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Research Article
In-vitro Antioxidant and Antimicrobial Activity of *Bougainvillea glabra* Flower

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Abstract
The aim of the present study is to assess the phytochemical nature, antioxidant and antimicrobial activities of methanolic extract of *Bougainvillea glabra* flower. Antioxidants play an important role in protecting cellular damage by reactive oxygen species. The fractions of the flower extract were screened for antioxidant activities using DPPH radical scavenging activity, reducing power assay, total antioxidant capacity and reduction of ferric ions by o-phenanthroline color method. Antimicrobial activities of different solvent fractions were tested against gram positive and gram negative bacterial strains by observing the zone of inhibition using disc diffusion method, where eImipenem (10 μg $disc^{-1}$) was used as the standard. The bacterial strains used in the study were *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The extracts revealed the presence of phytochemicals. Almost all three fractions exhibited remarkable antioxidant and antimicrobial activity in terms of all the assays tested. Water fraction showed DPPH radical scavenging activity with IC_{50} value of 135.73 μg mL^{-1}. Not surprisingly, the n-hexane fraction showed excellent antioxidant activity for reduction of ferric ions by *o*-phenanthroline color method. All three types of fractions showed inhibitory effect against all tested bacteria except *P. aeruginosa*. These findings indicate compounds isolated from carbon tetrachloride and water fractions possess pharmacological properties and potential to develop natural compounds based pharmaceutical products. All fractions of *B. glabra* were found to be the most effective free radical quencher, a potent source of natural antioxidants and antimicrobial agents, thus justifying their traditional use in green therapeutics.

Key words: *Bougainvillea glabra*, phytochemical screening, antioxidant, antimicrobial, DPPH

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants have always been a part of medicinal science from the beginning of human civilization to the present modern world of synthetic medicines. Even in the presence of variety of effective synthetic drugs, use of medicinal plants for maintaining human health has acquired a lot of importance in the present era. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids and phenolic compounds. Free radicals are chemical species, which contain one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radicals are generated as part of the body's normal metabolic process and play a dual role in our body as both deleterious and beneficial species. Excess production of Reactive Oxygen Species (ROS) and/or a decrease in antioxidant levels may lead to the tissue damage and different diseases. Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by ROS. Recent investigations suggest that the plant origin antioxidants with free radical scavenging properties may have great therapeutic importance in free radical mediated diseases. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effect, while relatively plant based medicines confer fewer side effects than the synthetic drug in some instances (Tapsell *et al*., 2006).

In recent years, the incidence of multiple drug resistant human pathogenic microorganisms has been increased day by day largely due to indiscriminate use of commercial antimicrobial drugs commonly prescribed in the treatment of infectious diseases. This in turn has pushed scientist to explore new antimicrobial substances from various sources like medicinal plants. According to the estimation of World Health Organization (WHO), in developing countries 80% of the population still depends on traditional medicines, mostly plant drugs for their primary care needs (Ikegami *et al*., 2003; Izzo, 2004). Different plant extracts have long been used for a wide variety of medicinal purposes as they represent reservoir of effective chemotherapeutics and can provide valuable sources of natural antimicrobials (Balandrin *et al*., 1985; Satish *et al*., 1999; Jones, 1996). Hence, the study for screening of antimicrobial properties of herbs is a crying demand which may help to find out proper treatment of several diseases caused by microorganisms.

Bougainvillea glabra also called as paper flower is a climbing evergreen woody ornamental (Mishra *et al*., 2009) shrub which inhabited to warmer climates is a native to Brazil and now also seen in areas like Middle East, Bangladesh, India, Pakistan, North America etc. (Bhat *et al*., 2011). *Bougainvillea glabra* from the family of Nyctaginaceae belongs to the genus Bougainvillea and this genus has 18 species of plants of which three of them *B. glabra, B. spectabilis* and *B. peruviana* have gained a lot of importance in the horticulture field (Bhat *et al*., 2011; Adebayo *et al*., 2005). Its stems are thin, with recurred prickles and leaves covered with small hairs. It produces abundant flowers with white and purple bracts. There are many varieties with different colors: red, orange, yellow, violet etc.

In traditional uses, the plant is used in variety of disorders like diarrhea, reduces acidity, cough and sore throat decoction of dried flowers for the blood vessels and leucorrhoea and decoction of the stem in hepatitis. The main part used is leaves (Yosef *et al*., 2001). *Bougainvillea glabra* is reported to have a wide range of medicinal properties like anti-viral (Bolognesi *et al*., 1997), anti-diabetic (Bhat *et al*., 2011; Adebayo *et al*., 2009; Saikia and Lama, 2011; Malomo *et al*., 2006), anti-fertility (Mishra *et al*., 2009), anti-inflammatory (Adebayo *et al*., 2005), anti-microbial activity (Edwin *et al*., 2007) and also considered to be larvicidal (Saikia and Lama, 2011). Since, there is no work available in the antioxidant property of flower. Considering all these facts, we planned for an investigation on *B. glabra* flower to evaluate its phytochemical nature and antioxidant activity, using extraction of dried flowers. Beside this we also planned to evaluate the antimicrobial activity of different fractions of *B. glabra* flower on both gram positive and gram negative bacteria.

MATERIALS AND METHODS

Collection and preparation of sample: The flowers of *B. glabra* were collected from Sonapur, Noakhali, Bangladesh. The collected flowers were washed with water so that the sticked dirt particle had been washed and then sun-dried for one week. Dried flowers were pulverized in a grinder to make delicate powder and then stored in airtight container until use.

Proximate analysis: Proximate analysis of a substance constitutes different classes of nutrients present in the samples such as moisture, ash and acid insoluble ash (Dev *et al*., 2015).

Determination of moisture content: Accurately weighed 5 g of powdered flower of *B. glabra* was taken in a crucible. It was

kept in a hot air oven at 105-110°C until free from moisture. The percentage of moisture content was then calculated with reference to the air-dried sample.

Determination of total ash value: Accurately weighed 5 g of powdered flower of *B. glabra* was taken in a dried silica crucible. It was incinerated at 450°C temperature, until free from carbon and then cooled. The weight of total ash was taken and the percentage of it was calculated with reference to the air-dried sample.

Determination of acid insoluble ash value: The total ash obtained was boiled for 5 min with 25 mL of 2 N HCl, filtered and the insoluble matter was collected on ashless filter paper. Then, it was washed with hot water, ignited in tarred crucible for 15 min at temperature not exceeding 450°C, cooled and weighed the obtained residue. The percentage of acid insoluble ash was calculated with reference to the air-dried sample.

Extraction: The powdered sample was extracted with 95% methanol in room temperature for two weeks with occasional shaking and stirring. It was then filtered through a fresh cotton material and finally with a Whatman No.1 filter paper. The 95% methanol extract was further partitioned successively with n-hexane and carbon tetrachloride. These fractions were concentrated with a rotary evaporator under reduced pressure and dried using oven dryer at 35-40°C. Dried extracts were stored for further use.

Preliminary phytochemical screening: Phytochemical screening of methanolic extracts was tested for the presence of alkaloid, flavonoid, reducing sugar, saponin, phenolic compound, tannin, protein and amino acid (Hossain, 2015).

Antioxidant activity: In order to investigate the antioxidant properties of the examined extracts, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power assay, total antioxidant capacity determination and reduction of ferric ions by *o*-phenanthroline color method were performed.

DPPH radical scavenging activity: The capacity to scavenge the stable free radical DPPH was monitored according to the method of Takao *et al*. (1994) adopted with suitable modifications from Kumarasamy *et al*. (2007). The DPPH (8 mg) was dissolved in methanol (100 mL) to obtain a concentration of 80 μg mL^{-1}. The absorbance was measured at 517 nm. Lower the absorbance, higher the free radical scavenging activity. Ascorbic acid and butylated hydroxytoluene (BHT) were used as reference standards. The 95% methanol was used as a blank. Scavenging activity was calculated as the percentage inhibition using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Reducing power assay: The reducing powers of different fractions were determined by the method of Oyaizu (1986) with modifications. The absorbance was measured at 700 nm against a blank in a spectrophotometer. Increased absorbance of the reaction mixture indicated the increased reducing power. The BHT was used as the standard. Three replicates were made for each test sample.

Total antioxidant capacity: The total antioxidant activity of the fractions was evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al.* (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. The absorbance was measured at 695 nm against blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

Reduction of Fe^{3+} ions by o-phenanthroline method: A reaction mixture containing 1 mL *o*-phenanthroline (5 mg in 10 mL methanol), 2 mL ferric chloride 0.2 mM (3.24 mg in 100 mL distilled water) and 2 mL of various concentrations of the extracts was incubated at ambient temperature for 10 min, then the absorbance was measured at 510 nm. Ascorbic acid and gallic acid were used as reference standards (Afroze and Hossain, 2015).

Antimicrobial activity

Microorganisms: To carry out antimicrobial assay of *B. glabra* flower extract, four different ATCC bacterial cultures were used that were collected from the Department of Microbiology, Dhaka Shishu (Children) Hospital and from the Department of Microbiology, University of Dhaka. The cultures were *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10707, *Pseudomonas aeroginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

Antimicrobial assay: The disc diffusion method was performed to determine the antimicrobial activity according

to the National Committee for Clinical Laboratory Standards (NCCLS) (Hossen *et al*., 2015). At first, sterile paper discs (4 mm) soaked with the previously prepared solutions of all extracts (n-hexane, carbon tetrachloride and water fraction) at a concentration of 20 mg mL^{-1} (by dissolving 20 mg of each extract into 1 mL of distilled water) were air dried for 3 h. Then the prepared Mueller-Hinton Agar (M-H) plates were used to make bacterial lawn using young bacterial cultures (0.5 McFarland standard turbidity) of reference bacterial strains by dipping sterile swabs. Finally, previously prepared paper discs of each extract were placed on swab plates. Imiperem antibiotic disc was used as a positive control for all organisms. The agar plates were incubated at 37°C for 24 h and the zone of inhibition were observed.

RESULTS

Proximate analysis: The flower of *B. glabra* was subjected to evaluate its moisture content, total ash and acid insoluble ash value (Table 1).

Preliminary phytochemical screening: Secondary metabolites are very important for the plant. The results of the phytochemical analysis revealed the presence of some secondary metabolites such as alkaloid, reducing sugar, flavonoid, saponin, phenolic compound, tannin, protein and amino acid (Table 2).

Antioxidant activity

DPPH radical scavenging activity: The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the *in-vitro* antioxidant activity of pure compounds as well as plant extracts. In our results, the water fraction showed the highest DPPH scavenging activity (89.76±0.012%) at concentration of 256 µg mL^{-1} while the lowest scavenging value was of n-hexane fraction (01.73±0.002%) at 8 µg mL^{-1} as presented in Fig. 1 and Table 3. For each fraction, the IC_{50} value was calculated from the curves plotted. Lower IC_{50} value indicates better DPPH radical scavenging activity. Water fraction of *B. glabra* exhibited the lowest IC_{50} value (135.73 µg mL^{-1}). On the other hand, n-hexane fraction exhibited the highest IC_{50} value (3446.53 µg mL^{-1}). Ascorbic acid (AA) and BHT were used as standard drug in this method.

Reducing power assay: The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 2 shows that the water fraction reduced the

Table 1: Moisture content and ash value of *B. glabra* flower

	Ash value (%)	
Moisture content (%)	Total ash	Acid insoluble ash
11.33	6.67	4.67

Table 2: Qualitative chemical analysis of different solvent fractions of *B. glabra* flower

	Fractions		
Phytochemical composition	n-Hexane	CCl_4	Water
Alkaloid	–	+	+
Reducing sugar	–	+	+
Flavonoid	–	+	–
Saponin	–	–	+
Phenolic compound	–	+	+
Tannin	–	+	+
Protein and Amino acid	–	+	+

+: Present and -: Absent

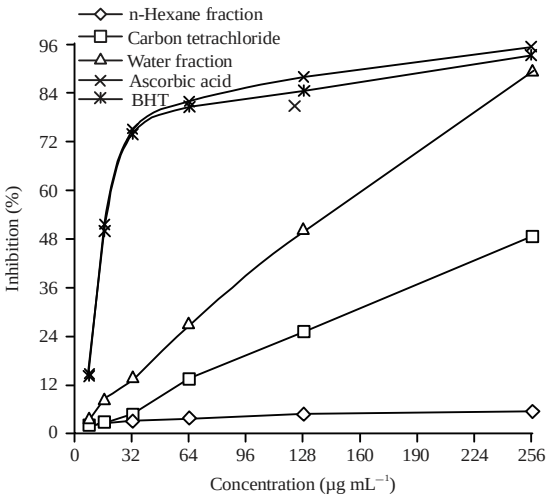

Fig. 1: DPPH radical scavenging activity of different fractions and standard antioxidants

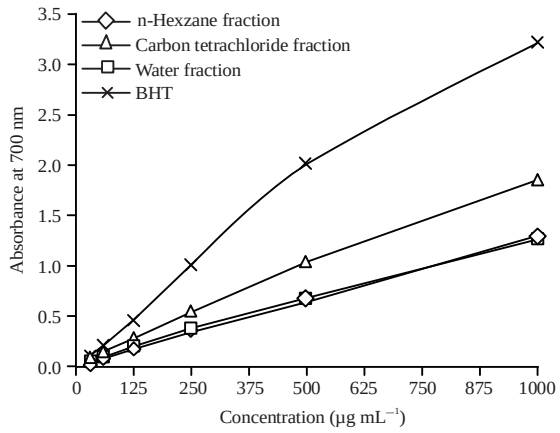

Fig. 2: Reducing power assay of different fractions of *B. glabra* with standard antioxidant

Table 3: DPPH radical scavenging activity of different fractions and standard antioxidants

	Fractions of *B. glabra*			Standard	
Concentration (μg mL^{-1})	n-Hexane	CCl_4	Water	AA	BHT
256	05.51±0.001	48.98±0.040	89.76±0.012	95.75±0.001	93.86±0.002
128	04.88±0.002	25.20±0.001	50.24±0.006	88.35±0.033	84.88±0.023
64	03.78±0.004	13.54±0.017	26.93±0.007	82.36±0.059	80.94±0.006
32	03.15±0.002	04.88±0.009	13.70±0.007	75.12±0.003	74.02±0.013
16	02.52±0.003	02.83±0.001	08.50±0.018	51.34±0.004	50.24±0.012
8	01.73±0.002	01.89±0.016	03.78±0.004	14.65±0.043	13.70±0.044
IC_{50} value	3446.53	259.40	135.73	04.85	10.38

Table 4: Zone of inhibition of the different fractions against four strains of bacteria

	Zone of inhibition in millimeters (Concentration of each extract was 20 mg mL^{-1})					
				Negative control		
Bacteria	n-Hexane fraction	CCl_4 fraction	Water fraction	n-Hexane	CCl_4	Positive control (Imipenem)
E. coli	16	16	15	0	0	28
P. aeruginosa	6	6	0	0	0	24
B. cereus	12	14	14	0	0	26
S. aureus	20	17	22	0	0	34

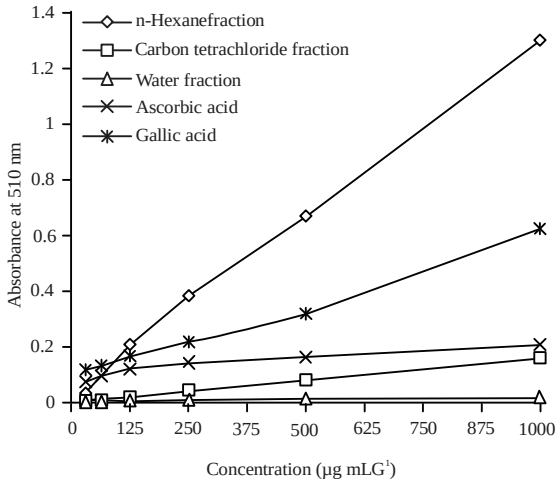

Fig. 3: Comparative analysis of *B. glabra* fractions and standard antioxidants for Reduction of Fe^{3+} ions by o-phenanthroline method

Fe^{3+} to ferrous ion (Fe^{2+}) more effectively (1.852) as compared to the n-hexane and carbon tetrachloride fractions (1.290 and 1.265), respectively at 1000 μg mL^{-1} concentration. BHT was used as standard antioxidant for comparison.

Total antioxidant capacity determination: Total antioxidant activities of all fractions were evaluated spectrophotometrically by the phosphomolybdenum method. The antioxidant activities of the fractions were compared with the standard antioxidant ascorbic acid. The total antioxidant capacity of various solvent fractions of *B. glabra* were found to decrease in this order: n-hexane (581 AAE/g)>carbon tetrachloride (121 AAE/g)>water (22 AAE/g). It means that the n-hexane and carbon tetrachloride fractions of *B. glabra* contain as much quantity of antioxidant compounds as equivalents of ascorbic acid to effectively reduce the oxidant in the reaction matrix.

Reduction of Fe^{3+} ions by o-phenanthroline method: The ferric ion reduction is extensively used to evaluate antioxidant activity. In this method, Fe^{2+} reacts rapidly with 1,10-o-phenanthroline and forms a red colored complex which is exceptionally stable. This complex has strong absorption in the visible spectrum at a wavelength of 510 nm. Gallic acid and ascorbic acid have been used as standard drug in this method. Not surprisingly, the n-hexane fraction showed appreciable antioxidant activity (Fig. 3). Interestingly, the value (1.299) is significantly higher than the standard antioxidants (0.627 and 0.210) tested at 1000 mg mL^{-1}. Additionally, the carbon tetrachloride fraction (0.162) also contributed good antioxidant activity at 1000 mg mL^{-1}.

Antimicrobial activity: The n-hexane, carbon tetrachloride and water fractions of *B. glabra* flower were subjected to antibacterial assay against both gram positive and gram negative bacteria namely, *S. aureus, B. cereus*, *E. coli* and *P. aeruginosa.* This study observed and compared the antimicrobial activity of three different extract of *B. glabra* flower on both gram positive and gram negative bacteria. Imipenem was used as a positive control whereas n-hexane and carbon tetrachloride was used as negative control.

The results of antibacterial assay are shown in Table 4. All three types of fraction of *B. glabra* showed inhibitory effect against all of the bacteria used I n this study except *P. aeruginosa* (Fig. 4). The ranges of zone were from 6-22 mm.

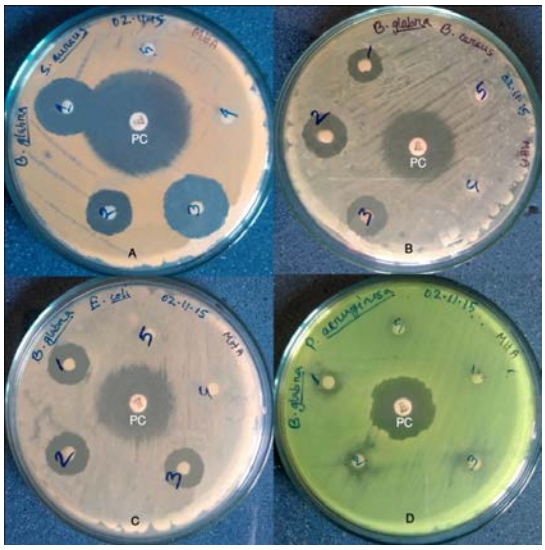

Fig. 4: Zone of inhibition of different extracts of *B. glabra* on the test bacteria. A = *S. aureus*, B = *B. cereus*, C = *E. coli*, D = *P. aeruginosa*, 1 = n-Hexane fraction, 2 = CCl_4 fraction, 3 = Water fraction, 4 = n-Hexane control, 5 = CCl_4 control and PC = Positive control

DISCUSSION

Proximate analysis is a method for the quantitative analysis of different macronutrients. From Table 1, it is clear that the flower of *B. glabra* contains 11.33% moisture. The low moisture content of the flower would hinder the growth of microorganism and storage life would be high (Adeyeye and Ayejuyo, 1994). Moreover, knowing the moisture content of a substance helps to determine if that substance is suitable for a specific use. The ash content of 6.67% indicates that the flower is comparatively rich in mineral elements. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Total ash value determination is important because it indicates the quality and purity of a crude drug.

The phytochemical analysis revealed that the amount of common bioactive components like alkaloid, reducing sugar, flavonoid, saponin, phenolic compound, tannin, protein and amino acid were concentrated in medium polar fraction (i.e., methanol) same as to Joshny *et al.* (2012). Search for effective, non-toxic natural compound with antioxidative activity has been intensified in recent years (Mariajancyrani *et al.*, 2013). On the basis of our results, bougainvillea appears to have potential for treatment of oxidative stress related diseases. Alkaloids have pharmacological applications as anesthetics and CNS stimulants (Madziga *et al.*, 2010). Flavonoids are most commonly known for their antioxidant activity and act as transformers which modify the body's reactions to carcinogens, viruses and allergens. They possess anti-cancerous, anti-inflammatory, anti-microbial and anti-allergic activity (Balch and Balch, 2000) and may, therefore be useful in therapeutic roles (Jisaka *et al.*, 1992). Tannins are used as antiseptic and this activity is due to presence of the phenolic group. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinnorhoea and diarrhoea. Phenols represent a host of natural antioxidants, defence against pathogens and herbivore predators and thus are applied in the control of human pathogenic infections (Maurya *et al.*, 2008).

The DPPH scavenging activity is based on the ability of sample to donate hydrogen which reacts with the DPPH radical. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom or transfer electron to DPPH, thus neutralize the free radical character and then this gives rise to the reduced form DPPH (non-radical) with the loss of the violet color. The water fraction of *B. glabra* showed good antioxidant activity (135.73 μg mL^{-1}) than carbon tetrachloride and n-hexane fractions (259.40 and 3446.53 μg mL^{-1}). The result of DPPH scavenging activity implies that the plant extract may be useful for treating radical related pathological damages (Wang *et al.*, 1998).

In reducing power assay, depending on the reducing power of antioxidant compounds, yellow color of the test solution changes to various shades of green and blue. The

presence of radicals (i.e., antioxidant) causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. This result is in agreement with that of Yen and Duh (1993), who reported that the reducing power of peanut hull extract increased with increase in concentration and correlated well with the extent of antioxidant activity. Similarly, Duh (1998), found that the antioxidant properties of mung bean hull and burdock extracts were shown to be concomitant with the development of reducing power.

Total antioxidant activity mainly concentrates on the thermodynamic conversion and measures the number of electrons or radicals donated or quenched by a given antioxidant molecule. It is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acidic pH. The TAC of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity). In our study, the medium-polar and non-polar fractions showed highest antioxidant activity.

The *o*-substituted phenolic compounds were found more active than unsubstituted phenol. A highly positive relationship between total phenols and antioxidant activity appears to be seen in many plants (Oktay *et al*., 2003). It was found that most of the phenolic compounds present in the plants possess antioxidant properties due their redox properties, which make the phenol as reducing agent, hydrogen donator and singlet oxygen quencher (Kahkonen *et al*., 1999). The presence of reductants such as antioxidant substances in the samples causes a reduction of the Fe^{3+} to Fe^{2+} form. Therefore, the ability of a compound to transfer electron is a significant indicator of its potential as an antioxidant (Sudha *et al*., 2011). The degree of coloration indicates the reduction potential of the compounds.

The antimicrobial activity of different fractions of *B. glabra* flower extract was varied on different types of bacteria. In this study, *S. aureus* showed highest sensitivity with zone of inhibition range from 17-22 mm whereas *P. aeruginosa* showed lowest sensitivity with zone of inhibition range from 0-6 mm compared to Perales and Leysa (2012). According to Hassan *et al*. (2009) could be attributed to presence of higher bioactive compounds in flower extracts. Furthermore, the sensitivity and susceptibility of the microbes to the plant extracts varied. In particular, the fungal strains were highly sensitive and susceptible to the plant extracts than the bacterial strains. Varying degrees of solubility of the active constituents with the solvents might be a reason behind the difference in efficacy of different solvent fractions (Al-Reza *et al*., 2009).

CONCLUSION

In light of the results of present study, it can be concluded that the plant extracts possess moderate to good antioxidant activity comparable to that of standard drugs BHT, ascorbic acid and gallic acid, which led us to the inference that the plant extract may contain bioactive compounds which may aid ongoing anticancer drug discovery from floristic resources. The result also suggested that different solvent fractions of *B. glabra* flower under study showed antimicrobial activity. The antimicrobial action of various fractions of *B. glabra* flower may indicate their potential as antimicrobial herbal remedies. Hence, further studies are suggested to be undertaken to pinpoint the exact compound (s) and to better understand the mechanism of such actions scientifically. This will emphasize on the isolation and characterization of active principles responsible for these activities of *B. glabra*.

REFERENCES

Adebayo, G.I., O.T. Alabi, B.V. Owoyele and A.O. Soladoye, 2009. Anti-diabetic properties of the aqueous leaf extract of *Bougainvillea glabra* (Glory of the Garden) on alloxan-induced diabetic rats. Rec. Nat. Prod., 3: 187-192.

Adebayo, J.O., A.A. Adesokan, L.A. Olatunji, D.O. Buoro and A.O. Aoladoye, 2005. Effect of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. Biokemistri, 17: 45-50.

Adeyeye, E.I. and O.O. Ayejuyo, 1994. Chemical composition of *Cola acuminata* and *Garcinia kola* seeds grown in Nigeria. Int. J. Food Sci. Nutr., 45: 223-230.

Afroze, F. and M.T. Hossain, 2015. Proximate analysis, phytochemical screening and antioxidant activity of *Psidium guajava* leaves growing in coastal area of Bangladesh. World J. Pharm. Pharm. Sci., 4: 140-151.

Al-Reza, S.M., V.K. Bajpai and S.C. Kang, 2009. Antioxidant and antilisterial effect of seed essential oil and organic extracts from *Zizyphus jujuba*. Food Chem. Toxicol., 47: 2374-2380.

Balandrin, M.F., J.A. Klocke, E.S. Wurtele and W.H. Bollinger, 1985. Natural plant chemicals: Sources of industrial and medicinal materials. Science, 228: 1154-1160.

Balch, J.F. and P.A. Balch, 2000. Prescription for Nutritional Healing. Penguin Putnam Inc., New York, pp: 267-270.

Bhat, M., S.K. Kothiwale, A.R. Tirmale, S.Y. Bhargava and B.N. Joshi, 2011. Antidiabetic properties of *Azardiracta indica* and *Bougainvillea spectabilis*: *In vivo* studies in murine diabetes model. Evid.-Based Complement. Altern. Med., Vol. 2011. 10.1093/ecam/nep033

Bolognesi, A., L. Polito, F. Olivieri, P. Valbonesi and L. Barbieri *et al*., 1997. New ribosome-inactivating proteins with polynucleotide: adenosine glycosidase and antiviral activities from *Basella rubra* L. and *Bougainvillea spectabilis* Willd. Planta, 203: 422-429.

Dev, U.K., M.T. Hossain and M.Z. Islam, 2015. Phytochemical investigation, antioxidant activity and anthelmintic activity of *Mikania micrantha* leaves. World J. Pharm. Res., 4: 121-133.

Duh, P.D., 1998. Antioxidant activity of burdock (*Arctium lappa* Linne): Its scavenging effect on free-radical and active oxygen. J. Am. Oil Chem. Soc., 75: 455-461.

Edwin, E., E. Sheeja, E. Toppo, V. Tiwari and K.R. Dutt, 2007. Anti-diarrhoeal, anti ulcer and antimicrobial activities of leaves of *Bougainvillea glabra* Choisy. Ars Pharmaceutica, 48: 135-144.

Hassan, H.S., M.I. Sule, M.A. Usman and A. Ibrahim, 2009. Preliminary phytochemical and antimicrobial screening of they stem bark extracts of *Bauhinia rufescence* Lam using some selected pathogens. Bayero J. Pure Applied Sci., 2: 53-55.

Hossain, M.T., 2015. Antioxidant, cytotoxicity, membrane stabilization and anthelmintic activity of ethanolic extract of *Sarcochlamys pulcherrima* leaves. Int. J. Green Herbal Chem., 4: 274-283.

Hossen, F., M.M.O. Rashid, M.M. Alam, M.S. Akter, M.T. Hossain and K.N. Akhter, 2015. Antibacterial activity of *Areca catechu* L. fruit extract loaded silver nanoparticles. World J. Pharm. Pharm. Sci., 4: 258-266.

Ikegami, F., Y. Fujii, K. Ishihara and T. Satoh, 2003. Toxicological aspects of Kampo medicines in clinical use. Chemico-Biol. Interact., 145: 235-250.

Izzo, A.A., 2004. Drug interactions with St. John's Wort (*Hypericum perforatum*): A review of the clinical evidence. Int. J. Clinc. Pharmacol. Thera., 42: 139-148.

Jisaka, M., H. Ohigashi, T. Takagaki, H. Nozaki and T. Tada *et al*., 1992. Bitter steroid glucosides, vernoniosides A_1, A_2 and A_3 and related B_1 from a possible medicinal plant, *Vernonia amygdalina*, used by wild chimpanzees. Tetrahedron, 48: 625-632.

Jones, F.A., 1996. Herbs-useful plants. Their role in history and today. Eur. J. Gastroenterol. Hepatol., 8: 1227-1231.

Joshny, J., R.D. Devi and B.N.V. Hari, 2012. Phytochemical and *in-vitro* anthelmintic activity of hydro alcoholic extract of *Bougainvillea glabra*. Int. J. Pharm. Pharm. Sci., 4: 115-117.

Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen, 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962.

Kumarasamy, Y., M. Byres, P.J. Cox, M. Jaspars, L. Nahar and S.D. Sarker, 2007. Screening seeds of some Scottish plants for free radical scavenging activity. Phytother. Res., 21: 615-621.

Madziga, H.A., S. Sanni and U.K. Sandabe, 2010. Phytochemical and elemental analysis of *Acalypha wilkesiana* leaf. J. Am. Sci., 6: 510-514.

Malomo, S.O., J.O. Adebayo, R.O. Arise, F.J. Olorunniji and E.C. Egwim, 2006. Effects of ethanolic extract of *Bougainvillea spectabilis* leaves on some liver and kidney function indices in rats. Phytochem. Pharmacol., 17: 261-272.

Mariajancyrani, J., G. Chandramohan, M.F. Beevi and A. Elayaraja, 2013. Preliminary phytochemical investigation and antioxidant activity of *Bougainvillea glabra* choicy leaves. Sch. Acad. J. Biosci., 1: 72-75.

Maurya, R., G. Singh and P.P. Yadav, 2008. Antiosteoporotic agents from natural sources. Nat. Prod. Chem., 35: 517-545.

Mishra, N., S. Joshi, V.L. Tandon and A. Munjal, 2009. Evaluation of anti-fertility potential of aqueous extract of *Bougainvillea spectabilis* leaves in Swiss albino mice. Int. J. Pharm. Sci. Drug Res., 1: 19-23.

Oktay, M., I. Gulcin and O.I. Kufrevioglu, 2003. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT-Food Sci. Technol., 36: 263-271.

Oyaizu, M., 1986. Studies on product of browning reactions: Antioxidant activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr., 44: 307-316.

Perales, Y.J. and M. Leysa, 2012. Phytochemical screening and antibacterial acitivity of *Bougainvillea glabra* plant extract as potential sources of antibacterial and resistance-modifying agents. Proceedings of the International Conference on Life Science and Engineering, October 27-28, 2012, Hong Kong, China, pp: 121-125.

Prieto, P., M. Pineda and M. Aguilar, 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem., 269: 337-341.

Saikia, H. and A. Lama, 2011. Effect of *Bougainvillea spectabilis* leaves on serum lipids in albino rats fed with high fat diet. Int. J. Pharm. Sci. Drug Res., 3: 141-145.

Satish, S., K.A. Raveesha and G.R. Janardhana, 1999. Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. Lett. Applied Microbiol., 28: 145-147.

Sudha, G., M.S. Priya, R.I. Shree and S. Vadivukkarasi, 2011. *In vitro* free radical scavenging activity of raw pepino fruit (*Solanum muricatum*). Int. J. Curr. Pharma. Res., 3: 137-140.

Takao, T., F. Kitatani, N. Watanabe, A. Yagi and K. Sakata, 1994. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotechnol. Biochem., 58: 1780-1783.

Tapsell, L.C., I. Hemphill, L. Cobiac, C.S. Patch and D.R. Sullivan *et al*., 2006. Health benefits of herbs and spices: The past, the present, the future. Med. J. Aust., 185: S4-S24.

Wang, M., J. Li, M. Rangarajan, Y. Shao, E.J. LaVoie, T.C. Huang and C.T. Ho, 1998. Antioxidative phenolic compounds from sage (*Salvia officinalis*). J. Agric. Food Chem., 46: 4869-4873.

Yen, C.C. and P.D. Duh, 1993. Antioxidant properties of methanolic extracts from peanut hull. J. Am. Oil Chem. Soc., 70: 383-386.

Yosef, S., L.J. Raymond and C.M. Gunter, 2001. Sand fly feeding on noxious plants a potential method for the control of leishmaniasis. Am. J. Trop. Hyg., 65: 300-303.