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Original Article

Phytochemical and biological attributes of *Bauhinia variegata* L. (Caesalpiniaceae)

Atributos fitoquímicos e biológicos de *Bauhinia variegata* L. (Caesalpiniaceae)

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Abstract

Bauhinia variegata plant is a very popular and traditionally potent ethnomedicine. Therefore, it is need of hour to study ameliorative characteristics of *B. variegata* for novel secondary metabolites. The current study was designed to explore antiproliferative potential of *B. variegata* due to scant reports on this aspect. Extracts of various parts (flowers, leaves, bark, stem, and roots) were prepared by successive maceration using organic solvents in increasing order of polarity (*n*-hexane, ethyl acetate, methanol, and water). The determination of polyphenolic contents was done by using colorimetric methods while antioxidant potential was measured using reducing power assay. Brine shrimp lethality assay was performed for determining preliminary cytotoxicity and antiproliferative activity against breast cancer MCF-7 cell line using MTT protocols. Moreover, antimicrobial activities were detected by using disc diffusion assay. The alpha-amylase assay was performed to monitor the antidiabetic potential of the plant. In case of phytochemical analysis methanolic extract of leaves and bark showed highest phenolic and flavonoids contents. *n*-Hexane and ethyl acetate extracts of stem and roots exhibited more than 90% mortality with LD_{50} ranges between 1-25 µg/mL when studied by brine shrimp lethality assay. *n*-Hexane and ethyl acetate extracts of roots and stem also showed antiproliferative activity against human breast cancer MCF-7 cell line with IC_{50} values ranges between 12.10-14.20 µg/mL. Most of the extracts displayed moderately high antibacterial and antifungal activities. The *n*-hexane extract of roots showed antidiabetic activity with 60.80 ± 0.20% inhibition of alpha-amylase. In sum, these preliminary results will be useful for further compound isolation from selected plant parts for the discovery of antibacterial, antidiabetic, and anticancer lead candidates.

Keywords: *Bauhinia variegata*, antiproliferative activity, MCF-7, antioxidant activity, antidiabetic activity, antimicrobial activity.

Resumo

A planta *Bauhinia variegata* é uma etnomedicina muito popular e tradicionalmente potente. Portanto, as características de melhoria de *B. variegata* foram estudadas. Foi avaliada a determinação dos teores antioxidantes e polifenólicos. O ensaio de letalidade do camarão de salmoura foi realizado para determinar a citotoxicidade preliminar e a atividade antiproliferativa contra linhas de células de câncer de mama MCF-7 usando protocolos de MTT. Além disso, foram detectadas atividades antimicrobianas. O ensaio da alfa-amilase foi realizado para monitorar o potencial antidiabético da planta. Dentre vinte amostras diferentes, o extrato metanólico (EM) da casca apresentou os maiores teores fenólicos totais. A EM das folhas apresentou excelente conteúdo de flavonoides, atividade antioxidante significativa foi exibida pelo extrato hexânico do caule. O extrato do caule de hexano exibe 77,40% como citotóxico em DL50 10,50 µg/mL quando avaliado através do ensaio de letalidade de artêmia. Extratos de hexano e acetato de etila de raiz e caule mostraram atividade antiproliferativa contra a linhagem celular MCF7 de câncer de mama humano (IC50 12,10-14,20 µg/mL). Para potencial antimicrobiano importante, vários extratos exibiram excelentes atividades antibacteriana e antifúngica, enquanto o extrato de n-hexano da raiz mostrou atividade antidiabética (60,80 ± 0,20% na concentração de 200 µg/mL). Em suma, estes resultados preliminares serão úteis para isolamento adicional de compostos a partir de partes de plantas selecionadas para a descoberta de candidatos a antibacterianos, antidiabéticos e anticâncer.

Palavras-chave: *Bauhinia variegata*, atividade antiproliferativa, MCF-7, atividade antioxidante, atividade antidiabética, atividade antimicrobiana.

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1. Introduction

Plants are well known for providing ordinary products for sustaining human health (Lewis and Elvin-Lewis, 1995; Mahmood et al., 2011). Worldwide, by approximately 80% of individuals use traditional medicines in one or another form even in developed countries (Ihsan-ul-Haq et al., 2013). More than 30% of all pharmacological preparations are based on the active ingredients of plants (Shinwari and Gilani, 2003), the investigation of such plant sources is crucial for an improved understanding of their efficacy, safety, and pharmacological properties (Verpoorte, 2000). The plant parts that are commonly used are flowers, leaves, bark, stem, and roots (Ali et al., 2015). The investigation of medicinal plants continues to yield new and valuable remedies (Patil, 2011).

To support the ethnomedical use of such folkloric medicines, *Bauhinia variegata* was selected for the current study. *B. variegata* is a flowering plant that belongs to the family Leguminosae (subfamily: Caesalpiniaceae) and is native to Southeast Asia, southern China, and western India. It is also known as the orchid tree or mountain ebony, and its local name in Pakistan, is 'kachnar'. It is cultivated as a decorative tree due to the intricate appearance of its flowers (Sudheerkumar et al., 2015). In March and April, the outgrowths of kachnar are roasted and eaten as a delicious food (Basumatari and Das, 2017). The bark is used for salivation, sore throat, cough, haemorrhoids, hematuria, menorrhagia, enlargement of the glands of the neck, and tumors (Sudheerkumar et al., 2015), while decoction of the bark is beneficial for treating ulcers and skin diseases, and the dried buds are useful for treating diarrhoea, worms, haemorrhoids, and dysentery. Leaves are used in diabetes and inflammatory disorders (Basumatari and Das, 2017). The decoction of the kachnar roots is used for indigestion and its flowers have laxative activity. Stem is used as anti-inflammatory in skin diseases. A preparation known as kachnara guggula is useful for treating tumors, ulcers, and skin diseases as well as for supporting the healthy function of the thyroid and the lymphatic system (Nadkarni, 1996).

The current study explored the biological activities of *B. variegata* by employing four solvents of different polarities for successive extraction. Phytochemical screening of various extracts was conducted through the analysis of the phenolic and flavonoid content. The current study provides a more complete understanding of the potent activities of *B. variegata*, which serves as a foundation for the further investigation of valuable compounds for drug development.

2. Materials and Methods

2.1. Plant collection and identification

Fresh plant parts were collected from a village area of Murree District Rawalpindi, Punjab Pakistan in March 2017. The plant was identified as *Bauhinia variegata* (Caesalpiniaceae) by Dr. Abdul Nazir, Assistant professor (Department of Environmental Sciences) COMSATS University Islamabad, Abbottabad Campus, and voucher no. for the identified plant is CUHA-195. Five plant parts, flowers, leaves, bark, stem, and roots were collected. These plant parts were dried at room temperature. After adequate drying, plant material was ground to obtain a fine powder. The powdered material of plant parts was weighed and stored for further usage.

2.2. Chemicals

Methanol, ethyl acetate, ethanol, dimethyl sulfoxide (DMSO), and *n*-hexane were purchased from Merck (Germany). Aluminum chloride, potassium dihydrogen phosphate, 2, 2-diphenyl, 1-picry hydrazyl (DPPH), sulfuric acid, Folin-Ciocalteu reagent, ferrous chloride, trichloroacetic acid, ammonium molybdate, sodium hydroxide, di-potassium hydrogen phosphate, roxithromycin, doxorubicin, clotrimazole, cefixime, taxol, ascorbic acid, gallic acid, and quercetin were purchased from Sigma (USA). ISP4 medium was prepared in the lab. The sabouraud dextrose agar (SDA) was purchased from Oxoid, England.

2.3. Extract preparation

For extraction purpose, different solvents like *n*-hexane, ethyl acetate, methanol, and water were used. 150 grams of dried powdered material of each plant part was soaked in 450 mL of the respective solvent. Soaked plant material was macerated for three days with regular shaking at room temperature. After 3 days, the samples were filtered using Whatman's filter paper no.1, and the obtained solvent was collected. This procedure was repeated second and third days. Filtrates were pooled, concentrated and crude extracts were prepared by evaporating solvents using rotary evaporator. Prepared extracts were stored in 20 mL glass vials. Extract yield was calculated by Equation 1:

$$Extract\ recovery\,(\%) = \frac{A}{B} x100 \quad (1)$$

where "A" is dried extract total weight obtained after drying and "B" is ground plant material total weight taken for each extraction.

2.4. Extract solutions preparation

Stock solutions were prepared in DMSO at 4.0 mg/mL (for phytochemical assay) and 20 mg/mL (for biological assay). These stock solutions were ultrasonicated to make clear solution for further use.

2.5. Phytochemical and biological evaluation

2.5.1. Total phenolic contents determination (TPC)

The Folin-Ciocalteu reagent method of TPC estimation was adopted as described by (Fatima et al., 2015). An aliquot of 20 µl (4 mg/mL DMSO) solution was poured into the labeled 96 well plate then the reagent Folin-Ciocalteu 90 µl was added. After that mixture was incubated for five minutes, and Na_2CO_3 (90 µl) was added to the respective wells. Using a microplate reader, (Biotech USA, microplate

reader Elx 800) absorption was taken at 630 nm each well. The reference standard was gallic acid. Dilutions of positive control (6.25-50 µg/mL) were used to obtain the calibration curve. Analysis was repeated three times and the results were expressed as (µg GAE/mg DW).

2.5.2. Total flavonoid contents determination (TFC)

The aluminum chloride colorimetric method was used to estimate total flavonoid contents (Fatima et al., 2015). In 96 well plate, 20 µl stock solution (4 mg/mL DMSO) was poured. Aluminum chloride (10 µl) 10% w/v and (10 µl) potassium acetate, followed by the distilled water 160 µl was added into each well. The mixture was incubated at room temperature for 40 minutes. After incubation at 415 nm absorbance was recorded. Diluting the quercetin concentrations at 2.5, 5, 10, 20, 40 µg/mL calibration curve was plotted. The experiment was repeated three times and the experimental results were mentioned as (µg QE/mg DW).

2.5.3. Determination of the free radical scavenging activity (FRSA)

The antioxidant potential of crude extracts was determined by monitoring their quenching ability of stable 2,2-diphenyl, 1-picrylhydrazyl (DPPH) free radical (Ul-Haq et al., 2012). The spectrophotometric method was adopted to measure the percent free radical scavenging activity (%FRSA), and to monitor 50% scavenging (inhibition) concentration (SC_{50}). The quenching ability of DPPH was mentioned as IC_{50} which is the required concentration to inhibit free radical generation by 50%. Four dilutions of the samples (20 µl), to achieve concentrations of 200, 66.66, 22.22, 7.40 (µg/mL), were mixed with 180 µl (9.2 mg/100 mL) methanol DPPH solution in polystyrene 96 well plates. The absorbance of the dilutions was measured at 517 nm after 30 minutes of incubation at 37°C using a microplate reader. The following Equation 2 was used to calculate percent free radical scavenging activity (% FRSA):

$$\%FRSA = \left(1 - Ab_S / Ab_c\right) * 100 \quad (2)$$

where Abs is the DPPH solution with sample absorbances and Abc is the negative control containing the reagent absorbance without sample. Positive control was ascorbic acid, and the experiment was repeated three times. For the crude extracts, IC_{50} was calculated for those samples showing maximum radical scavenging activity by adopting the dilution method.

2.5.4. Determination of total antioxidant capacity (TAC)

Using a phosphomolybdenum assay, total antioxidant capacity was determined. A reagent in (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) 900 µl was combined with 100 µl of extract sample. DMSO was considered a blank solution. In a water bath, the mixture was kept for 90 minutes. After cooling, with a PDA spectrophotometer (8354 Agilent Technologies, Germany) at 695 nm, absorption of standard and test samples was measured. The test was repeated three times. A standard curve was made using different dilutions of ascorbic acid. Results of activity were reported as (µg AAE/mg DW) as reported earlier (Fatima et al., 2015).

2.5.5. Determination of total reducing power (TRP)

To measure the reducing capacity of samples potassium ferricyanide assay was performed (Fatima et al., 2015). Phosphate buffer 400 µl and (1%) potassium ferricyanide was added with an aliquot extract of 200 µl (4 mg/mL DMSO) then the mixture was incubated at 40°C for 35 minutes. After adding (10%) of trichloroacetic acid 400 µl in the mixture at room temperature for 10 minutes the reaction mixture was centrifuged at 3000 rpm. Distilled water 500 µl and (0.1%) of $FeCl_3$ 100 µl was mixed with (500 µl) separated supernatant and at 700 nm absorption was taken. The controlled substance was ascorbic acid. The calibration curve was obtained by performing dilutions from 6.25 to 100 µg/mL. The sample reducing power was shown as (µg AAE /mg DW).

2.5.6. Cytotoxicity testing of the brine shrimp lethality assay

96 well plate had been successfully used to make a brine shrimp lethality assay based on a predefined protocol (Bibi et al., 2011). *Artemia salina* test eggs were incubated in seawater at 30°C for two days adding 6 mg/L dry yeast. Two chambers containing a specially designed tank were used for this purpose. Eggs were laid in the larger portion and covered with aluminum foil. With the help of a lamp, the smaller portion was lighted. Pasteur's pipet was used for transferring harvest nauplii to a small beaker. Shrimp larvae were transferred from the beaker to 96 well microplate and seawater was added to the 96 well plate in such a way that the entire concentration of DMSO remained less than 1%. With concentrations of various dilutions, output was tested. Doxorubicin was a controlled drug whereas DMSO was blank control. After 24 hours, the mortality rate was measured by looking at the total number of surviving shrimps with the help of microscopic method. All tests were performed in triplicate.

2.5.7. Cytotoxicity determination against human breast cancer MCF-7 cell line

The MCF-7 breast cancer cell line (ATCC # TIB202) was utilized to determine the cytotoxicity of extracts following the already reported protocol (Bibi et al., 2011). Breast cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM) media along with 10% fetal bovine serum and 10% antibiotic. These cells then maintained at 37°C and in an atmosphere with 5% CO_2. In each well of 96 microplates, approximately 190 µl of MCF7 cells were shifted at concentration of $1x10^4$ cell/well. Test extracts were added in triplicate during the final screening. Taxol acts as positive control while 1% DMSO acts as a negative control. The plates were placed at 37°C for 4 h in a humid CO_2 incubator after adding 20 µl solution of MTT. After removing media, 100 µl of DMSO was added to each well of the plate to dissolve the formazan crystals. Using a microplate reader, absorption was observed at 540 nm. The assay was repeated three times. IC_{50} of samples was calculated.

2.5.8. Antimicrobial assays

2.5.8.1. Determination of antibacterial activity

To test the strength of antibacterial activity in samples of *B. variegata*, a disc diffusion method was used (Fatima et al., 2015). Freshly prepared bacterial strains of *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumoniae,* and *P. aeruginosa* were streaked to make lawn on the nutrient agar plates. Test samples 5 µl, cefixime, and roxithromycin 5 µl, as the reference standard and negative standard, were loaded into complete sterile discs and packaged these all plates. After 24 hours of incubation at 37°C, the diameter of the sample inhibition zone and control drugs were measured. The area represented by diameter greater than 10 mm zone of inhibition was considered significant for the test results of the samples to determine MIC. The microtiter plates were stored at 37°C to incubate for getting results. The lowest concentration of the inhibitory growth was expressed as its MIC. The experiment was performed thrice.

2.5.8.2. Determination of antifungal activity

The power of antifungal activity in samples of *B. variegata* was investigated by the agar disc diffusion method (Fatima et al., 2015). The fungal species, *F. solani*, *A. fumigatus*, *A. flavus*, *A. niger* and *Mucor.* species were mixed in Tween 20. Standard turbidity 100 µL of pre-adjusted mold was wrapped in dextrose agar. Standard drug clotrimazole and sample each 5 µl were applied on the sterile disc arranged on media plates. Plates were kept at 30°C for 48 hours and the zone of inhibition around the disc was measured in mm. The samples exhibiting an inhibition area ≥10 mm diameter were tested to detect minimum inhibitory concentrations (MIC) at the lowest concentration range.

2.5.9. Determination of antidiabetic activity

The antidiabetic potential was determined by the alpha-amylase inhibition assay (Kim et al., 2000). A reaction mixture containing 10 µl sample (4 mg/mL DMSO), phosphate buffer 15 µl, alpha-amylase enzyme 25 µl, and 40 µl starch was prepared and placed for 30 minutes on 96 well plates. The reaction was stopped by adding 1M, 20 µl of HCl at 50°C. Then iodine reagent 90 µl was added to each sample. The void was corrected by adding DMSO to the sample followed by an enzyme solution.

2.6. Statistical analysis

Results of *B. variegata* obtained from phytochemical, antimicrobial, and cytotoxic procedures were repeated thrice and expressed as mean ± SD. Statistical analysis were performed by one way analysis of variance (ANOVA) by using Graph Pad prism8 package.

3. Results

Successive maceration method of extraction was used with four solvents (*n*-hexane, ethyl acetate, methanol, and water). Each plant part (powder of each part) had been subjected to maceration with the above sequence of solvents and supernatant collected, concentrated, and saved for further use. Extract recovery of *B. variegata* was given in Table 1. The results showed maximum extract yield in the distilled water, methanol while the lowest yield was observed in *n*-hexane, ethyl acetate extracts w/w, respectively.

3.1. Determination of TPC and TFC

Among twenty different extract samples, the highest TPC (60.62 ± 0.29) was observed in methanol extract (ME) of stem while the lowest TPC was shown by *n*-hexane extract of bark. ME of leaves expressed high flavonoid contents with (55.47 ± 0.19 µg QE/mg DW) whereas the lowest TFC was observed in the *n*-hexane extract of bark as presented in Figure 1.

3.2. Biological evaluation

3.2.1. Antioxidant assays

3.2.1.1. FRSA determination

The results of FRSA showed that water and methanolic extract of all plant parts possess significant free radical scavenging potential with values ranging between 85 to 93%. Methanolic extract of stem showed 93.43 ± 0.19% with IC_{50} value of 51 µg/mL (Figure 2).

3.2.1.2. Total Antioxidant capacity and Total Reducing Power estimation

Total antioxidant capacity (TAC) assay results indicated that methanolic extract showed highest values for all plant

Table 1. Extract recovery and respective solvents used for extraction of *Bauhinia variegata*.

Plant parts	Extract (*n*-Hexane)		Extract (Ethyl-acetate)		Extract (Methanol)		Extract (Water)	
	Weight (grams)	% Recovery	Weight (grams)	% Recovery	Weight (grams)	% Recovery	Weight (grams)	% Recovery
BVF	2.0	1.33	7.0	4.66	15.0	10.00	20.0	13.33
BVL	5.0	3.33	11.0	7.33	21.0	14.00	26.0	17.33
BVB	6.2	4.13	9.0	6.00	16.0	10.66	19.5	13.00
BVS	1.0	0.66	4.0	2.66	10.0	6.66	13.0	8.66
BVR	1.7	1.13	5.3	3.53	25.0	16.66	32.0	21.33

Flowers (F); Leaves (L); Bark (B); Stem (S); Root (R); and BV = *Bauhinia variegata*.

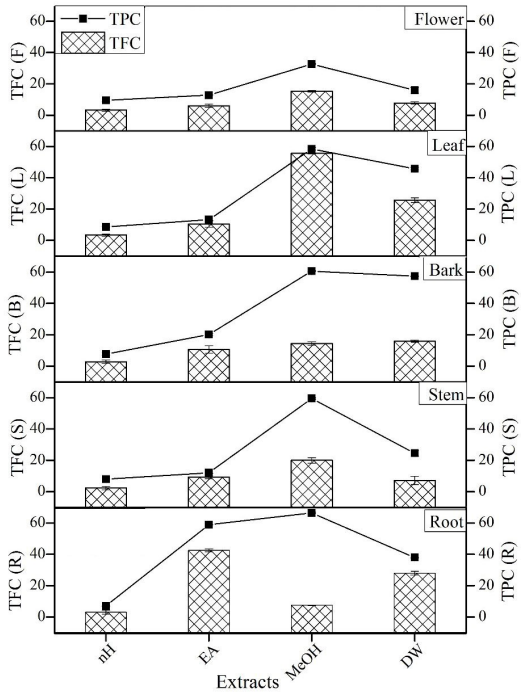

Figure 1. Total phenolic content (TPC) and total flavonoid content (TFC) of *B. variegata* in different extracts. Values are presented as mean ± Standard deviation (n = 3). nH = *n*-hexane; EA = ethyl acetate; MeOH = methanol; DW = distilled water; (S) = stem; (L) = leaf; (F) = flower; (B) = bark; (R) = root.

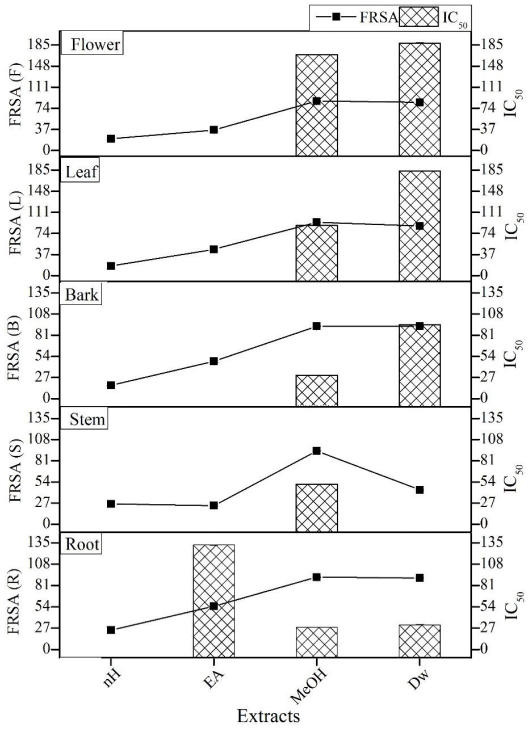

Figure 2. Free radical scavenging activity (FRSA) of free radical in different extracts. Ascorbic acid used as standard drug with an IC_{50} 21.8 ± 0.28 µg/mL. All results are shown in triplicate as mean ± SD. nH = *n*-hexane; EA = ethyl acetate; MeOH = methanol; DW = distilled water; IC_{50} = concentration for 50% inhibition; (S) = stem; (L) = leaf; (F) = flower; (B) = bark; (R) = root.

part extracts. However, methanolic extract of stem showed highest TAC of 99.97 ± 3.97 µg AAE/mg DW (Figure 3).

The trend of total reducing power results also showed similar pattern in case of each plant part extract. Methanolic extract of stem possessed significant TRP with values of 274.87 ± 2.30 µg AAE/mg DW as depicted in Figure 3. However, less polar extracts showed low reducing potential.

3.2.2. Cytotoxicity assessment

3.2.2.1. Brine shrimp lethality assay

The cytotoxicity of plant extracts was determined by using brine shrimp lethality assay. It was observed that most of the samples showed significant percent mortality in low and moderately high polar extracts. *n*-Hexane and ethyl acetate extracts of stem and roots exhibits more than 90% mortality with LD_{50} ranges between 1.58 and 13.79 µg/mL, respectively. However, *n*-hexane and ethyl acetate extracts of roots also showed significant mortality with LD_{50} 10.4 to 24.02 µg/mL, respectively. Overall results of this assay showed that samples possess cytotoxicity (Figure 4).

3.2.2.2. Cytotoxicity against human breast cancer MCF-7 cell line

According to results of cytotoxicity assay against MCF7 cell line, seven out of total twenty samples showed significant antiproliferative activity. *n*-Hexane and ethyl acetate extracts of stem showed 80.41 and 93.02% inhibition, respectively. Similarly, *n*-hexane and ethyl acetate extracts of bark and root showed more than 70% of inhibition with IC_{50} values ranging between 12 to 15 µg/mL. However, in case of leaf methanolic extract showed 74.41% inhibition against cancer cell line.

Taxol was a standard control having an IC_{50} value of 2.9 ± 0.025 µg/mL. Results of the antiproliferative activity of *B. variegata* extracts are summarized in Table 2.

3.2.3. Antimicrobial activity determination

3.2.3.1. Antibacterial assay

The antibacterial activity was determined by calculating the zone of inhibition. Extracts showed more activity against *B. subtilis*. Methanol, ethyl acetate, and *n*-hexane extracts producing the greater inhibition i.e., 26 ± 0.32, 21 ± 0.23, and 19 ± 0.13 mm. The activity of the extracts was compared with roxithromycin as standard which produced a zone of inhibition 28 ± 0.43 mm at 10 µg/disc concentration. The results are shown in Table 3.

3.2.3.2. Antifungal assay

The antifungal potential of *B. variegata* was also checked against five different strains of fungus: *F. solani*, *A. fumigatus*, *A. flavus*, *A. niger,* and *Mucor* species, and the activity of the extract was determined by determining the inhibition in mm around the disc. The antifungal activity was found

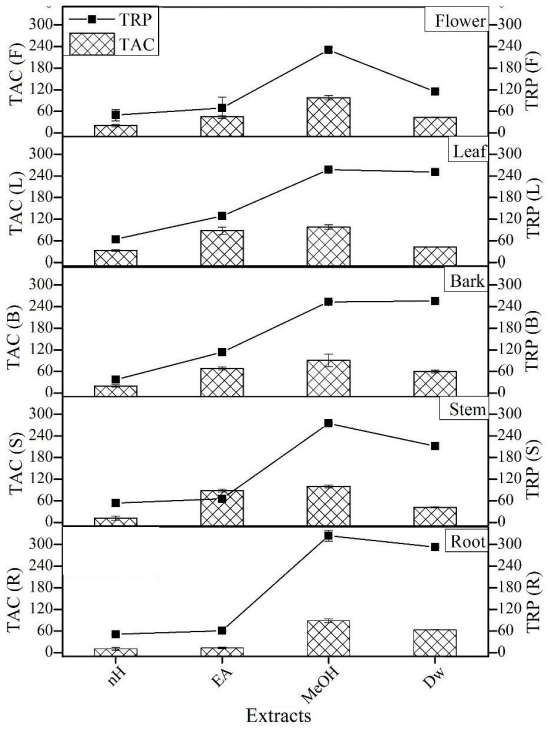

Figure 3. Total antioxidant capacity (TAC) and total reducing power (TRP) of different extracts. Values are presented as mean ± Standard deviation (n = 3). nH = *n*-hexane; EA = ethyl acetate; MeOH = methanol; DW = distilled water; (S) = stem; (L) = leaf; (F) = flower; (B) = bark; (R) = root.

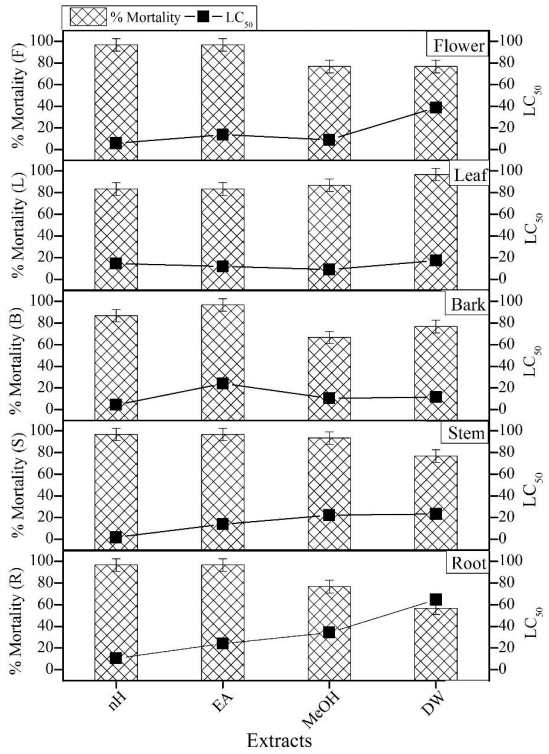

Figure 4. Brine shrimp lethality assay of *B. variegata* extracts. Doxorubicin is used as a standard with an LC_{50} 5.63 ± 0.25 µg/mL. All values are obtained thrice and shown as mean ± SD. nH = *n*-hexane; EA = ethyl acetate; MeOH = methanol; DW = distilled water; LC_{50} = concentration for 50% lethality; (S) = stem; (L) = leaf; (F) = flower; (B) = bark; (R) = root.

against *F. Solani*, *A. niger*, and *Mucor*. species with the zone of inhibition ranging between 16 to 18 mm. In this assay all the samples showed minor to moderate activity.

The non-polar extracts showed more antifungal activity as compared to polar one. Clotrimazole showed the zone of inhibition values of 32 ± 0.12 with minimum inhibitory concentration of 2.5 µg/disc (Data not shown).

3.2.4. Antidiabetic activity assessment

3.2.4.1. Alpha-amylase inhibition assay

The alpha-amylase inhibition assay indicated *n*-hexane extract of root displayed better activity with the value of 60.80 ± 0.20% followed by ethyl acetate extract of root showed 30.50 ± 0.20%. These activities were not strongly comparable with acarbose (standard drug) which showed the effect of 89 ± 0.41% and IC_{50} = 33.53 µg/mL. Assay results of *B. variegata* are shown in Figure 5.

4. Discussion

The demand for medicinal plants for disease treatment is increasing because of their valuable compounds, which can cure many diseases and assist physicians in managing the burden of disease (Petrovska, 2012). The extract was obtained using a single solvent extraction process. The successive use of mono solvent system for extraction provided the benefit of different polarities of the components for separation rather than to use the mixture of solvent of different polarity. This method also helped to understand extraction potential, biological and phytochemical nature of extracts. The extraction results implied that the maximum extraction yield can be obtained by using variant polarity solvents. Polar solvents presented a higher extraction yield than non-polar solvents. Therefore, to obtain the maximum extract yield, the solvent chosen for extraction is a critical factor (Fatima et al., 2015). However, the maximum extract yield does always the component's biological potential. The maximum extract yield was obtained in water extract of leaves, followed by methanolic extract of roots; the lowest yield was obtained in *n*-hexane extract of stem, providing further evidence that more polar solvents yield more compounds compared with less polar solvents in extraction (Pin et al., 2010).

Phytochemical screening provides an idea of the medicinal value of a plant. In the present study, extracts were screened, and the results confirmed the presence of flavonoids and phenols. Phenolics are the molecules with the highest capacity to neutralize free radicals. Therefore, in food research, the quantification of phenolics is a routine practice (Sánchez-Rangel et al., 2013). Using the standard curve equation, the total phenolic content was estimated as the gallic acid equivalent. All extracts exhibited excellent results, yielding values ranging from

Table 2. Cytotoxicity against human breast adenocarcinoma MCF-7 cell line of different solvent extracts of *B. variegata*

Extract	% Inhibition (20 μg/mL)	IC_{50} (μg/mL)	Extract	% Inhibition (20 μg/mL)	IC_{50} (μg/mL)
BVF-H	40.30 ± 0.21	>20	BVB-M	53.50 ± 0.52	>20
BVF-EA	72.06 ± 0.25	15.5 ± 0.014	BVB-A	69.55 ± 0.36	17.2 ± 0.065
BVF-M	55.35 ± 0.34	>20	BVS-H	80.41 ± 0.15	14.1 ± 0.024
BVF-A	63.61 ± 0.15	>20	BVS-EA	93.20 ± 0.18	12.1 ± 0.054
BVL-H	41.99 ± 0.16	>20	BVS-M	65.31 ± 0.34	15.3 ± 0.027
BVL-EA	60.58 ± 0.45	>20	BVS-A	68.30 ± 0.17	15.6 ± 0.034
BVL-M	74.41 ± 0.27	14.8 ± 0.072	BVR-H	79.86 ± 0.38	12.8 ± 0.045
BVL-A	62.85 ± 0.69	>20	BVR-EA	71.35 ± 0.61	15.5 ± 0.017
BVB-H	80.40 ± 0.42	13.7 ± 0.037	BVR-M	70.94 ± 0.54	16.3 ± 0.043
BVB-EA	76.39 ± 0.18	14.7 ± 0.024	BVR-A	59.22 ± 0.63	>20
Taxol	99.54 ± 0.43	2.9 ± 0.025	Taxol	99.54 ± 0.43	2.9 ± 0.025

All values are repeated three times and results are mentioned as mean ± SD. The drug Taxol was used as a positive control. >20 = No activity. BV = *Bauhinia variegata*; Flower (F); Leaves (L); Bark (B); Stem (S); Root (R); *n*-Hexane = H; Ethyl acetate = EA; Methanol = M; Water (aqueous) = A.

Table 3. Antibacterial activity of *B. variegata* extracts tested against bacterial strains.

Extract	*Diameter of growth zone inhibition (mm ± SD at 100 μg/disc, MIC: μg/mL)									
	S. aureus	**MIC**	***B. subtilis***	**MIC**	***P.aeruginosa***	**MIC**	***K.pneumoniae***	**MIC**	***E. coli***	**MIC**
BVFH	16.77±0.75	100	12.78±0.25	100	10.53±0.50	-----	16.77±0.25	100	12.50±0.30	100
BVFEA	17.03±0.65	>100	14.50±0.61	100	16.97±0.45	100	12.67±0.35	>100	15.53±0.47	100
BVFM	10.43±0.60	-----	10.53±0.60	-----	20.73±0.25	>100	11.60±0.66	-----	10.70±0.26	-----
BVFW	10.33±0.67	-----	07.90±0.50	-----	04.23±0.87	-----	07.67±0.31	-----	03.97±0.25	-----
BVBH	10.27±0.42	-----	10.55±0.19	-----	10.80±0.20	-----	22.93±0.12	>100	10.97±0.25	-----
BVBEA	14.87±0.40	100	16.00±0.20	100	12.77±0.25	100	20.60±0.53	>100	13.73±0.25	>100
BVBM	14.03±0.25	100	12.20±0.64	100	10.63±0.32	-----	12.10±0.26	100	10.73±0.25	-----
BVBW	06.23±0.75	-----	10.70±0.30	-----	03.10±0.36	-----	06.67±0.65	-----	02.97±0.45	-----
BVLH	12.77±0.25	>100	09.63±0.55	-----	09.70±0.52	-----	17.03±0.25	>100	11.73±0.64	-----
BVLEA	18.43±0.55	100	12.15±0.53	100	16.07±0.31	100	19.60±0.36	>100	13.50±0.50	>100
BVLM	12.93±0.12	>100	10.53±0.60	-----	12.13±0.32	>100	10.57±0.51	-----	08.33±0.58	-----
BVLW	06.13±0.32	-----	08.63±0.35	-----	06.97±0.15	-----	05.10±0.36	-----	02.93±0.31	-----
BVSH	18.57±0.51	100	08.75±0.58	-----	12.13±0.40	100	18.53±0.50	>100	12.70±0.36	33.3
BVSEA	04.83±0.15	-----	08.60±0.68	-----	08.90±0.36	-----	12.43±0.51	>100	12.20±0.30	100
BVSM	10.07±0.31	-----	11.12±0.35	-----	10.80±0.20	-----	07.63±0.55	-----	07.33±0.31	-----
BVSW	12.03±0.45	33.3	05.37±0.57	-----	12.10±0.36	100	06.50±0.87	-----	04.73±0.25	-----
BVRH	16.27±0.32	100	12.55±0.19	>100	13.80±0.20	>100	17.93±0.12	100	12.97±0.25	100
BVREA	12.57±0.40	>100	14.00±0.20	100	12.77±0.25	100	14.60±0.53	>100	14.73±0.20	>100
BVRM	14.33±0.25	100	11.20±0.64	-----	10.63±0.32	-----	12.10±0.26	100	09.73±0.30	-----
BVRW	06.23±0.75	-----	10.70±0.30	-----	08.10±0.36	-----	08.67±0.65	-----	06.97±0.45	-----
Cefixime	-----	-----	-----	-----	21.50±0.50	1.11	20.60±0.53	3.33	21.60±0.53	3.33
Roxithromycin	23.50±0.50	3.33	21.88±0.76	3.33	-----	-----	-----	-----	-----	-----
DMSO	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

*Zone of inhibition including the diameter of the disc (6 mm). The final concentration of standard used was 20 μg/disc. Values are presented as ZOI ± SD, the assay was run in triplicate. No activity in disc diffusion assay or not active (zone ≥10 mm) for MIC determination.

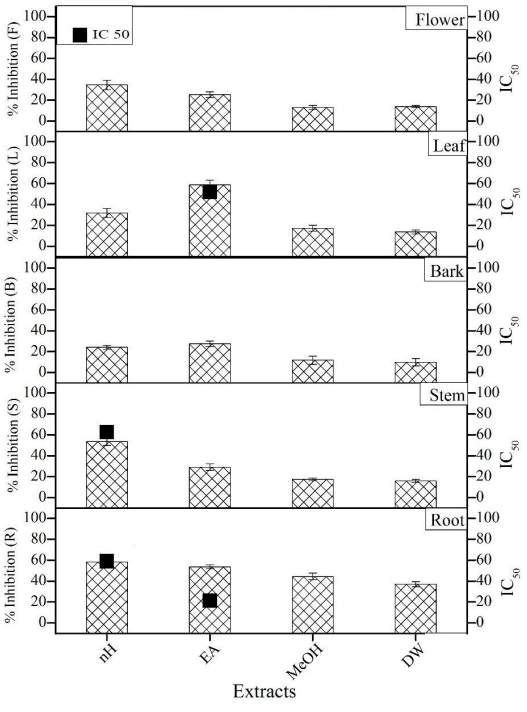

Figure 5. *B. variegata* extracts result for α-amylase inhibition assay. The standard drug used is Acarbose IC_{50} 33.43 ± 0.28 µg/mL. All procedures are repeated three times and results are mentioned as mean ± SD. nH = *n*-hexane; EA = ethyl acetate; MeOH = methanol; DW = distilled water; IC_{50}= concentration for 50% inhibition; (S) = stem; (L) = leaf; (F) = flower; (B) = bark; (R) = root.

(61.62 ± 10 to 7.70 ± 5 µg AAE/mg). The methanolic extracts had the highest values. The total phenolic content is considered a marker for the evaluation of the effects on oxidative status (Inderjit and Nilsen, 2003). Substantial free radical scavenging activity has been noted for many phytochemicals. The current results demonstrated that the phenolic content of the plant varies with the solubility and polarity of solvents. The total flavonoid content was estimated using a method like that used for phenolic content estimation. Considerable total flavonoid content was observed in all extracts. The methanolic extracts had the highest values. Flavonoids inhibit lipid peroxidation in plants because they are a rich source of free radical scavenging and protect the organism against cell damage (Halliwell, 2008). Therefore, the current study results indicate that phenolics and flavonoids play a valuable role in protecting against oxidative reactions.

The total reducing potential of all extracts of *B. variegata* was estimated by calculating the transformation of Fe^{3+} to Fe^{2+} (Oyaizu, 1988). The results revealed that methanol extracts provided the highest reducing power. It can be concluded that the reducing power of *B. variegata* may be due to the hydrogen-donating characteristic of the plant through the reaction with free radicals and the blocking of the radical chain reaction (Mahmood et al., 2011; Zhu et al., 2015).

All extracts of *B. variegata* exhibited excellent results in terms of free radical scavenging potential. To evaluate the free radical scavenging activity of extracts, the total antioxidant capacity was determined. During this process, Mo (VI) was reduced to Mo (V) in the samples, and phosphomolybdenum, a green color complex with a maximum absorbance of 695 nm, was formed (Jayaprakasha and Patil, 2007). Fresh DPPH solution with the absorption of 517 nm developed a deep purple color, which automatically fades when antioxidants are present. Antioxidant molecules can quench DPPH free radicals through electron donation. Generally, this assay is performed for free radical scavenging potential determination. DPPH of *B. variegata* extract was studied using the stable free radical solution of methanol, showing excellent DPPH values for ethyl acetate and methanol extracts. In the reaction mixture, the presence of antioxidants developed a brighter color and a higher optical density (Tan and Shahidi, 2013). In the current study, *n*-hexane extract of roots and bark exhibited strong activity and yielded the lowest IC_{50} values.

The brine shrimp lethality assay was used to evaluate the cytotoxicity of extracts. Shrimp larval tissue responds similarly to mammalian cells (Bibi et al., 2011). This is a simple and feasible method to use a model organism for cytotoxicity study. Researchers are investigating the use of natural products for anticancer drug preparations (Gelani and Uy, 2016). Samples of *B. variegata* had revealed good cytotoxicity results; low or medium polarity extracts had shown more promising results. *n*-Hexane extract of the roots demonstrated strong cytotoxicity with LD_{50} value of 1.58 µg/mL (Figure 4). This is comparable with previously reported results that indicate that low polar extract samples could be a good source of new compounds, and the findings can be used to isolate and purify active constituents of *B. variegata*. The mortality rate due to cancer is predicted to exceed 10 million by 2020 (Soliman et al., 2013; Hussain et al., 2007) therefore, the cytotoxic activity of the plant as discussed can provide protection against cellular necrosis and a strategy to mitigate the disease burden.

n-Hexane and ethyl acetate extracts of the stem demonstrated 80.41 and 93.02% antiproliferative activity, respectively against human breast cancer MCF-7 cell line. However, the substantial activity of *n*-hexane extract requires further investigation for the discovery of an anticancer lead compound. Overall results of this assay showed that samples possess cytotoxicity (Figure 4). These finding are in accordance with previous report by Bibi and coworkers that *n*-hexane extract of *Aster thomsonii* showed significant cytotoxicity potential (Bibi et al., 2011). It is also reported that *n*-hexane extract of marine sponge showed strong lethality against brine shrimps (Gelani and Uy, 2016).

The limited ability of synthetic compounds to overcome resistance has increased the need for the discovery of new lead compounds (Nair et al., 2005). The identification of new antimicrobial drugs that are effective against emerging infectious diseases is crucial (Jones et al., 2008). Medicinal plants are explored to identify new lead compounds for developing drugs against microbial infections (Sukanya et al., 2009). To evaluate the antibacterial activity of *B. variegata* extracts, a disc

diffusion assay was performed against different Gram-positive and Gram-negative pathogenic species, most of the plant extracts displayed good antibacterial activity. The maximum activity was observed for non-polar extracts. Antibacterial activity depends on the composition of the medium, the strains of the pathogens, the testing methodology, and the plant material (Rios and Recio, 2005). The antibacterial activity of ethnopharmacological sources indicates that Gram-positive bacteria growth is inhibited by phenolics, flavonoids, and tannins (Ahmad and Beg, 2001; Madureira et al., 2012). The *B. variegata* phytochemical analysis results confirmed the presence of these secondary metabolites; thus, *B. variegata* can be used in the discovery of unique anti-infective drugs.

The presence of phenolics, flavonoids, essential oils, and tannins in plants is responsible for antifungal activity (Cowan, 1999). Flavonoids can form complexes with the cell wall of fungal proteins and destroy membranes because of their lipophilic characteristics (Arif et al., 2009). Tannins exhibit the same action, adopting a similar pathway (Sher, 2009). Plant extracts were further investigated for antifungal potential. The *n*-hexane extract of the stem demonstrated significant antifungal activity. The antifungal activity of *B. variegata* may be related to the presence of phenolic compounds.

Traditional medicines are prominently used for the treatment of chronic diseases, such as diabetes mellitus (Modak et al., 2007). Herbal medicines are preferred because of their low cost and safety. In folkloric medicine, *B. variegata* is known as an antidiabetic. Secondary metabolites, which reduce oxidative stress, could be responsible for alpha-amylase inhibition activity (Pahwa et al., 2015). All samples were investigated for alpha-amylase inhibition activity, and the *n*-hexane extract of the roots demonstrated moderate inhibition potential.

5. Conclusion

The study concludes that *n*-hexane and ethyl acetate extracts of bark, stem, and roots of *Bauhinia variegata* could be source of cytotoxic secondary metabolites. The biological and phytochemical investigation of plant extracts diverges the attention to isolate bioactive constituents that can be potential drug leads.

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