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Appraisal of antioxidant potential and biological studies of bogan bail (*Bougainvillea glabra*) leaf extracts using different solvents

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Abstract: Current research work was performed to evaluate the antioxidant, antidiabetic, thrombolytic, and cytotoxic potential of *Bougainvillea glabra* leaf extracts with different solvents. Extraction of leaves was carried out by maceration using solvents of various polarity such as *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and methanol. The highest values of total phenolic and flavonoid contents were assessed in methanolic extract, such as 58.57% and 29.41%, respectively. Antioxidant potential was measured by DPPH free radical, showing 65.16% scavenging activity in methanolic extract. Similarly, the reducing power of methanolic extract was also measured. Hemolytic activity of *B. glabra* leaf extracts was evaluated, and maximum percentage lysis was found as 6.86% in chloroform extract. The thrombolytic activity of *B. glabra* leaf extracts was evaluated against human red blood cells, and the maximum percentage of clot lysis was 59.10% in chloroform extract. Maximum antidiabetic activity (16.20%) was observed in methanolic extract. Overall, the presented results reveal that bogan bail extract is capable of being employed in nutra-pharmaceutical industry.

Keywords: *B. glabra*; scavenging; thrombolytic; antidiabetic; hemolytic

From the beginning of human civilisation to the present age, the medicinal constituents of plants have been characterised with a definite therapeutic potential and are of vital importance for synthetic medicines. The medicinal plants are most important for maintaining human health in the present era, even in the field of effective synthetic drugs. Non-synthetic and natural drugs have great importance in medical science; these drugs are prepared with plant constituents because of their low cost, non-toxic nature, and easy availability (Akhtar et al. 2020).

Many plants possessing flavonoids and phenolic compounds with profound antioxidant potential have gained a lot of importance in nutra-pharmaceuticals (Qadir et al. 2019, 2020). These have been reported to produce preventive effects against various complex diseases like cardiovascular disorders, ageing processes, inflammatory, and diabetic problems (Nostro et al. 2000).

Bougainvillea glabra also known as bogan bail, belonging to *Nyctaginaceae* family, is a green, ornamental, and woody shrub found in warm regions. It is native to Brazil and also introduced to areas like the Middle East, the Indian sub-continent, and North America. In Mandsaur, the traditional practitioners have a preference for *B. glabra* leaves for the treatment of a variety of disorders, such as diarrhoea and stomach

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acidity. In Panama, *B. glabra* is used in the treatment of low blood pressure. According to the phytochemicals screening tests, it has been revealed that phytochemicals such as alkaloids, tannins, reducing sugar, saponins, and cardiac glycosides are present in *B. glabra* leaf extracts that may be linked to therapeutic properties such as anti-pyretic, analgesic, antibacterial, anti--diarrheal, anti-inflammatory and antioxidant activities (Devasagayam and Sainis 2002). In line with potent medicinal activities, this work was planned to investigate the antidiabetic, cytotoxic, and biological effects of *B. glabra* leaf extracts in relation to different solvents.

MATERIAL AND METHODS

B. glabra leaves were collected from Layyah, Pakistan. A voucher specimen (CHEM-06/15) was allocated after plant recognition and authentication by taxonomist Dr. Muhammad Haneef (Department of Botany, University of Sargodha, Woman Campus Faisalabad, Pakistan). The leaves were washed properly with water, shade dried, and grounded very well (grinder AB-03; Absons, Pakistan). A 100 g powder of sample was macerated in 1 L of each solvent such as *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and methanol using an orbital shaker (model GFA-H-42/250; Pamico, Pakistan) for 24 h. Each filtrate was concentrated using a rotary evaporator (model DIN EN 60529; Heidolph, Germany), and the extract obtained was kept in the refrigerator at 4 °C. The percentage yield of extract was calculated on the basis of dry powder and solvents used.

Determination of total phenolics contents (TPC)

Total phenolics content (TPC) was calculated by the method of Chaovanalikit and Wrolstad (2004). The mixture containing 0.01 g mL^{-1} plant extract, 7.5 mL distilled water, and 0.5 mL Folin-Ciocalteu reagent were agitated in 1.5 mL of sodium carbonate 20% (w/v) and boiled at 40 °C for 20 min (water bath, model WNB14; Memmert, Germany). The absorbance was calculated at 755 nm using gallic acid as the standard.

Test for total flavonoid contents (TFC) determination

Total flavonoid content (TFC) was calculated by the method as explained by Dewanto et al. (2002). The plant extract (0.01 g mL^{-1}) and 5 mL distilled water along with reported quantities such as 0.3 mL of 5% $NaNO_2$, 0.3 mL of 10% $AlCl_3$ and 1 M of NaOH were mixed thoroughly in a test tube. Absorbance was measured at 510 nm. TFC was estimated as catechin equivalents (CE, g 100 g^{-1}).

Estimation of antioxidant activity

DPPH free radical determination. DPPH free radical scavenging activity was evaluated by the method of Iqbal et al. (2005). Plant extract (1 mg mL^{-1}) was diluted in methanol, and sample solution (1 mL) was added to DPPH (2.5 mL). DPPH absorbance of the sample (0.025 g L^{-1}) was calculated at 515 nm after 30 min. Three replicated readings were taken for each sample.

Reducing power determination. Reducing power was calculated by using the method of Yen et al. (2000) with slight modification. Solvent extracts of *B. glabra* leaves (2.5–10 mg mL^{-1}) were mixed with sodium phosphate buffer (5.0 mL, 0.2 M, and pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%). Incubation of mixture was done for 20 min at 50 °C (incubator, model 30-1060; Memmert, Germany) after the addition of trichloroacetic acid (5.0 mL, 10%). Absorbance was noted at 700 nm.

Hemolytic activity

The hemolytic activity of *B. glabra* leaves was evaluated by the method of Powell et al. (2000). For each assay, the plant extracts having a concentration 1 mg mL^{-1} were used to evaluate the hemolytic activity. Triton X-100 (0.1%) was used as a positive control, and phosphate buffer saline (PBS) was taken as a negative control. The absorbance was noted at 576 nm. The percentage lysis of red blood cells (RBCs) was calculated.

Thrombolytic activity

The thrombolytic activity was performed as described by Prasad et al. (2007). Blood (500 µL) was incubated at 37 °C for 40 min (incubator, model 30-1060; Memmert, Germany). After 40 min, blood clot formation was done; serum was separated out with the help of a micropipette. For this assay, 1 mg mL^{-1} of each extract was added in clotted blood to evaluate clot lysis properties of extracts. Streptokinase and distilled water were taken as positive and negative controls, respectively. The percentage clot lysis was calculated.

Antidiabetic activity

Sodium phosphate buffer (25 mL, 6.9 pH) was dissolved in 0.012 g of alpha-amylase. Solvent extract (1 mg mL^{-1}) was mixed with 500 µL of alpha-amylase solution. This mixture was incubated for 10 min at 25 °C (incubator, model 30-1060; Memmert, Germany). On cooling the mixture, 500 µL starch solution was added and incubated for 10 min at 25 °C. DNSA

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solution (1 mL) was added to it, and the mixture was heated for 5 min in the water bath, and absorbance was measured at 540 nm. The experiments were performed in triplicate. The mean and standard deviation were calculated statistically.

Statistical analysis

The experiments were performed in triplicate. The results were compiled by calculating the mean and standard deviation by using MS Excel 2016.

RESULTS AND DISCUSSION

Extract yield of *B. glabra* leaves. Extraction of leaves of *B. glabra* was carried out by employing five different solvents such as *n*-hexane, ethyl acetate, *n*-butanol, chloroform, and methanol. The highest percentage yield was observed in absolute methanolic extract, while the lowest was in *n*-hexane extract. The % yield of extracts in particular solvents is summarised in Table 1. The extract yield for powdered plant material with different polarity-based solvents were ranging from 0.49 to 1.87 g 100 g^{-1}. The findings reported in this study are very close to the results of Samad et al. (2007), showing good effectiveness of methanol for extraction of phytochemicals from different parts of plants. It might be concluded that the yield of plant extracts also depends on the nature of solvents being used in the extraction process (Riaz et al. 2012; Rizwan et al. 2012).

Table 1. The per cent yield of *B. glabra* leaves extracts

Plant extracts	Per cent yield (g 100 g^{-1})
n-hexane	0.49 ± 0.01
Chloroform	1.11 ± 0.04
Ethyl acetate	1.40 ± 0.02
n-butanol	1.44 ± 0.03
Methanol	1.87 ± 0.01

B. glabra – *Bougainvillea glabra* (bogan bail); *n* = 3

Antioxidant analysis. The results presented in this study revealed that total flavonoids, phenolics, and DPPH radical scavenging activity varied widely between different types of extracts as presented in Table 2. Overall, the TFC values in samples of solvent extracts were in the range of 6.91–29.41 (g 100 g^{-1}) CE. Total phenolic contents obtained for solvent extracts of *B. glabra* leaves ranged from 28.35 to 158.57 (g 100 g^{-1}) gallic acid equivalent (GAE). Highest TFC (29.41 g 100 g^{-1}) and TPC (158.57 g 100 g^{-1}) values were found in the methanolic extract, while *n*-hexane extract exhibited the lowest TFC (6.91 g 100 g^{-1}) and TPC (28.35 g 100 g^{-1}) values. Total phenolic contents of dry matter of *B. glabra* leaves extracts decreased in the following order: methanol > > *n*-butanol > ethyl acetate > chloroform > *n*-hexane.

The results of the antioxidant power of solvent extracts employed in this study varied according to the nature of the solvent used and particularly to the method of analysis. Scavenging activity of powdered plant material in different polarity-based solvents ranged from 57.87% to 89.64%. The higher scavenging activity was observed as 65.16% for methanol, while a lower activity (57.87%) in the case of *n*-hexane. In this assay, the synthetic antioxidant butylated hydroxyl toluene (BHT) was used as a positive control. According to earlier results reported by Kumar et al. (2012), the aqueous and methanolic extracts of *B. glabra* exhibited scavenging activity against DPPH radical. Similar investigations were carried out by Carlson et al. (2008), who indicated that the antioxidant potential is dependent on percentage scavenging.

Reducing power determination. The reducing power of solvent extracts was evaluated at different concentrations (Figure 1). It was observed that methanolic extract had the highest reducing power in comparison to other solvent extracts employed in the current study.

Table 2. TFC, TPC and DPPH percentage scavenging of *B. glabra* leaves extracts (*n* = 3)

Plant extracts	TFC (CE, g 100 g^{-1})	TPC (GAE, g 100 g^{-1})	DPPH percentage scavenging (%)
n-hexane	6.91 ± 0.01	28.35 ± 0.02	57.87 ± 0.38
Chloroform	7.45 ± 0.02	49.47 ± 0.04	59.40 ± 0.58
Ethyl acetate	10.18 ± 0.01	82.19 ± 0.05	61.20 ± 0.07
n-butanol	12.40 ± 0.02	133.6 ± 0.10	62.77 ± 0.06
Methanol	29.41 ± 0.04	158.57 ± 0.10	65.16 ± 0.05
BHT	–	–	89.64 ± 0.07

TFC – total flavonoid contents; TPC – total phenolics content; DPPH – 2,2-diphenyl-1-picrylhydrazyl; CE – catechin equivalents; GAE – gallic acid equivalents; BHT – butylated hydroxyl toluene; *B. glabra* – *Bougainvillea glabra* (bogan bail)

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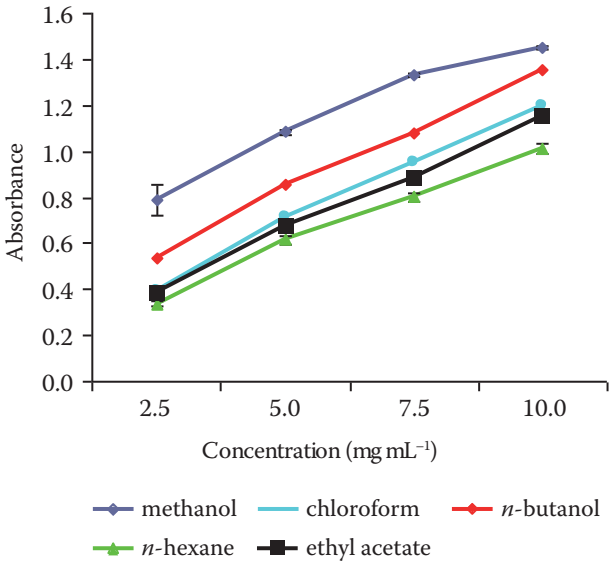

Figure 1. Reducing power of solvent extracts at different concentrations

The lowest reducing power was recorded for *n*-hexane extract. Effective results shown by methanolic extracts can be related to the strong extraction efficacy of methanol as compared to others. Reducing power is directly linked to the antioxidant potential of the plant extract as described earlier (Siddhuraju and Becker 2003). Therefore, the evaluation of the antioxidant potential of plants can be studied by using the reducing power assessment as a parameter.

Hemolytic and thrombolytic activities. Hemolytic and thrombolytic activities of *B. glabra* leaves were evaluated against human RBCs. The percentage hemolysis and thrombolysis of relative extracts are given in Table 3. Hemolytic activity of plant material ranged from 1.02% to 6.86%. Maximum hemolysis was obtained for chloroform extract, while on the other side, the minimum value of 1.02% was obtained for methanolic extract. Triton X-100 used as a positive control, demonstrated a maximum percentage lysis value 99.49%. In the case of thrombolytic activity, results also revealed that all extracts showed remarkable thrombolysis of blood clots ranging from 30.66–59.10%. The *in vitro* thrombolytic activity study summarised that *B. glabra* leaves showed the highest clot lysis (59.10%) in chloroform extract and lowest (30.66%) in *n*-butanol extract. Water was taken as a negative control (16% clot lysis), while streptokinase was taken as a positive control (80.19% clot lysis). The present results are very close to the findings of Ali et al. (2015), who investigated the significant thrombolytic effects of some medicinal plant extracts against blood clots.

Table 3. Per cent RBC lysis and clot lysis of *B. glabra* leaves extracts (n = 3)

Plant extracts	Per cent RBCs lysis	Per cent clot lysis
n-hexane	4.95 ± 0.02	55.20 ± 0.03
Chloroform	6.86 ± 0.04	59.10 ± 0.05
Ethyl Acetate	4.79 ± 0.02	34.51 ± 0.03
n-butanol	5.65 ± 0.04	30.66 ± 0.05
Methanol	1.02 ± 0.03	32.79 ± 0.03
Triton X-100	99.49 ± 0.19	–
Streptokinase	–	80.19

RBC – red blood cell; *B. glabra* – *Bougainvillea glabra* (bogan bail)

Table 4. Per cent antidiabetic activity of *B. glabra* leaves extracts (n = 3)

Plant extracts	Per cent antidiabetic activity
n-hexane	6.32 ± 0.024
Chloroform	10.83 ± 0.042
Ethylacetate	9.09 ± 0.023
n-butanol	15.02 ± 0.024
Methanol	16.20 ± 0.011

B. glabra – *Bougainvillea glabra* (bogan bail)

Antidiabetic activity. The percentage yield of extracts was measured as indicated in Table 4. Antidiabetic activity of the methanolic extract was found maximum (16.20%) as compared to other extracts. While a minimum antidiabetic potential (6.32%) was observed for *n*-hexane extract. The percentage of antidiabetic activity was compared to standard acarbose medicine (62.47%).

CONCLUSION

This study appraised the antioxidant, cytotoxic, and antidiabetic potential of bogan bail extract using a different range of particular solvents. It was also observed that the plant extract significantly exhibited phenolic and flavonoid contents, which are responsible for its antioxidant potential and hence lower hemolytic activity. Furthermore, the presence of some important bioactive such as phenolics lends better antidiabetic activity in bogan bail extract. Overall, methanolic extract offered better TPC (58.57%), TFC (29.41%), and antidiabetic potential (16.20%) as compared with other extracts. Higher antioxidant character and lower cytotoxic behaviour of bogan bail extract may be used as food content/medicine in nutra-pharmaceutical industries.

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