC–MS and FTIR analysis

GC-MS analysis of bioactive compounds was performed using Shimadzu QP2010 Plus with TD 20 thermal desorption system equipped with omegawax 100 columns. GC-MS involves an electron ionization system with high energy electrons (70 eV) and the constant flow rate of Helium was 1.21 mL/ min. The column temperature started from 50 °C to 280 °C with a gradual rise of 10 °C/min. The relative amount of chemical compounds present in the extracts was expressed at percent based on the area of the peak produced in the chromatogram. The National Institute of Standard and Technology (NIST) library and the WILEY 8 library were used for the identification of compounds. Fourier transform infrared spectrophotometer (FTIR) (PerkinElmer FTIR Spectrometer) was used for the analysis of dried plant samples. The frequency range for the spectrum analysis was from 4000 to 400 cm^{-1}.

Antioxidant activities

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays were performed according to the method reported by Brand-Williams (1995) [14] with some modifications. DPPH radical Stock was prepared by mixing DPPH (2.4 mg) and ethanol (10 ml) and stored in a refrigerator. The working solution of DPPH radical was prepared by diluting the stock with ethanol. It was diluted until an absorbance value of 0.98 ±0.02 at 517 nm was obtained. The 100 µl of plant extract having different concentrations were mixed with DPPH (3 ml) working solution and kept in dark for 30 min at room temperature. The absorbance of the reaction mixture was taken at 517 nm. Ascorbic acid was used as a standard antioxidant. For the blank, an equal volume of methanol is used in place of the plant sample. The inhibition percent was calculated with the following formula:

Percent inhibition = $[(A_{control}-A_{sample}) / A_{control})] \times 100$,

Where $A_{control}$ is the absorbance of the DPPH solution and A_{sample} is the absorbance of the solution DPPH with standards/ extracts.

Phosphomolybdate assay

The total antioxidant capacity of the extracts was determined by the phosphomolybdate assay as described by Prieto et al. (1999),[15] and [16] with some modifications. The plant extract of various concentrations (300 µl) was added to the 3 ml of phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Test Tubes containing the mixture were wrapped in aluminum foil and incubated at 95 °C for 90 minutes. The reaction mixture was allowed to cool to room temperature and its absorbance was recorded at 765 nm. The ascorbic acid was used as standard. For the blank, an equal volume of methanol is used in place of the plant sample. The total antioxidant capacity (TAC) was determined in mg of ascorbic acid equivalent (AAE) per g of extract.

Antibacterial Activities

The antibacterial activity of the extract was investigated with the help of using Kirby-Bauer single-disc susceptibility assay. Two bacterial strains *Pseudomonas aeruginosa* and *Bacillus subtillis* have been used for antibacterial activity of plant extract. The pathogenic bacterial strains were grown in the Luria-Bertani (LB) broth subculture medium at constant shaking at 110 rpm (37 °C) for 12 hours. Then inoculum was accrued with the aid of using centrifugation at 10,000 rpm for 10 minutes. The bacterial concentration was diluted (10^5 CFU mL^{-1}) for future use. Gentamycin and extract solvent were used as a positive and negative control. From the bacterial suspension of *Pseudomonas aeruginosa* and *Bacillus subtilis*, 20 µL inoculum was pipette out and dropped on petri plates. We unfold the cultures over the petri plate using a sterile glass spreader. For disc diffusion assay, the sterile clean clear paper disc with a diameter of 5 mm was used. Sterilized discs were dipped in different concentrations of extracts (25 to 1000µg/ml) and placed in the

center of the agar plate. The petriplates were then sealed with parafilm and incubated at 37 °C for 10-16h [17]. Minimum Inhibitory Concentration (MIC) and Zone of Inhibition (ZOI) of plant samples in different solvents were recorded. The plant samples were compared with standard gentamicin (GM).

Statistical analysis

Statistical analysis was performed using MS Excel 2019, Origin, and Statistical Package for the social sciences (SPSS) software. One-way ANOVA (LSD and Duncan multiple comparison tests) was applied to designate the significance level (p = 0.05). All the experiments were performed in triplicates (n = 3) and their values were expressed as mean ± SEM (standard error of means).

Results And Discussion

Plant extraction yield

The extraction yield was found in different solvent i.e. methanol (4.12 g), hexane (3.97g) and aqueous (3.51g) solvent (fig.1a). The percentage extraction yield (fig.1b) in different solvents (i.e. methanol, hexane and water) was found respectively i.e. methanol (16.48%), hexane (15.88 %) and aqueous (14.04 %).

Secondary metabolite screening

Various phytochemical analyses of plant extract in three solvents was performed for the detection of alkaloids, phenolic compounds, saponin, cardiac glycosides, flavanoids, tannin, terpenoids, anthroquinone phlobatannin and the results summarized in Table 1.

Bioactive compounds present in the extracts

Fourier Transform- Infrared spectroscopy (FTIR)

The results of the FTIR plot, peak values and functional groups are shown in Figure 2 and Table 2

The FT-IR spectrum of *Drymaria cordata* extract showed the peak at 3640.71 cm^{-1} which indicates the presence of O-H bending that confirms the presence of alcohol. The sharp peak at 3352.82 cm^{-1} was indicative of aliphatic secondary amine and these peaks confirmed the presence of N-H stretch. The presence of methylene was supported by a strong peak at 2921.11 cm^{-1} and 2862.06 cm^{-1}. N-H stretch was observed at 2347.14 and confirms the presence of amino-related components. The spectrum showed the peak at 2159.02 cm^{-1} which indicated the presence of Thiocyanate (-SCN) and C-N Stretch. The presence of a peak at 1736.89 cm-1 indicated the aromatic ring group. The peak at 1600.83 showed the presence of C= C Stretch and alkene. N-O Stretch indicates the presence of nitrate ions at 1365.02 peaks. The peaks obtained at 1199.84 cm^{-1} and 1048.22 cm^{-1} indicate the presence of C= C Stretch and aromatic group. At the 662.79 cm^{-1} showed the presence of C-O stretch and confirms the presence of thioether group.

GC-MS

GC-MS was used to analyze the bioactive compounds present in the crude extract. GC-MS chromatogram of plant extract depicts the presence of total of 37 bioactive compounds (Table 3). The retention time, molecular weight (MW), molecular formula (MF), the area percentage of bioactive compounds and their reported biological activities are provided in Table 2. Among the bioactive compounds, the most dominated compounds were hexanedioic acid (22.79), hexadecanoic acid (12%), nonadecadiene (10.45), hexadecen-1-ol (8.18), octadecadienoic acid (7.11%) and neophytadiene (6%). GCMS analysis revealed the presence of various compounds i.e. neophytadiene (6%) which is known for its various activities such as antibacterial, headache, rheumatism, skin disease and Diabetes [1819]; hexadecanoic acid (12%) used as antidiabetic, anti-inflammatory, hypocholesterolemic , and larvicidal [20 21]; octadecadienoic acid (7.11%) revealed its therapeutic values as anticancer and antidiabetic [2223]; nonadecadiene (10.45 %) is depicted as antimicrobial and immunity booster agent [24]; hexadecen-1-ol (8.18 %) is known for antidiabetic, antimicrobial and antioxidant activity [2526] and hexanedioic acid (22.79 %) revealed as potent antibacterial compound [27]. The therapeutic potential of the bioactive compounds supports the medicinal values of this plant in the traditional medicinal system. The pharmacological actions of medicinal plants are dependent on their different bioactive compounds. These various bioactive compounds are useful in diverse health complications and act as potent biochemical agents for the effective treatment against pathogens, oxidants and diabetes without any side effects. Extracts from the *Drymaria cordata* are rich sources of phytochemical compounds that could be used in the synthesis and development of the future drugs.

Antioxidant activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assays and Phosphomolybdate assay were performed to reveal antioxidant activity of plant extract. Both these methods are frequently used for antioxidant evaluation of plant extract and plant-based food products.

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assays

The antioxidant activity was analyzed by DPPH radical scavenging capacity of the extracts presented as percentage inhibition and IC50 values. Ascorbic acid was used as a standard for comparison with the extract. In the present study, all samples showed good trapping activity in a dose-dependent manner (25-1000 µg/ml). Plant extracts in three solvents i.e. methanol (DCM), hexane (DCH), and water (DCW) were compared with ascorbic acid. It was revealed that percentage inhibition is dependent on the concentration of extract. The scavenging activity is directly related to the increasing concentration

of extract. At 1000 µg/ml methanol extract (DCM) showed the maximum radical scavenging activity i.e. 71.57% in comparison to the other two exact i.e. hexane (DCH) (66.18 %) and water (DCW) (62.11 %) (Fig. 3). But ascorbic acid showed a maximum percentage inhibition (92.76 %) in comparison to all extract in different solvent. Comparative analysis was based on IC50 value for ascorbic acid (59.27 µg/ml), methanol (345.31 µg/ml), hexane (469.19 µg/ml) and water extract (529.17 µg/ml) (Fig. 4). The low IC_{50} value of the plant extract reflects the high radical scavenging activity. The IC_{50} value of the methanol extract was higher than hexane and water extract.

Phosphomolybdate assay

Phosphomolybdate assay was used to evaluate the total antioxidant potential of plant extracts and standard ascorbic acid. The standard calibration curve was obtained using ascorbic acid in terms of the ascorbic acid equivalent (mg AAE/g) (Fig. 5). Total antioxidant capacity was dependent on doses of drugs i.e. as we increase the concentration of plant extract (25 µg/ml-1000 µg/ml); a similar increment in scavenging capacity was observed. Methanol (DCM) extract showed the maximum radical scavenging activity i.e. (834.44 ± 4.01 mg AAE/g) in comparison to hexane (786.11± 2.4 mg AAE/g) and water extract (768.33 ± 3.47 mg AAE/g) at 1000 µg/ml (Fig. 6) .

It is a well-known fact that plant bioactive compounds constitute one of the major groups of plant-extracts acting as primary antioxidants against reactive free radicals. Phenols and flavonoids are one of the most widespread and diverse kinds of natural antioxidants that possess various radical scavenging and antioxidant activities. Phytochemical screening revealed that the extract of *Drymaria cordata* contains alkaloids, phenolic compounds, saponin, tannin and terpenoids. GCMS results of *Drymaria cordata* (Table 2) also revealed many bioactive compounds which are antioxidants in nature. It was concluded that the presence of these secondary metabolites confirms the presence of antioxidant potential of *Drymaria cordata* extract. DPPH and phosphomolybdate assay further confirm the radical scavenging capacity of extract. The reducing capacity is associated with the presence of antioxidants (reductones) components which initiate the effect of breaking the free radical chain mechanism. The free radical scavenging capacities were almost systematically higher in the methanolic extract than in hexane. This study confirms that the polar extract of *Drymaria cordata* has better antioxidant activities than the non-polar extract. It may be possible due to their higher polarity and better solubility of phenolic compounds in polar rather than non-polar [62].

Antibacterial activity

Disc diffusion assay was used to evaluate the antibacterial activity of DCM, DCH, DCW and gentamycin (positive control) against *Bacillus subtilis* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) bacterial strains. Bacterial inhibition zone diameter in millimeters was measured in the triplicates with different concentrations of extracts, gentamycin (25-1000 (µg/ml) and methanol (vol. Conc.) as shown in fig.7 (a) and (b).

Both zones of inhibition (ZOI) and minimum inhibitory concentration (MIC) was determined in triplicates. The gentamycin showed the highest ZOI in both the bacteria in all the concentrations which was followed by DCM, DCH, DCW and methanol. The mean of the zone of inhibition (ZOI) for each experiment was analyzed by using Origin Pro and are presented as mean ± SEM of three replicates. SPSS software was used for the One-way analysis of variance (ANOVA) applying Duncan's tests was performed to depict the significant ($p \leq 0.05$) differences between different concentrations. At concentrations i.e. 25 and 50(µg/ml) did not reveal any anti-bacterial activity, but gentamycin shows antibacterial activity against both bacteria at similar concentrations. In the case of *Bacillus subtillis*, the DCM showed an increasing tendency in ZOI i.e. 8 mm at 100 µg/ml to 15.17 mm at 1000 µg/ml. DCH and DCW also showed increasing trends in ZOI i.e. 5.67 mm at 100 µg/ml to 9.17 mm at 1000 µg/ml for DCH and 7.83mm at 250 µg/ml to 11.5 mm at 1000 µg/ml for DCW as shown in Fig. 8. But the gentamycin, as a standard drug shows its better ZOI (7.5 mm at 25 µg/ml to 22.67 mm at 1000 µg/ml) than extracts in all concentrations. In the case of *Pseudomonas aeruginosa*, when the DCM concentration was increased from 100 µg/ml to 1000 µg/ml, an increasing trend in ZOI was observed i.e. 6.83 mm to13.17 mm. Similarly, DCH and DCW also showed increasing trends in ZOI i.e. 6.83 mm at 250 µg/ml to 17.33 mm at 1000 µg/ml for DCH and 7.33mm at 250 µg/ml to 11.33 mm for DCW as shown in (Fig. 10). But the gentamycin, as a standard drug showed its better ZOI (7.83 mm at 25 µg/ml to 20.33 mm at 1000 µg/ml) than extracts in all concentrations. The DCM, DCH, DCW and gentamicin showed different antibacterial actions against the tested strains with the MIC values 75 µg/ml ,145 µg/ml,125 µg/ml and 33.33 µg/ml for *Bacillus subtillis* while as 70 µg/ml, 100 µg/ml, 120 µg/ml and 20 µg/ml for the *Pseudomonas aeruginosa*. This shows that the DCM has better antibacterial potency than the all extract as shown in Fig.9,11.

The results indicate that the plant extract can be used against bacterial strains resistant to antibiotics. Based on our experimental findings, we suggest that *Drymaria cordata* might be used as an effective crude drug in the management of antibiotic resistance of bacteria. The cell wall of bacteria plays an important role in maintaining the cell shape. Components of the bacterial cell wall produce different adsorption pathways for extract. Phytochemical screening revealed the existence of several classes of secondary metabolites such as alkaloids, coumarins, saponins, polyphenols, flavonoids, tannins, triterpenes and steroids. Several molecules are active on pathogenic bacteria. Such metabolites in the plant extracts may provide a preliminary explanation for their antibacterial activity. It was observed that each extract have a different antibacterial activity. These may be due to differences in their chemical composition and the mechanism of action of their bioactive constituents. The activity of extract depends not only on the existence of secondary metabolites in plant extracts but also on their concentration and possible interaction with other components. The antibacterial effect of tannins is attributed to their ability to react with proteins to form stable components that are insoluble in water. The bacterial cell wall is made up of proteins. Tannins can bind to proteins rich in proline and interfere with protein synthesis. The antimicrobial activity of saponins is responsible for the escape of proteins and enzymes from the cell. The antibacterial effect of alkaloids is their ability to interchange with the DNA of Gram positive and negative bacteria and to interfere with cell division. The activity of flavonoids is due to their ability to bind to intracellular cells and soluble proteins. The antibacterial properties of steroids are due to the complex with membrane lipids and exert its loss-inducing action. The problem of microbial resistance

increases over time and the prospects for the use of antimicrobial biologics in the future are still uncertain. Therefore, it is necessary to take measures to solve this problem and introduce the antibacterial activity of medicinal plants [63].

Conclusion

Medicinal plants play an important role in the treatment of various serious diseases and reduce the negative effects of conventional treatments. Our finding indicates that *Drymaria cordata* is an excellent source of natural antioxidants and can neutralize the damage caused by ROS generated in the cell during various diseases. Therefore, this plant could be recommended as a good natural source for further investigation of natural antioxidants and antibacterial drugs.

Declarations

Declaration of competing interest

Author declares no conflict of interest.

Ethical statement: This article does not contain any studies involving animals performed by any of the authors.

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Author contributions

AA designed and performed whole experiments related to this work. Prof. SK helps in the conceptualization and design of the work. DK help in data analysis and interpretation of work. AMA and AS help in drafting and reviewing the manuscript. Vandana helps in spectral analysis and the drafting of manuscripts.

Data availability

The data used to support the finding within this study is included within this article.

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Tables

Table 1: Phytochemical screening of extracts of *Drymaria cordata*

S.No.	Name of test and Secondary metabolite	Methodology	Results	Methanol	Hexane	Water	References
1	Wagner test and Alkaloids	Add 2ml extract + 1% HCl + steam + 1ml of the solution with 6 drops of Wagner's reagent	Brownish red Ppt	+++	+	+	(Chanda et al. 2006).
2	Kellar- Killiani test and Cardiac glycosides	50 mg methanolic extract + 2 ml of chloroform + H_2SO_4 to form a layer.	Brown ring at interphase	++	+	-	(Onwukaeme et al. 2007).
3	NaOH test and Flavanoids	Extract + dilute NaOH, + dilute HCl	Yellow solution on NaOH turns colorless on Hcl	++	+	+	(Onwukaeme et al. 2007).
4	Lead acetate test and Phenolic compounds	To the test solution, a few drops of 10% lead acetate solution were added.	Formation of white precipitate	++	+	+	(G.C. Bag et al. 2013)
5	Frothing test and Saponin	0.5ml extract + 5ml distilled water and shake well	Persistence of frothing	++	+	+	(Parekh and Chanda, 2007)
6	Braemer's test and Tannin	10% alcoholic $FeCl_3$ + 2-3ml of methanolic extract (1:1)	Dark blue or greenish grey coloration	+	++	+	(Kumar et al. 2007; Parekh and Chanda, 2007).
7	Salkowski test and Terpenoids	5ml extract + 2ml Chloroform + 3ml conc. H_2SO_4	Reddish Brown color of interface	++	+	++	(Edeoga et al. 2005)
8	Ammonia test and Anthroquinone	Add 1 ml of dilute (10 %) ammonia to 2 ml of chloroform extract.	A pink-red color in the ammoniacal (lower) layer	++	+	-	(Onwukaeme et al. 2007).
9	HCL test and Phlobatannin	Extract was boiled with 2 ml of 1% hydrochloric acid.	Formation of red precipitate	+	-	+	(G.C. Bag et al. 2013)
10	Iodine test and Starch	The aqueous extract 5ml was treated with the reagent of the starch (iodine).	Blue color indicates the presence of starch	-	-	-	(Zohra et al. 2012)

Table 2: FTIR peak values and functional groups

Peak value (cm^{-1})	Stretching type	Functional Groups
3640.7109	OH stretch	Phenols
3352.82718	N-H stretch	Aliphatic secondary amine, Hydroxy group
2921.11263	C-H stretch	Methylene (>CH2)
2862.0611	C-H stretch	Methylene
2347.14318	N-H stretch	Amino-related component, NH component
2159.02873	C-N Stretch	Thiocyanate (-SCN)
1736.8901	C= C Stretch	Aromatic Ring
1600.83721	C= C Stretch	Alkene
1365.02508	N-O Stretch	Nitrate ion , gem-Dimethyl
1199.8404	C= C Stretch	Aromatic, C-H in-plane bend
1048.22161	C= C Stretch	Aromatic, C-H in-plane bend
846.329229	C= C Stretch	Aromatic, C-H out-of-plane bend
662.790698	C-O stretch	Thioethers, Alkyne C-H bend

Table 3: Chemical compounds of extract from *Drymaria cordata*

S. No.	R. time	Area%	M. Wt (g/mol)	Formula	Nature Compound	Name	Biological Activity	Reference
1.	7.460	0.34	158	$C_{10}H_{22}O$	Fatty alcohol	1-Decanol	Antibacterial activity	28
2.	8.250	0.38	172	$C_{10}H_{20}O_2$	Acetals organic compounds	2-Hexene, 1-(1-ethoxyethoxy)-, 1-(1-Ethoxyethoxy)-2-hexene	NA	
3.	9.590	0.49	184	$C_9H_{12}O_4$	spiro-polytetra-hydrofuran	7,8-Dioxaspiro[bicyclo[3.2.1]octane-2,2'-oxolane]-5'-one	Anticancer Activity	29
4.	9.967	1.10	224	$C_{16}H_{32}$	aliphatic hydrocarbons	1-Hexadecene	Antimicrobial, Antioxidant , Antidiabetic Activities	30 31
5.	10.232	0.24	472	$C_{20}H_{40}O_5Si_4$	Noradrenalin Metabolite	3,4-Dihydroxymandelic acid-tetratms	Antioxidant Activities	32
6.	12.231	1.11	242	$C_{16}H_{34}O$	Alcoholic compound	1-Hexadecanol	Antimicrobial compound	33
7.	12.685	6.65	278	$C_{20}H_{38}$	sesquiterpenoids	Neophytadiene	antibacterial compound, headache, rheumatism, skin disease, Diabetes	18 19
8.	12.765	0.43	268	$C_{18}H_{36}O$	ketones	2-Pentadecanone	Wound healing in and antibacterial activities	34
9.	12.943	1.20	278	$C_{20}H_{38}$	sesquiterpenoids	Neophytadiene	antibacterial compound, headache, rheumatism, skin disease, Diabetes	18 19
10	13.137	2.95	296	$C_{20}H_{40}O$	Phytol	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Anti-inflammatory, antioxidant, antimicrobial, , anticancer, anti-diuretic, Antidiabetic ,Immunostimulatory	25 26
11	13.353	0.48	232	$C_{17}H_{28}$	Alkene	1,8,11,14-Heptadecatetraene	NA	
12	13.564	0.48	266	$C_{18}H_{34}O$	Acetate	E,E-3,13-Octadecadien-1-ol	antioxidant and antitumor activities	35
13.	13.611	12.07	270	$C_{17}H_{34}O_2$	Fatty acid	Hexadecanoic acid, methyl ester Palmitic acid	Antibacterial, Antifungal, Antidiabetic Anti-inflammator, Antioxidant , Hypocholesterolemic , Nematicide , Pesticide , Antiandrogenic , Hemolytic, Mosquito larvicidical	20 21
14	13.692	1.46	292	$C_{18}H_{28}O_3$	Aromatic acid ester	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro methyl ester	anti-neuroinflammatory activity, Antidiabetic compound	36 37
15	14.092	0.87	256	$C_{16}H_{32}O_2$	Fatty acid	n-Hexadecanoic acid	antioxidant, anticholesteremic, , anti-inflammatory antitumorand immunostimulant properties	38

16	14.276	0.72	266	$C_{19}H_{38}$	fatty acids	1-Nonadecene	antituberculosis antifungal activity, antidiabetic and antitumor activities	39
17	15.246	7.11	294	$C_{19}H_{34}O_2$	Ester	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Anticancer activity, Antidiabetic, antioxidant	22 23
18	15.306	10.45	278	$C_{19}H_{34}O$	Fatty Acids	(Z,Z)-6,9-cis-3,4-epoxy-nonadecadiene	Antimicrobial, Immunity booster,	24
19	15.419	8.18	296	$C_{20}H_{40}O$	Diterpenoid	Phytol, 2-Hexadecen-1-ol	Antidiabetic, Antimicrobial and antioxidant	40
20	15.540	1.65	298	$C_{19}H_{38}O_2$	Fatty acids	Methyl stearate	GABA aminotransferase inhibitor, Anti-inflammatory,Antidiabetic, intestinal, Lipid metabolism regulator, Gastrin inhibitor, Antihelmintic (Nematodes), Antinociceptive	41 42
21	16.139	0.37	266	$C_{19}H_{38}$	fatty acids	1-Nonadecene	Antituberculosis, antifungal activity, antidiabetic and antitumor activities	39
22	16.608	1.27	402	$C_{20}H_{34}O_8$	organooxygen	Tributyl acetyl citrate	Pharmaceutical Excipient	43
23	17.072	1.13	312	$C_{19}H_{36}O_3$	Fatty acid	Glycidyl palmitate	Anti-staphylococcal activity	44
24	17.550	0.69	324	$C_{21}H_{40}O_2$	Terpenes	4,8,12,16-Tetramethylheptadecan-4-olide	Inhibitors for HER2 (Growth promoting protein), Anticancer compound	45
25	17.822	22.79	370	$C_{22}H_{42}O_4$	Adipic acid	Hexanedioic acid, Bis(2-ethylhexyl) ester	antibacterial activity	27
26	18.560	0.53	299	$C_{16}H_{29}NO_4$	Lactone	Oxacyclohexadecan-2-one	COX-2 and LOX-3 Inhibitors (Osteoarthritis)	46
27	18.714	1.37	326	$C_{22}H_{46}O$	aliphatic alcohol	Behenic alcohol	Antiviral, antifungal, antibacterial properties	47
28	18.937	0.29	242	$C_{15}H_{30}O_2$	fatty acid	Tetradecanoic acid, Methyl ester	Larvicidal and repellent Activity, Antidiabetic	48 49
29	20.456	1.01	382	$C_{25}H_{50}O_2$	fatty acid	Tetracosanoic acid, methyl ester	Larvicidal and repellent Activity, Antidiabetic	48 49
30	21.045	0.32	410	$C_{30}H_{50}$	Triterpene	Squalene	Emollient and antioxidant and antitumor activities	50
31	21.437	0.83	618	$C_{20}H_{23}F_{17}O_2$	carboxylic acid	Heptadecafluorononanoic acid, undecyl ester	Apoptosis	51
32	21.707	1.28	326	$C_{22}H_{46}O$	saturated alcohol	1-Docosanol	Antiviral	52
33	22.131	0.29	230	$C_{14}H_{30}O_2$	Alkane	Dodecane, 1,1-dimethoxy-Lauraldehyde	antibacterial activity, Anti-oxidant and antifungal activity, Diabetic Peripheral Artery Disease	53 54
34	25.437	2.09	412		stigmastanes	Chondrillasterol	antimicrobial activity,	

				$C_{29}H_{48}O$			Antidiabetic	55 56
35	28.342	2.45	506	$C_{34}H_{66}O_2$	fatty acid phytyl ester	Phytyl Tetradecanoate	NA	
36	29.637	3.46	758	$C_{54}H_{110}$	Alkane	Tetrapentacontane	Antioxidant, Antimicrobial, antidiabetic, anticancer activities activity	57 58
37	32.017	1.47	392	$C_{26}H_{48}O_2$		9,12-Octadecadienoic acid, (Z,Z)-, octyl ester	antioxidant activities, Antitumor, Antidiabetic	59 60 61

Figures

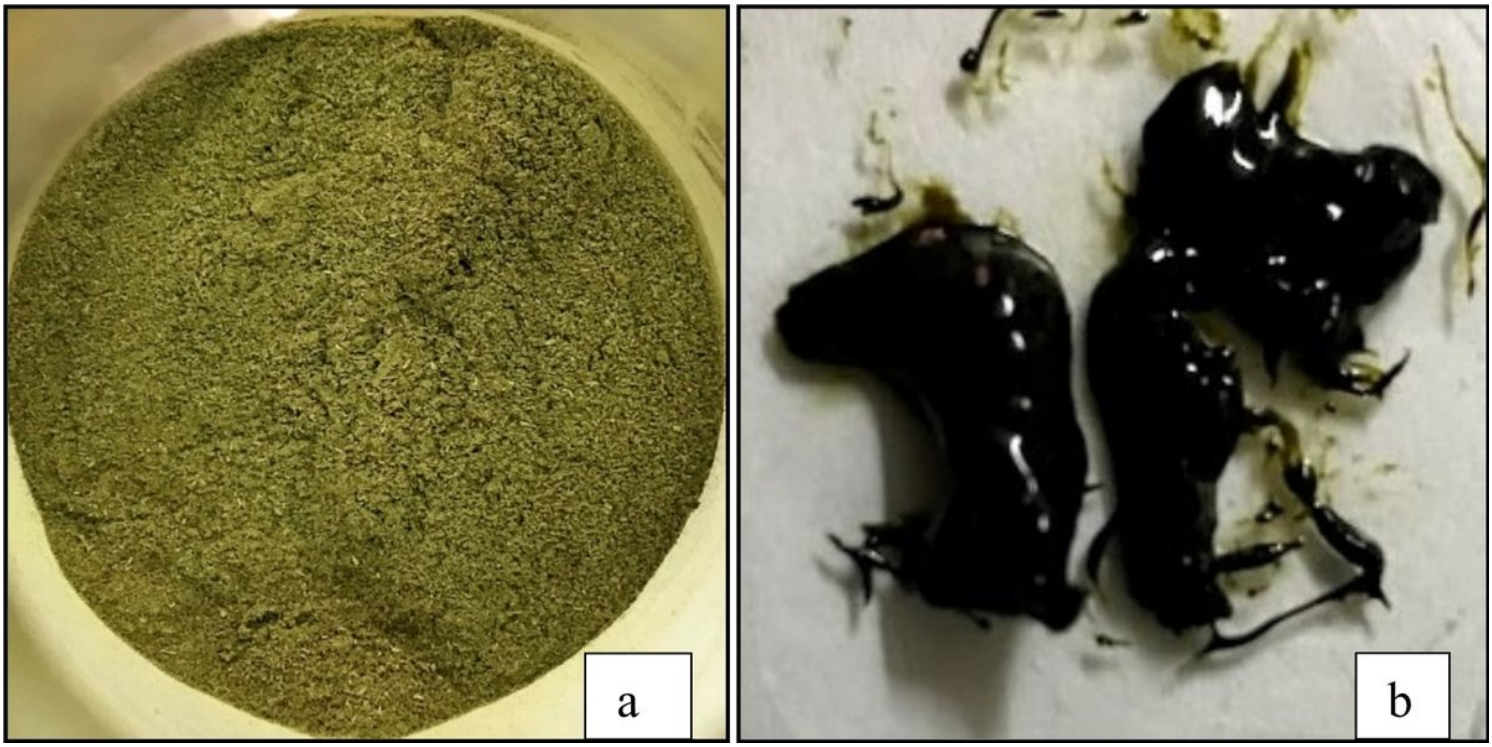

Figure 1

(a) Dried plant material (b) Plant extract

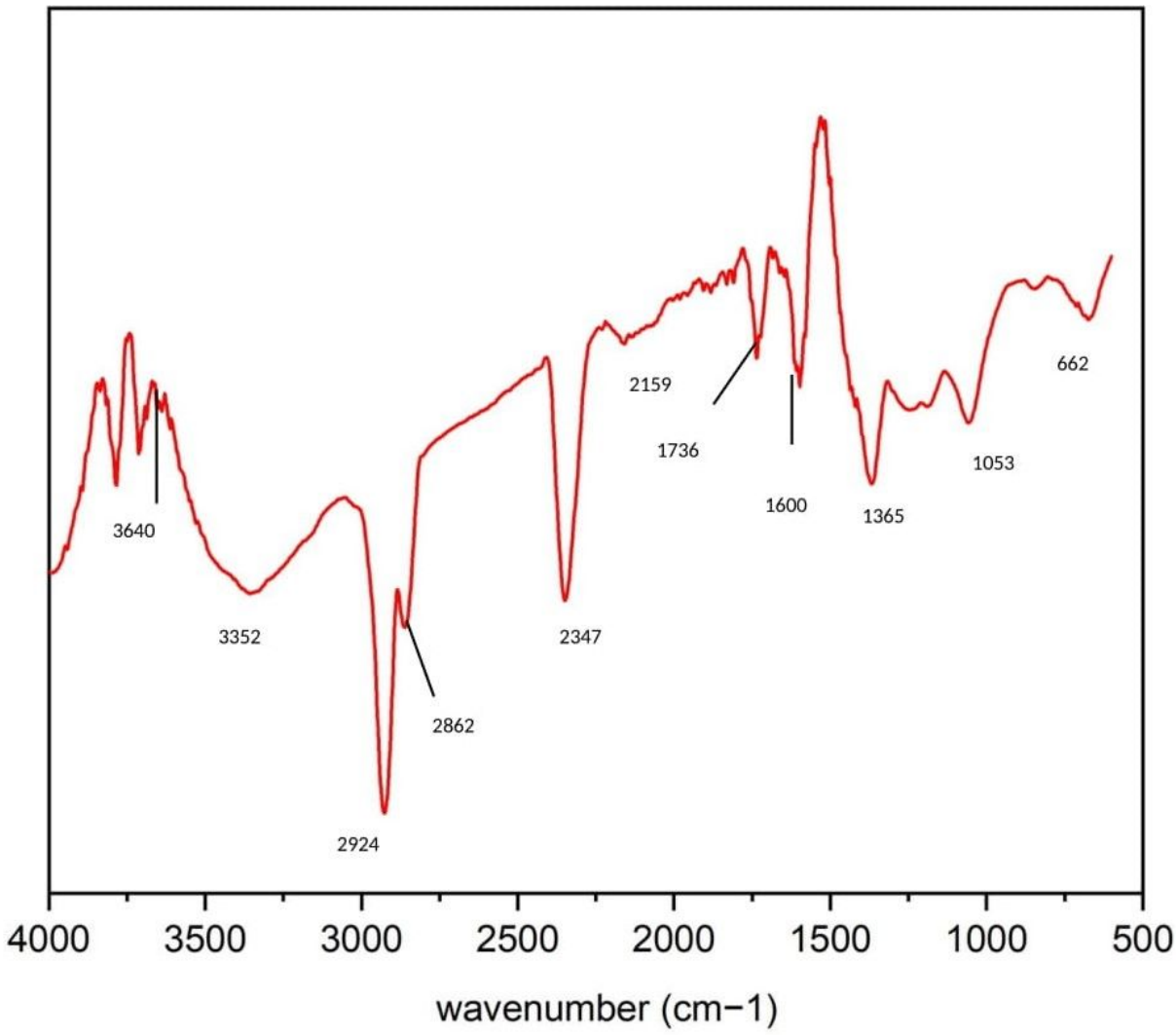

Figure 2

FTIR plot of *Drymaria cordata* extract

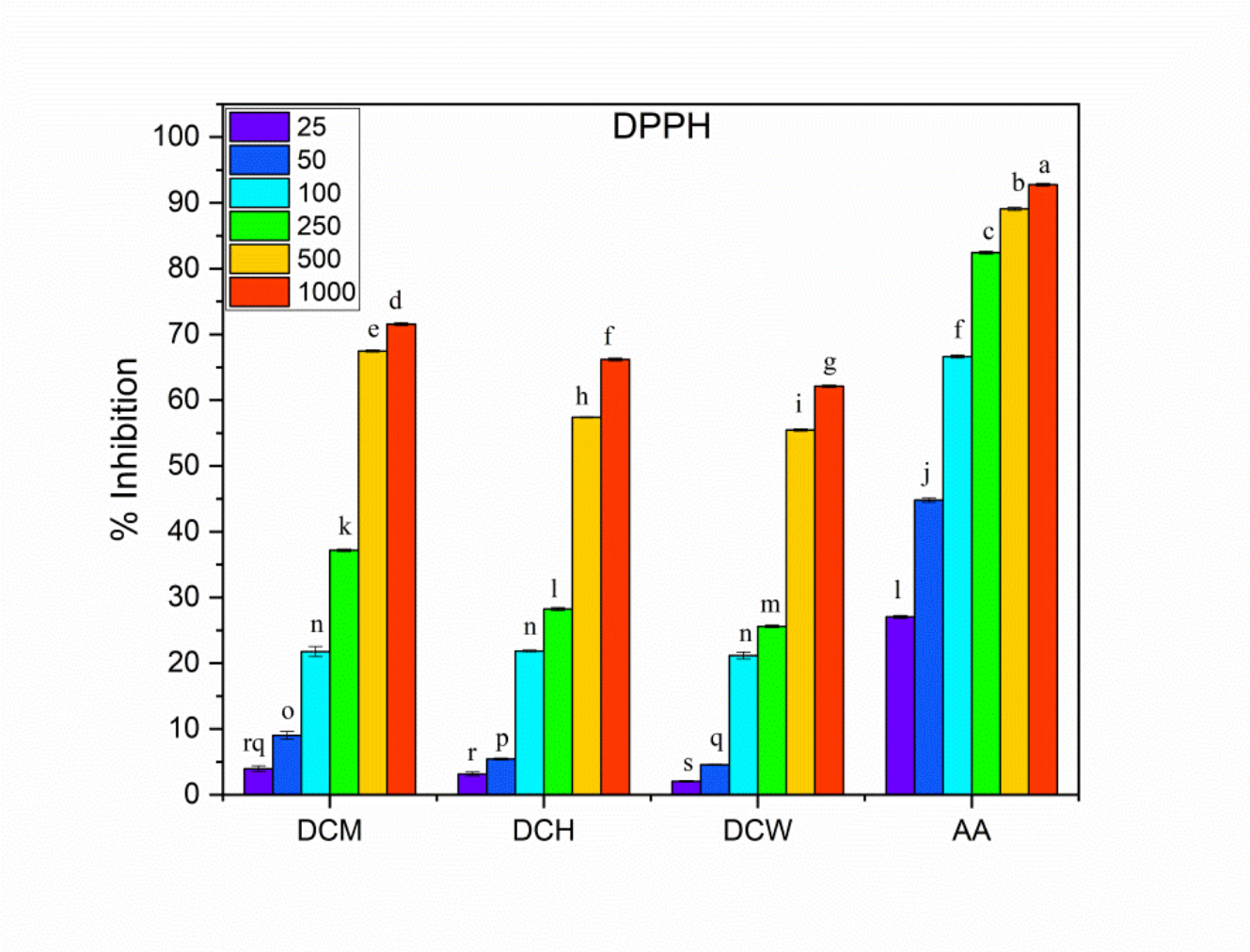

Figure 3

DPPH scavenging activity

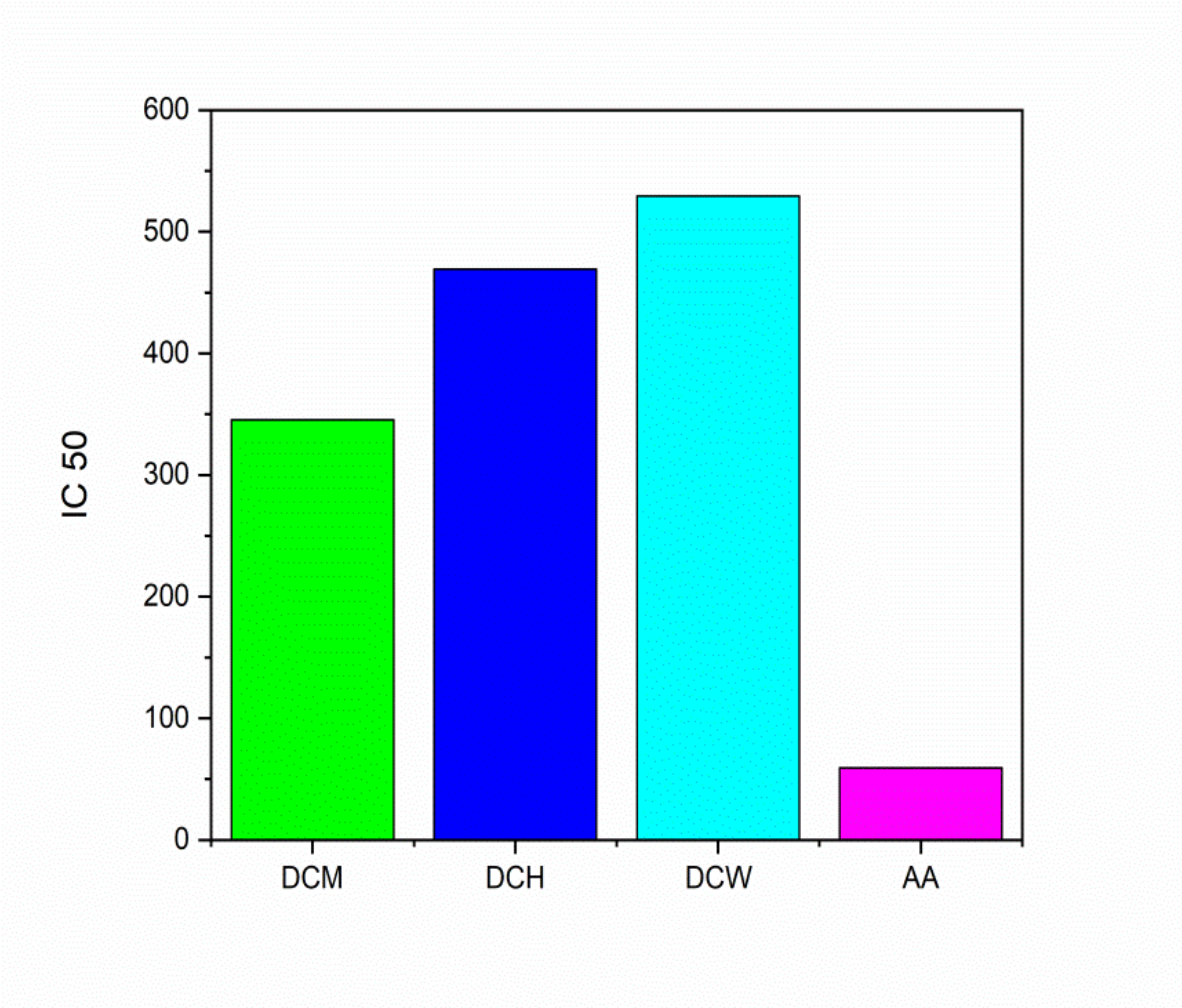

Figure 4

IC 50 values

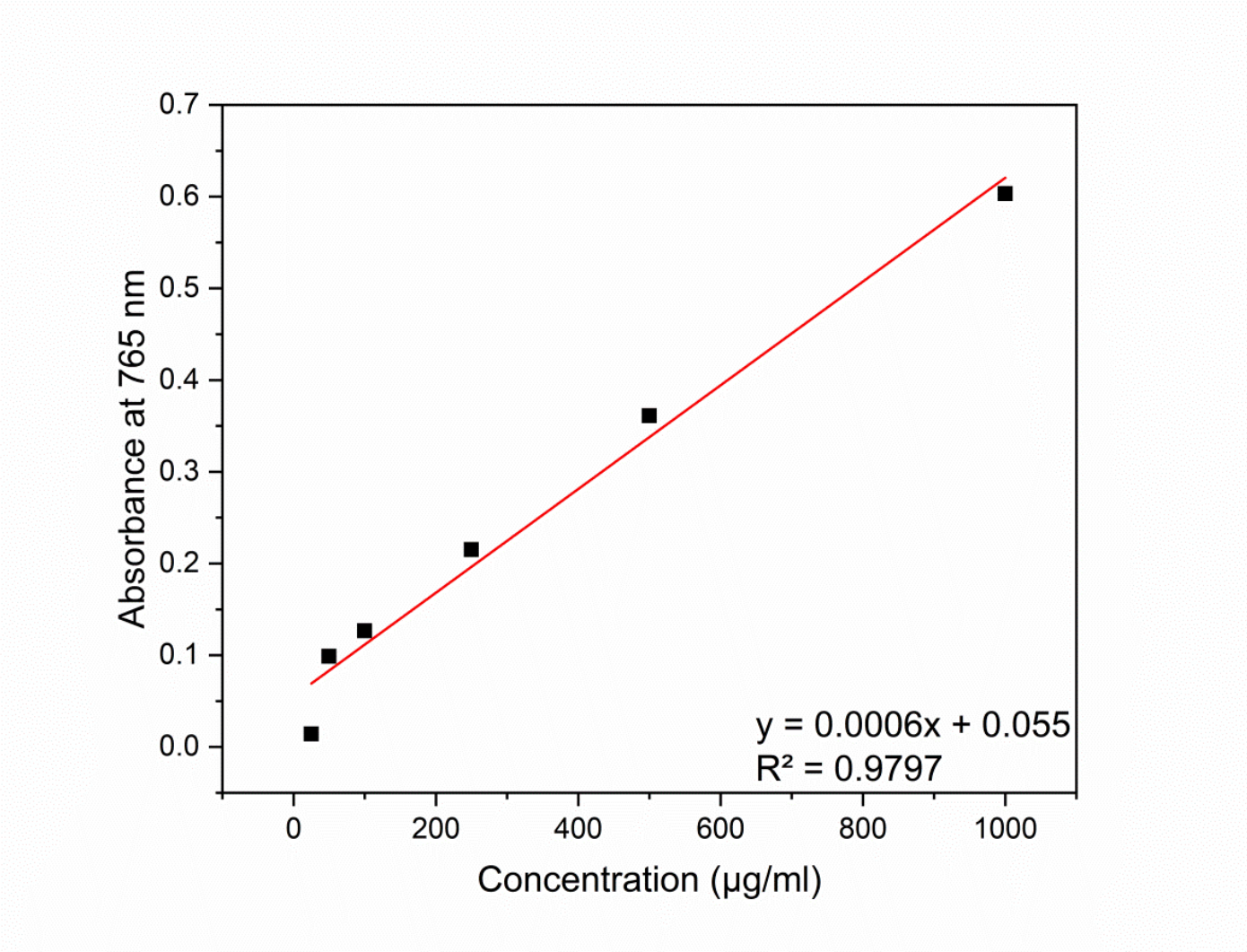

Figure 5

Standard curve of ascorbic acid

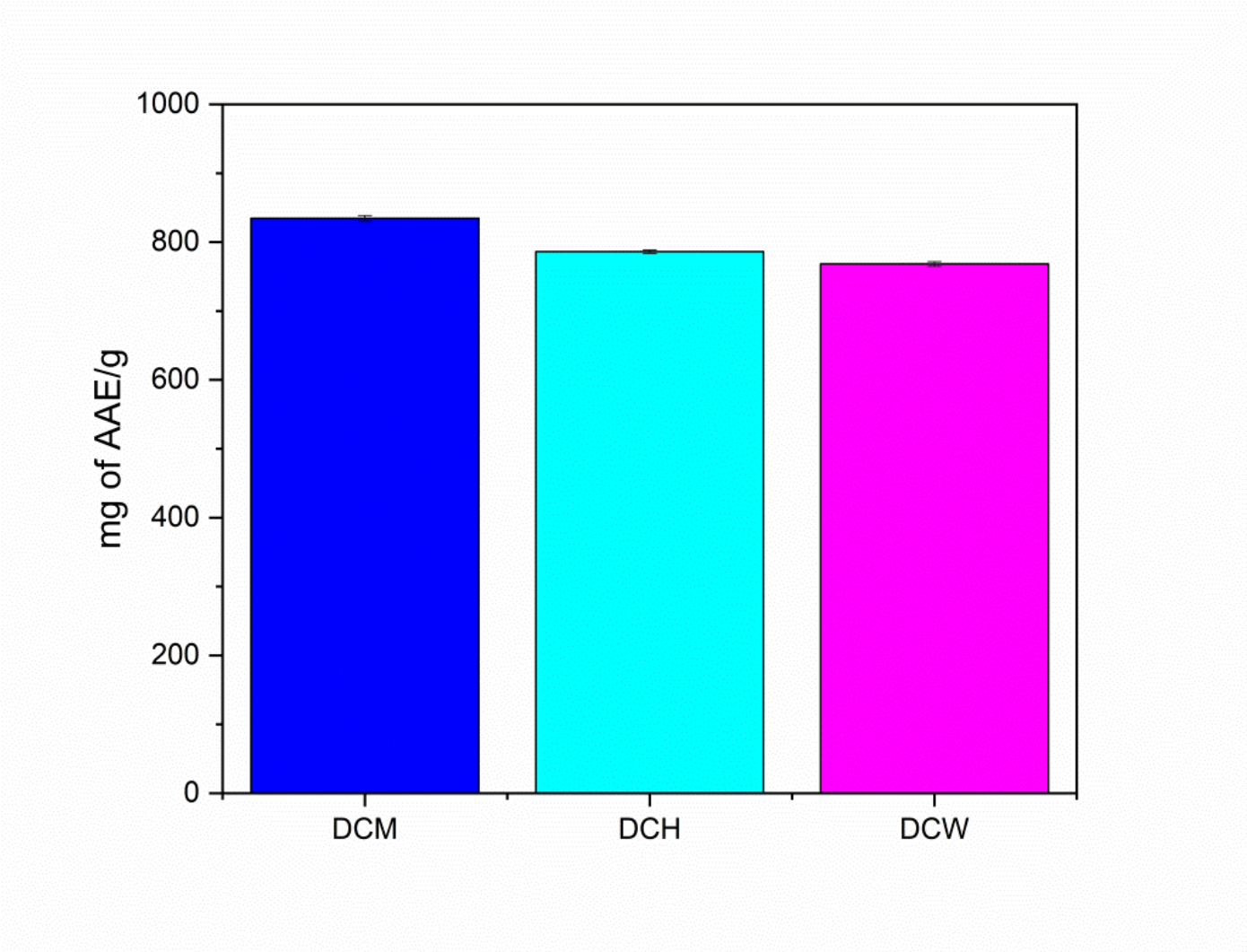

Figure 6

Antioxidant capacity of plant extracts

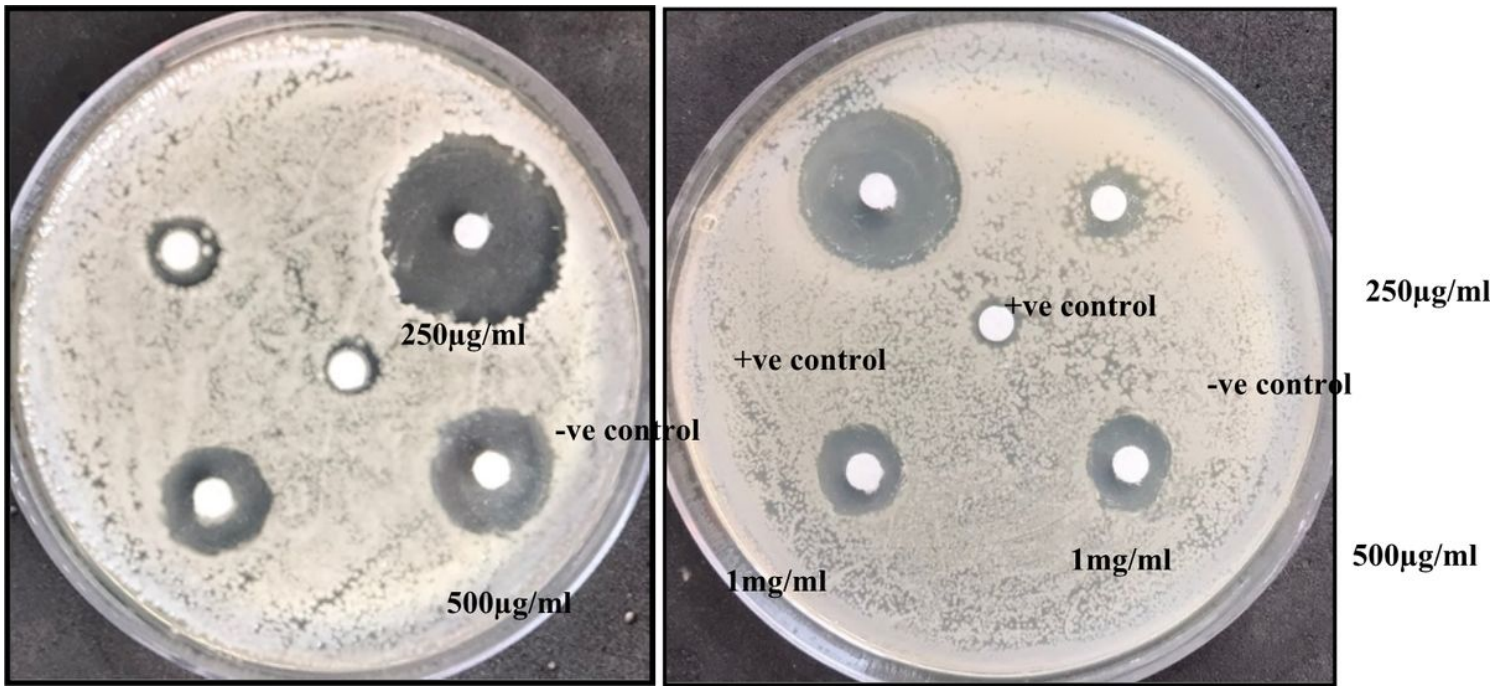

Figure 7

(a) *Bacillus subtilis* (b) *Pseudomonas aeruginosa*

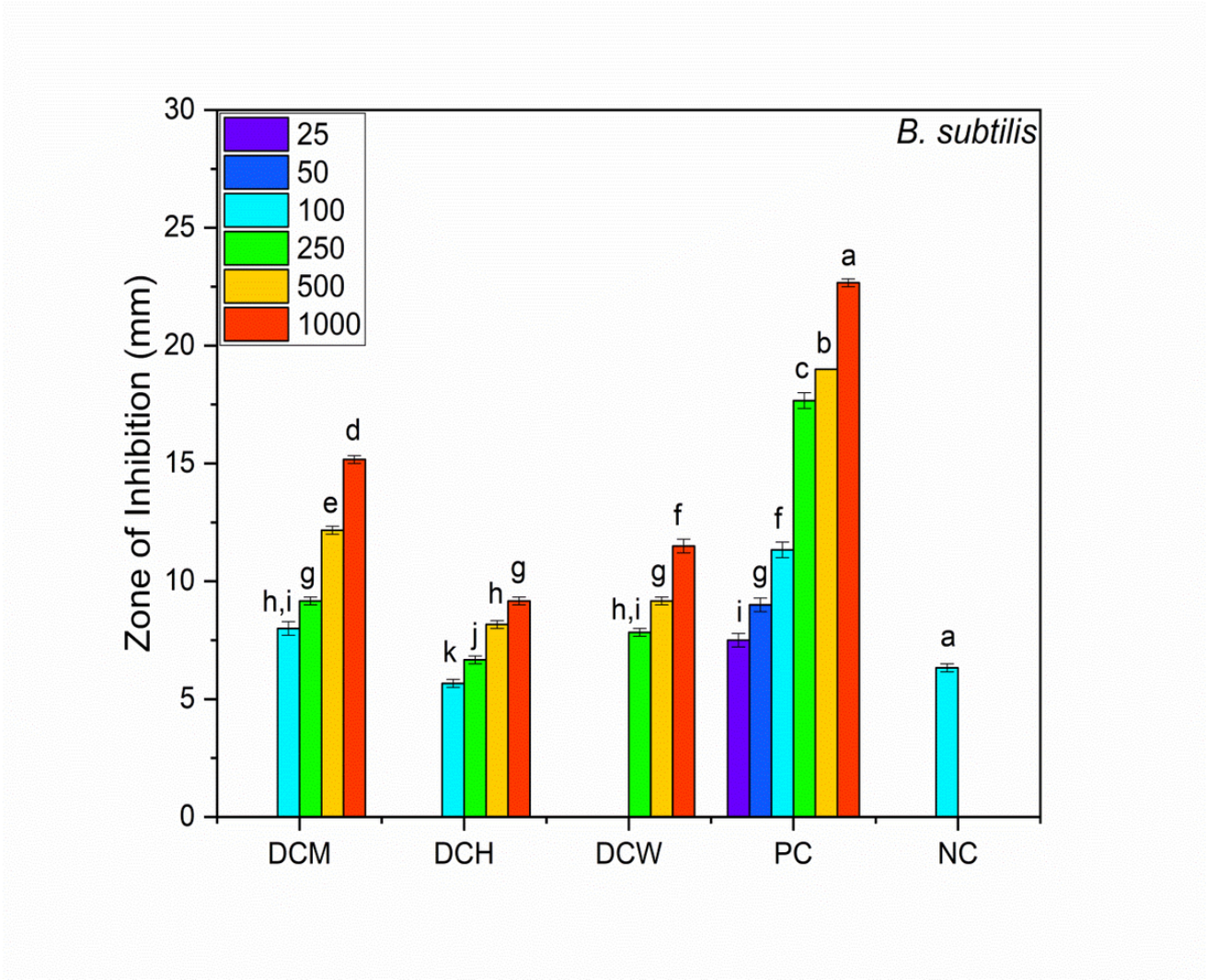

Figure 8

zone of inhibition (*Bacillus subtilis*)

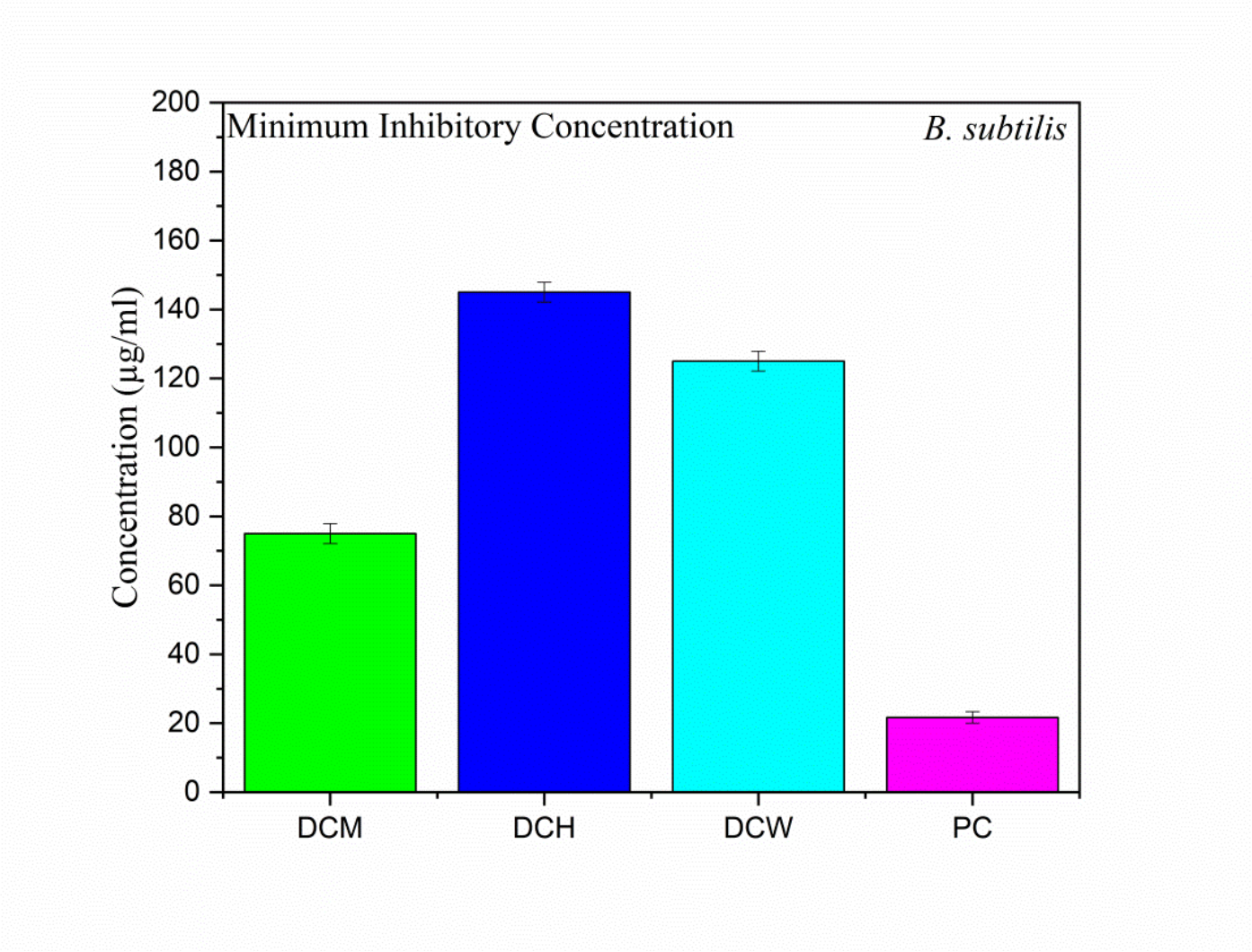

Figure 9

Minimum Inhibitory Concentration (MIC) (*Bacillus subtillis)*

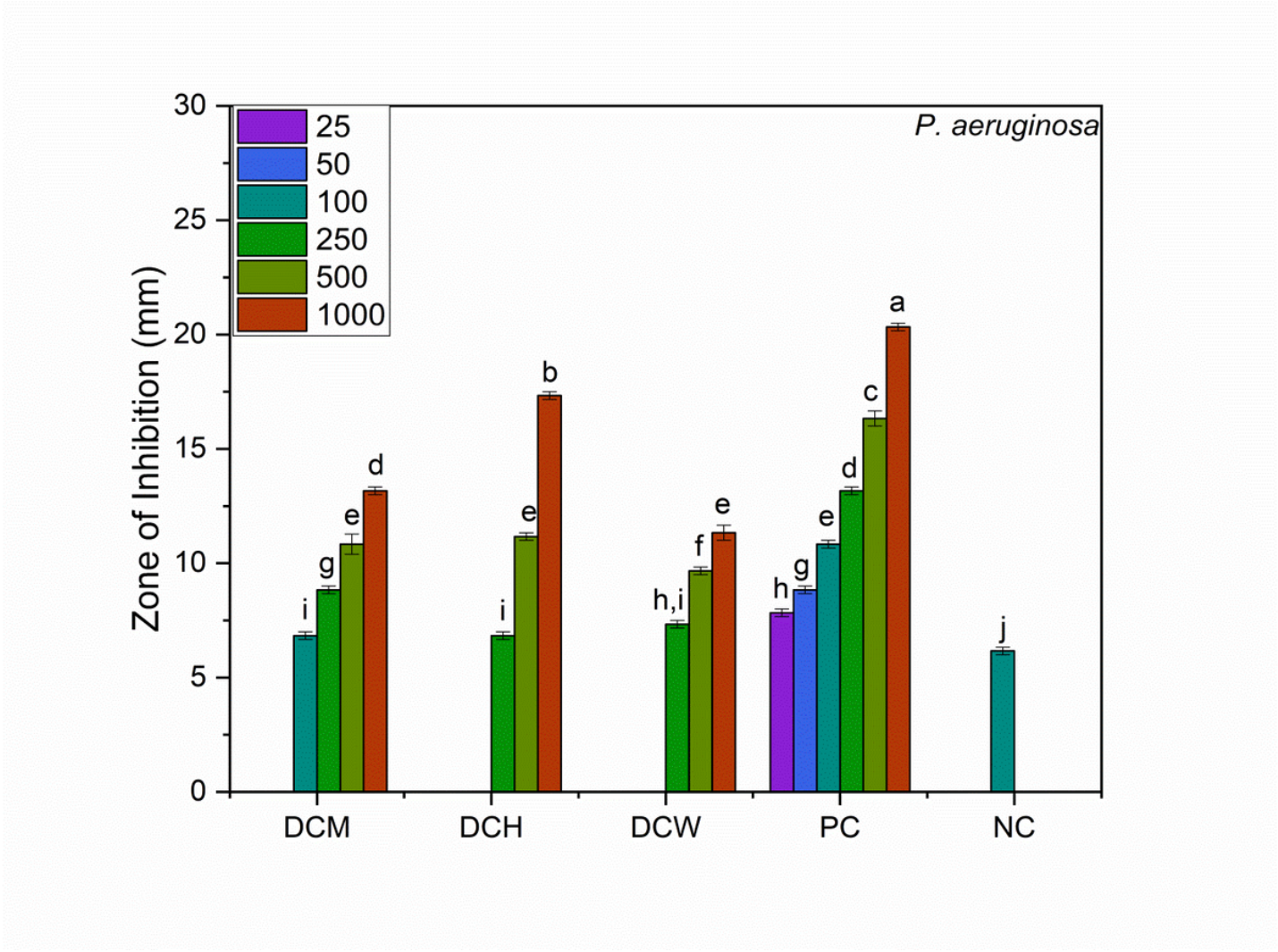

Figure 10

zone of inhibition (*Pseudomonas aeruginosa*)

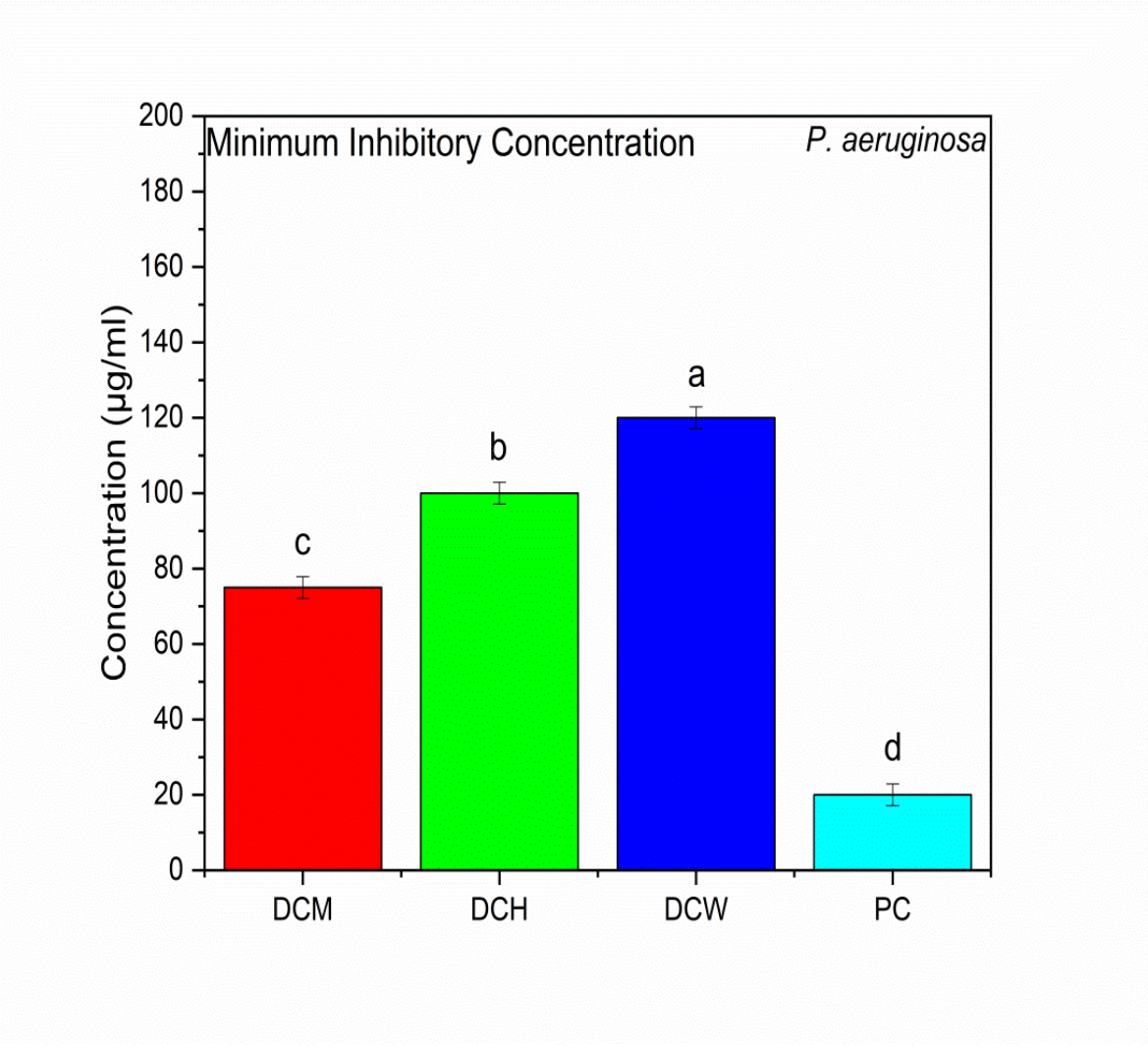

Figure 11

Minimum Inhibitory Concentration (MIC) (*Pseudomonas aeruginosa)*

Supplementary Files

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