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Research Article

EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF BOUGAINVILLEA SPECTABILIS

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ABSTRACT

Objective: The aim of the present study was to evaluate the antioxidant and antibmicrobial activity of various solvent extracts of *Bougainvillea spectabilis* leaves. *B. spectabilis* are popular ornamental plants in most areas with warm climates.

Methods: Antimicrobial activity of different solvent extracts of *B. spectabilis* leaves were tested against Gram positive and Gram negative bacterial strains as well as *Aspergilli* sp. by observing the Minimum inhibitory concenteration and zone of inhibition. The antifungal activity of compounds was studied by Micro Broth Dilution, Disc Diffusion assay while antibacterial activity was studied by Resazurin based Microtitre Dilution Assay. The bacteria used in the study were *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Vibrio cholerae* and *Streptomyces.* Antioxidant activity was done using metal chelating assay, superoxide radical scavenging activity and Nitric oxide radical Scavenging activity.

Results: Maximum zone of inhibition (13.5 mm) was observed by methanolic extract against *K. pneumoniae*. All the plant extracts were found to be active against all the fungal species tested. *T*he antimicrobial activity of amphotericin B standard showed inhibitory effect on *E. coli* and *K. pneumoniae* at extremely low concentration. Also tetracycline standard showed inhibitory activity against all the *Aspergilli* sp. at 1.95 µg/ml. The aqueous extracts of the plant exhibited a potential antioxidant activity as tested by all the three assays.

Conclusion: *B. spectabilis* might be considered as a potential source of metabolites which could be developed as precursors for antimicrobial and antioxidant drugs.

Keywords: *Bougainvillea spectabilis,* Antioxidant, Antimicrobial, Extracts, Drugs

INTRODUCTION

Antibiotic resistance has become a global concern [1] as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug-resistant pathogens [2]. As well as in an effort to minimize the undesirable effects of synthetic food preservatives in human health, food industries and scientists have recently turned their interest to new alternates. Medicinal plants are well known for their antioxidant and antimicrobial properties, as they are rich in phenolic substances, usually referred to as polyphenols, which are ubiquitous components of plants and herbs [3] .The importance of medicinal plants as a source of active drugs emerged from the chemical profile that produces a clear physiological action on the biological system. Flavonoids, alkaloids, tannins and phenolic compounds have been established as the most important bioactive compounds of plants [4] .

In many parts of the world medicinal plants are still against bacterial, viral and fungal infections. India is endowed with a rich wealth of medicinal plants which in turn are a source of genetic diversity [5]. India is also a major exporter of raw MAPs and processed plant based drugs. Exports of crude drugs from India in 1994-95 were valued at US$ 53,219 million. The growing interest in the investigation of antioxidants and antimicrobial agents from plants are due to two reasons. First, there is epidemical and clinical evidence suggesting that consumption of vegetables and fruits reduce the risk of developing chronic diseases (eg. Cancer) and secondly, phytochemicals are safer than synthetic chemicals. Screening of various plant extracts for antioxidants, lipid peroxidation and antimicrobials activity were reported [6-9].

The genus *Bougainvillea*, in the Nyctaginaceae (4 O' clock) family of plants, has 14 species, with three that are horticulturally important *Bougainvillea spectabilis*, *B. glabra* and *B. peruvina*. Bougainvilleas are popular ornamental plants in most areas with warm climates. *B. spectabilis* is a large climber with distinctive cured thorns and hair on stems and leaves. The plant, *B. spectabilis* was selected based on its use in ayurvedic and traditional system of medicine. In some areas like the Western Europe, North Africa, Middle East and the Indian Sub-continent, *B. Spectabilis*, is used to shorten the life span of sand flies that cause leishmaniasis [10]. *B. spectabilis* is also used in herbal combination for the treatment of diabetes [11]. Information also exists about the use of *Bougainvillea glabra* in the cure of ulcer, diarrhoea, and having anti-microbial activities [12]. Information from some traditional medical practitioners show that some beetles that feed on any *Bougainvillea* stems are dried crushed into powder and added as main ingredient in popular herbal combinations used in the treatment of diabetes mellitus. Out of an estimated 250000 higher plants, less than 1% has been screened pharmacologically [13] So, the present study was taken with the objective to prepare various extracts of this plant and to evaluate the antioxidant and antimicrobial activities of plant.

MATERIALS AND METHODS

Plant Material

The plant i.e *Bouganvillea spectabilis* was procured from Rohtak, Haryana, India. *B. spectabilis* leaves were collected, washed with fresh water and dried under shade at room temperature. The leaves were powdered and stored in sterile containers for further use.

Preparation of crude extract

Many compounds of the plants are separated from crude sources by solvent extraction. The most common and popular method is the Soxhlet's extraction. In hot extraction, the solvent in the receiver flask were kept in heating mantle and temperature was fixed below the boiling point of the solvent taken for the extraction. The solvent forms vapours at the elevated temperature. The process was continued till the extraction was complete. In this method, approximately 50 gm of each plant leaves were extracted separately in different solvents according to increasing polarity like petroleum ether, chloroform, acetone, methanol and water. The extracts were filtered and filtrate evaporated under reduced pressure to obtain crude extract of the plant.

Aqueous Extraction

In this method 50 gm of the crushed plant parts were dipped separately in 500 ml of the distilled water for 48 hours at room

temperature in a conical flask and shaken periodically. The extract filtered and filtrates evaporated under reduced pressure on the water bath to obtain the crude extract.

Calculation of Percent yield of the extract

The percent yield of the crude extract in different solvent was calculated by the following formulae:

$$\textbf{Percent yield} = \frac{\text{Weight of the crude extract obtained in gm} \times 100}{\text{Total weight of Plant powder in gm}}$$

Test organisms

Clinical isolates of *Aspergillus* species and pathogenic bacteria were obtained from Mycology department, Vallabhbhai Patel Chest Institute, Delhi and IMTECH, Chandigarh and employed in the current study. Three *Aspergilli* strain of *A. fumigatus* (ITCC 4517), *A. flavus* (ITCC 5192) and *A. niger* (ITCC 5405) and five bacterial strains of *Escherichia coli* (ATCC 25922), *Vibrio cholerae* (ATCC 39315), *Klebsiella pneumoniae* (ATCC 700603), *Staphylococcus aureus* (ATCC 259323), *Streptomyces* (MTCC 4066).

Antimicrobial evaluation of various plant extracts

The antifungal activity of compounds was studied by Micro Broth Dilution, Disc Diffusion and Spore-germination-inhibition assay [14] while antibacterial activity was studied by Resazurin based Microtitre Dilution Assay [15]. Brief description of these assays is given below:

Micro broth dilution assay

The spores ($1x10^6$) of *Aspergillus* were harvested from 96 hours cultures and were treated with different concentrations of plant extracts. The plates were incubated at 37 ^{0}C and examined macroscopically after 48 hours for the growth of *Aspergillus* mycelia.

Disc Diffusion Assay

The disc diffusion test performed in radiation sterilized Petri plates of 10.0 cm diameter (Tarson). The disc of the sample placed on the surface of the agar plates already inoculated with Aspergillus spores ($1x10^6$) or bacterial culture. The plates incubated at 37^{0}C and examined at 48 hours for zone of inhibition, if any, around the discs.

Resazurin based Microtitre Dilution Assay

Resazurin based MDA was performed in 96 well plates under aseptic conditions. A volume of 100 µl of test materials in 10% (v/v) DMSO or sterile water (usually a stock concentration 25 mg/ml for crude extracts) added into the first row of the plate. To all wells of plate 50 µl of nutrient broth and 50 µl of normal saline was added. Serial dilutions were performed using a multichannel pipette such that each well had 100 µl of the test material in serially descending concentrations. Tips were discarded after use. 10 µl of resazurin indicator solution was added in each well. Finally 10 µl of bacterial suspension was added to each well to achieve a concentration of 5×10^6 CFU/ml. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad-spectrum antibiotic as positive control (usually tetracycline in serial dilution). The plates were prepared in triplicate and placed in an incubator set at 37°C for 18–24 hours. The colour change was then assessed visually. Any colour change from purple to pink or colourless was recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial strain.

Antioxidant assays

Metal chelating activity

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis et al., (1994) [16]. To investigate 0.5 ml of extract, 1.6 mL of deionised water and 0.05 mL of $FeCl_2$ (2 mM) was added. After 30s, 0.1 ml ferrozine (5 mM) was added. Ferrozine react with the divalent iron to form stable magenta complex species that were soluble in water. Then after 10 min at room temperature, the absorbance of the Fe^{2+} –Ferrozine complex was measured at 562 nm. All test and analyses were done in triplicate and the percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula given below.

$$PI = A_{(Control)} - A_{(Sample\ or\ Standard)} / A_{(Control)} \times 100.$$

Where $A_{(Control)}$ = Absorbance of control reaction

$A_{(Sample\ or\ Standard)}$ = Absorbance of sample extract or standard

Super oxide-radical scavenging assay

The scavenging activity of extracts towards superoxide anion radicals was measured by the method of Liu *et al*., (1997) [17]. The superoxide anions were generated in a non- enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 µM) solution, 0.75 ml of NADH (936 µM) solution and 0.3 mL of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The super oxide anion scavenging activity was calculated according to the following equation:

$$\text{Scavenging effect \%} = A_{(Control)} - A_{(Sample\ or\ Standard)} / A_{(Control)} \times 100.$$

Where $A_{(Control)}$ = Absorbance of control reaction

$A_{(Sample\ or\ Standard)}$ = Absorbance of sample extract or standard

Nitric oxide radical scavenging activity

Nitric oxide radical inhibition was estimated by the use of Griess Illosvoy reaction. In this investigation, Griess Illosvoy reagent was generally modified by using naphthyl ethylene diamine dihydrochloride (0.1 % w/v) instead of the use of 1- naphthylamine (5 %). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and the extract (0.2-1.0 mg/ml) were incubated at 25^{o}C for 150 minutes. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml sulfanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min for completion of the reaction process of diazotization. Further, 1 ml of the naphthyl ethylene diamine dihydrochoride was added, mixed and was allowed to stand for 30 min at 25^{o}C. The absorbance was taken at 546 nm. Ascorbic acid was used as a standard. The percent inhibition (PI) was calculated using the formula.

$$PI = A_{(Control)} - A_{(Sample\ or\ Standard)} / A_{(Control)} \times 100.$$

Where $A_{(Control)}$ = Absorbance of control reaction

$A_{(Sample\ or\ Standard)}$ = Absorbance of sample extract or standard

RESULTS

Various extracts of the plant were prepared, filtered and evaporated as per standard procedures mentioned in materials and methods. The percentage yield of plant extracts are shown in **Table 1**.

Table 1: The percentage yield of plant extracts in various solvents

Plant Extract	Percentage Yield
Water	6.86
Methanol	3.81
Acetone	1.42
Chloroform	2.51
Petroleum ether	0.63

Antimicrobial activity

The various extracts of the medicinal plant *B. spectabilis* were observed for antibacterial activity (MIC) if any, against *Escherichia coli, staphylococcus aureus,* Antibacterial activities at specific doses are mentioned in **Table 2**. Petroleum ether and Chloroform extract

of the plant have shown MIC at a concentration of 6 mg/ml against *S. aureus* whereas all other extracts did not showed any activity at this concentration against any bacteria. Antibacterial activity in disc diffusion assay was observed in chloroform and acetonic extracts of the plant against all the test bacterial species. Maximum zone of inhibition (13.5 mm) was observed by methanolic extract against *K. pneumoniae* (**Table 3**). The antifungal activity (MIC) against *A. fumigatus, A. flavus* and *A. niger* are given in **Table 4**. All the plant extracts were found to be active against all the fungal species tested. *T*he antimicrobial activity of amphotericin B standard showed inhibitory effect on *E. coli* and *K. pneumoniae* at extremely low concentration (**Table -5**). Also tetracycline standard showed inhibitory activity against all the *Aspergilli* sp. at 1.95 µg/ml.

Table 2: MIC(mg/ml) of *B. spectabilis* extracts against various bacteria by Resazurin based MDA

Solvent Name	*S. aureus*	*V. cholerae*	*Streptomyces*	*E. coli*	*K. pneumoniae*
Petroleum ether	6.0	-	-	-	-
Chloroform	6.0	-	-	-	-
Acetone	-	-	-	-	-
Methanol	-	-	-	-	-
Water	-	-	-	-	-

Table 3: Zone of Inhibition (mm) of *B. spectabilis* extracts against various bacteria by disc diffusion assay

Solvent Name	*S. aureus*	*V. cholerae*	*Streptomyces*	*E. coli*	*K. pneumoniae*
Petroleum ether	7.0	9.5	-	9.0	11.0
Chloroform	9.0	11.5	11.0	12.5	13.0
Acetone	12.0	12.5	7.0	9.5	11.5
Methanol	8.5	12	-	8.0	13.5
Water	-	-	-	12.5	9.0

Table 4: MIC(mg/ml) of *B. spectabilis* extracts against various fungi by microbroth dilution assay

Solvent Name	*A. fumigatus*	*A. flavus*	*A. niger*
Petroleum ether	0.375	0.375	0.75
Chloroform	0.375	0.75	1.5
Acetone	1.5	0.187	0.375
Methanol	0.75	0.375	1.5
Water	1.5	1.5	1.5

Table 5: MIC(mg/ml) of standard drug Tetracycline against various bacteria by Resazurin based MDA

Name of bacteria	MIC against Tetracycline (mg/ml)
Escherichia coli	0 .078
Staphylococcus aureus	0.312
Vibrio cholerae	0.0172
Streptomyces	0.312
Klebsiella pneumoniae	0.005

Antioxidant activity

Plant extract and phytoconstituents found effective as radical scavengers. Plant extracts of *B. spectabilis* examined for its antioxidant potential by three assay.

Metal chelating activity

Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. The formation of Ferrozine-Fe^{2+} complex is not complete in the presence of plant extract, indicating their ability to chelate the iron. The absorbance of Ferrozine-Fe^{2+} complex decreased linearly in a dose-dependent manner (0.2 to 1 mg/ml). The standard compound Ascorbic acid did not exhibit any metal chelating activity at all the tested concentrations. Reaction of ascorbic acid with $FeCl_2$ might enhance the degradation of ascorbic acid and increase the ascorbyl acid radical concentration [18].The metal chelating activity of the leaves extract of *B. spectabilis* was found as: methanol shows higher activity at 0.2 mg/ml whereas at other higher concentrations (aqueous extracts shows higher activity. While in aqueous extract was found to possess maximum chelating activity than other tested extracts as indicated in **Table 6**.

Superoxide radical scavenging activity

In PMS/NADH-NBT system, superoxide anions derived from dissolved oxygen by PMS/NADH coupling reaction reduce NBT. The decrease in the absorbance at 560 nm with the antioxidants thus indicates the consumption of superoxide anions in the reaction mixture. In the present investigation, the reference compound, ascorbic acid, showed a pro-oxidant effect. Ascorbic acid is a potent reducing agent and acts as a free radical scavenger. However, it may act as a pro-oxidant in the presence of metals [19]. So we have used another reference compound synthetic antioxidant butylated hydroxyl toluene (BHT) which showed remarkable superoxide radical scavenging activity. In case of leaves extracts of *B. spectabilis*, superoxide radical scavenging activity was found in the following order: aqueous > methanol >acetone > petroleum ether>choloroform (**Table 7**)

Nitric oxide radical scavenging activity

Despite of the possible beneficial effects of nitric oxide, its contribution to oxidative damage is increasingly becoming evident. This is due to the fact that nitric oxide can react with superoxide to form peroxynitrile anions, which is a potential strong oxidant that can decompose to produce hydroxyl radical and nitrogen dioxide

[20]. Incubation of solutions of sodium nitroprusside in phosphate buffer saline at 25^{0}C for two hours resulted in a linear time-dependent nitrite production, which is reduced by the tested extracts of *B. spectabilis*. This may be due to the antioxidative principles (chiefly phenolics) in the extracts which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. In the present study, nitric oxide radical scavenging potential of leaves of *B. spectabilis* were tested. Different extract showed different inhibition of nitric oxide radicals as shown in **Table 8**. As at 0.2 and 0.6 mg/ml aqueous shows maximum inhibition followed by methanol, acetone, choloroform and petroleum ether extract. In contrast at 0.4 mg/ml petroleum ether shows maximum inhibition followed by aqueous, acetone, methanol, choloroform. At 0.8 mg/ml methanol shows maximum inhibition followed by acetone, choloroform and petroleum ether.

DISCUSSION

The conventional methods used to assess the antimicrobial activity of different antimicrobial agents are by the determination of the Minimum Inhibitory Concentration (MIC) either by broth dilution or by agar diffusion method. These methods are well established procedures for which there are accepted guidelines including those endorsed by National Committee for Clinical Laboratory Standards. However, when examining the antimicrobial activity of poorly soluble experimental substances, solubilized in DMSO, the above mentioned methods are not suitable [21]. In the present study, we have used Agar disk diffusion assay for calculation of inhibition zone and Microbroth dilution method for MIC (Minimum Inhibitory Concentration) calculation. In Disc Diffusion Assay, it was assumed that during incubation, the test sample diffuses out from the disk into the agar medium, creating a circular concentration gradient that decreases logarithmically with increased distance from the disk. As the test sample diffuses out from the disc, the bacteria multiply creating a lawn of visible growth on the agar except in area (zone) around the disc where diffused molecules possessed properties to inhibit bacterial growth [22]. In the present study, leaves, extracts of *B. spectabilis* were investigated for their antimicrobial effect against several gram negative and gram positive bacterial strains. In classifying the antibacterial activity as gram positive and gram negative, it would generally be expected that a much greater number would be active against gram positive than gram negative bacteria. However, in this study, a large number of extracts were active against both gram positive and gram negative while a relatively less number were active against gram positive bacteria alone. The activity against both the types of bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins. In some extracts, resistance to the Gram-negative bacteria is mainly due to the outer membrane, which acts as an efficient permeability barrier against macromolecules and hydrophobic substances [23]. To destabilize the lipopolysaccharide layer of outer membrane of Gram negative bacteria, the use of several chelating agents, such as EDTA and other substances, have been proposed [24]. Unfortunately, those agents that cause outer membrane permeabilization are often too toxic to be used as food ingredients. More interesting for the destabilization of outer membranes of Gram-negative bacteria in food products without safety implications seems to be citric acid as chelating agent [25] , and high homogenization pressure [26, 27] . The present study reveals that the use of polar solvents (water and methanol) in the preparation of plant extracts provides more consistent antibacterial activity as compared to aqueous extracts. This observation clearly indicates that the polarity of antibacterial compounds make them more readily extracted by polar solvents, and using organic solvents also does not negatively affect their bioactivity against bacterial species. The data also showed that some antimicrobial substances could only be extracted by methanol and water solvents, suggesting that polar solvents are clearly better solvents of antimicrobial agents. However, a number of reports on the antimicrobial effects of leaves of *B. spectabilis* have been observed in various parts of world [28]. Our results remarkably varied from the earlier reported studies on these plants. This could indicate the influence of soil nature, elevation and other environmental factors on the nature and level of antimicrobials synthesized in secondary metabolites of the plant. *B. spectabilis* leaves collected from the Chennai, Tamilnadu state was found to possess antimicrobial activity against *K. pneumoniae* and *S. aureus* (zone of inhibition is greater than 10mm). These leaves extracts was completely found to be ineffective against *V. cholerae* [28]. However, in our study, leaves extract was least effective against bacteria *K. pneumoniae*. In accordance to above reported study, we have also not observed any inhibition activity against *V. cholerae* and reported effective against *S. aureus* (greater than10mm) These differences could be due to the nature and level of the antimicrobial agents present in the extracts and their mode of action on the different microorganisms. However, in our study, acetone extract of leaves was found to be effective against *K. pneumoniae* (11.5 mm), *E. coli* (9.5 mm), *S. aureus* (12 mm), *V. cholerae* (12.5mm), S*treptomyces* (7mm) which are collected from the Rohtak district of Haryana. The extracts exhibit lower antioxidant activity than the standard oxidant (Ascorbic acid) or positive control taken the various assays used. It can be explained by the fact that we know that standard antioxidant used are in purified form whereas the crude extracts are the mixture of various compounds. Further, it has also been observed that certain other compounds may be of help in enhancing the potency of the active compounds resulting in an additive or synergistic positive effect while others may neutralize or inhibit the same [29].

CONCLUSION

This study is a preliminary evaluation of antimicrobial and antioxidant activity of the plant *B. spectabilis*. It indicates that, the plant have the potential to generate novel metabolites. The plants demonstrating broad spectra of activity may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animals and human health and provide biochemical tools for the study of infectious diseases. Future investigations will focus the research on the *in vivo* antimicrobial activities and on chemical identification of the antimicrobial ingredients in the screened efficacious extracts of *B. spectabilis*. Based on these conclusions, further purification and testing of the efficacious plant extracts of *B. spectabilis* is recommended, in order to identify the major active ingredient responsible for the antimicrobial and antioxidant activities of these extracts.

Table 6: Metal chelating activity (%) of leaf extracts of *B. Spectabilis*

	Leaf extracts					
Concentration (mg/ml)	**PE**	**CE**	**AcE**	**ME**	**AE**	**AS**
0.2	4.30 ± 0.38	3.09 ± 0.47	7.21 ± 0.71	45.55 ± 0.18	44.44 ± 0.13	63.40±68
0.4	8.08 ± 0.12	7.18 ± 0.48	11.39 ± 0.18	46.11 ± 0.71	52.23 ± 0.58	65.30±62
0.6	12.4 ± 0.18	8.14 ± 0.52	16.50 ± 0.78	51.10 ± 0.11	58.34 ± 0.87	68.60±28
0.8	17.3 ± 0.93	14.64 ± 0.37	25.1 ± 0.61	56.66 ± 0.78	59.83 ± 0.98	73.45±34
1.0	20.4 ± 0.83	18.30 ± 0.19	26.84 ± 0.57	58.11 ± 0.90	62.44 ± 0.18	78.53±58

Values are expressed as mean ± S.D., PE -Petroleum ether, CE- Chloroform extract,

ACE -Acetone extract; ME- Methanol extract, AE- Aqueous extract; AS- Ascorbic acid

Table 7: Superoxide radical scavenging activity (%) of leaf extracts of *B. spectabilis*

	Leaf extracts					
Concentration (mg/ml)	**PE**	**CE**	**ACE**	**ME**	**AE**	**BHT**
0.2	6.33 ± 0.20	2.5 ± 0.26	14.48±0.40	27.29±0.13	32.17±0.19	44.11 ± 0.84
0.4	14.51 ± 0.40	7.71 ± 0.25	20.62 ± 0.46	35.54 ± 0.44	39.38 ± 0.35	54.82 ± 0.21
0.6	20.66 ± 0.27	14.49 ± 0.40	23.47 ± 0.38	41.69 ± 0.28	43.74 ± 0.21	66.52 ± 0.45
0.8	25.63 ± 0.16	15.57 ± 0.39	28.67 ± 0.59	45.42 ± 0.22	47.14 ± 0.15	73.50 ± 0.45
1.0	28.32 ± 0.50	20.55 ± 0.40	31.59 ± 0.43	48.63 ± 0.21	55.56 ± 0.41	75.78 0.36

Values are expressed as mean ± S.D.; PE- Petroleum ether, CE- Chloroform extract, ACE- Acetone extract; ME -Methanol extract, AE- Aqueous extract; BHT- Butylated hydroxytoluene

Table 9: Nitric oxide radical scavenging activity (%) of leaf extracts of *B. spectabilis*

	Leaf extracts					
Concentration (mg/ml)	**PE**	**CE**	**ACE**	**ME**	**AE**	**CUR**
0.2	0.52± 0.10	2.73 ± 0.12	1.56 ± 0.24	36.46 ± 0.28	42.74 ± 0.33	65.37 ± 0.54
0.4	4.10 ± 0.36	5.05 ± 0.10	5.58 ± 0.30	40.24 ± 0.19	51.46 ± 0.32	76.33 ± 0.32
0.6	12.84 ± 0.10	7.55 ± 0.10	8.25 ± 0.19	43.67 ± 0.32	53.78 ± 0.52	81.11 ± 0.11
0.8	19.94 ± 0.11	10.38 ± 0.15	9.59 ± 0.22	49.25 ± 0.41	61.25 ± 0.25	85.78 ± 0.28
1.0	26.14 ± 0.17	11.45 ± 0.31	11.59 ± 0.31	54.23 ± 0.22	64.65 ± 0.27	89.38 ± 0.06

Values are expressed as mean ±SD; PE- Petroleum ether, CE- Chloroform extract, ACE- Acetone extract; ME- Methanol extract, AE- Aqueous extract; CUR- Curcumi

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