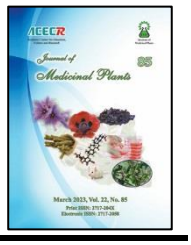
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Research Article

Quantitative analysis and biological activities of *Bauhinia vahlii* Wight & Arn. growing in Nepal

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ABSTRACT

Background: The genus *Bauhinia* consists of about 300 species and is widely distributed in most tropical countries of the world. **Objectives:** The antioxidant, antibacterial, antidiabetic, and quantitative analysis of stem, leaf, and seed extracts of *B. vahlii* (the synonym name of *Phanera vahlii*) were investigated. **Methods:** The antioxidant activity of the crude extracts and fractions was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. The antidiabetic activity was investigated by α-amylase inhibition assay. The toxicity of plant extracts was assessed by brine shrimp lethality assay (BSLA) using *Artemia salina* as a biological test organism. **Results:** The aqueous fraction of the leaf showed a high total phenolic content of 168.47 ± 1.94 mg GAE/g. The total flavonoid content was found maximum in an aqueous fraction of leaf with 158.51 ± 2.99 mg QE/g. The stem extract showed potent antioxidant activity with IC_{50} 1.91 ± 0.33 µg/ml as compared to the standard quercetin IC_{50} 3.46 ± 0.40 µg/ml. The DCM (Dichloromethane) fraction of the leaf exhibited noteworthy *α*-amylase inhibition properties with IC_{50} 112.70 ± 2.0 µg/ml as compared to the standard acarbose of 9.34 ± 2.0 µg/ml. The antimicrobial results showed that the methanolic extract of stem and seed exhibited the zone of inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae* but failed against *E. coli*. The methanol and aqueous extract of the leaf showed toxicity against brine shrimp nauplii with LC_{50} 257.63 µg/ml and 100 µg/ml, respectively. **Conclusion:** This study showed *Bauhinia vahlii* is rich in flavonoid and phenolic content and the plant may be a rich source of natural antioxidants and antidiabetic agents that could be isolated as the drug candidate.

Abbreviations: DPPH, 2,2-Diphenyl-1-Picrylhydrazyl; IC_{50}, Median Inhibitory Concentration; ROS, Reactive Oxygen Species; NTFP, Non-timber Forest Produces; FCR, Folin Ciocalteu Reagent; DMSO, Dimethyl Sulfoxide; LC_{50}, Median Lethal Concentration; ZOI, Zone of Inhibition; GAE: Gallic Acid Equivalent; QE, Quercetin Equivalent; PPA, Porcine Pancreatic Amylase; SD, Standard Deviation; CKD, Chronic Kidney Disease

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1. Introduction

Nepal, a relatively small country, occupying just 0.1 % of the world's total landmass, is surprisingly rich in natural resources because of its diverse climate, geology, topographical area, and meteorological conditions. The people of Nepal living in a remote area where access to modern medicine is limited rely on traditional medicine such as medicinal plants for primary healthcare [1]. The majority of villages of Nepal lack access to modern medicine so there is a long history and tradition of using medicinal and aromatic plants as part of the ayurvedic system and as home remedies.

The global trend of synthetic drugs has turned to herbal drugs which are referred to as a return to nature to prevent simple to life-threatening diseases. Commercially available drugs have shown significant importance in the control of infectious diseases, oxidative stress, and diabetic complications in the human body. But the synthetic drugs impart side effects, low efficacy, and are expensive. Numerous cellular processes inside the human body and other exogenous factors produce highly toxic free radicals such as hydrogen peroxide, superoxide, and hydroxy radicals in excess, thereby causing biomolecular damage which in turn causes diseases like diabetes mellitus, cancer, atherosclerosis, heart and neurodegenerative conditions, acute and chronic kidney disease (CKD) [2]. Antioxidants can neutralize free radicals, chelate metal ions, inhibit lipid peroxidation, have reducing properties, and promote and prevent disease through reducing oxidative stress caused by reactive oxygen or nitrogen species in the human body [3]. Natural antioxidants are advantageous because they are less harmful, more efficient, and cost-effective. Diabetes is an endocrine metabolic disorder characterized by fasting blood glucose levels above or equal to 7 mmol/L [4]. It is a complicated disease caused by a disruption of carbohydrate, protein, and fat metabolism, resulting in macro and microvascular changes that lead to secondary complications [10]. The effects of diabetes mellitus include hyperglycemia, lipedema, and oxidative stress, and the disease can lead affected individuals to long-term problems such as organ failure, skin diseases, kidney damage, hardening of arteries and blood vessels, heart disease, stroke, nephrotic syndrome, cataract and retinopathy [6]. Several studies have suggested that dietary supplementation with antioxidants and antiglycation nutrients may be safe and effective for treating complications related to diabetes [7]. Salivary and pancreatic α-amylase degrade and hydrolyzes α-(1,4) glycosidic bonds present in the complex polysaccharides, which eventually increases the blood sugar level. Common antidiabetic agents such as sulfonylureas, non-sulfonylureas, and secretagogues, frequently lower the breakdown of long-chain carbohydrates [8-10].

The genus *Bauhinia* comprises approximately 300 species across tropical and subtropical regions, belonging to the family Leguminosae (Caesalpinioideae) [11]. *Bauhinia vahlii* Wight & Arn. (this name is a synonym of *Phanera vahlii* (Wight & Arn.) Benth.) is included in the genus *Bauhinia* and is a folk medicinal plant that has been cultivated in different regions of the world [12]. The different species of *Bauhinia* such as *B. reticulata*, *B. rufescens* and *B. variegata* have traditionally been used against roundworm infections, conjunctivitis, anthrax, ulcerations, dysentery, blood poisoning, leprosy, as well as lung and skin diseases, However, extracts of the bark of *Bauhinia variegata* are used to treat cancer [13]. The *Bauhinia vahlii* is used for the cure of diabetes, skin diseases, eye diseases, anemia, cardiac disorders, and so on

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[14-16]. The ripe raw seeds and cooked or roasted mature seeds of *B. vahlii* are taken as food by the tribes living in Dhading Nepal [15]. A literature survey revealed that some of the biological activities in this genus are reported from around the globe but the particular species growing in Nepal is not well explored for its antidiabetic, antioxidant, and toxicity activities. To the best of our knowledge, the quantitative analysis of plant secondary metabolites with their biological activities and toxicity study is the first report in *B. vahlii* plant growing in Nepal.

2. Materials and methods

2.1. Chemicals and reagents

The solvents used in this study for the polarity-based extraction **(***n*-hexane, dichloromethane, ethyl acetate, and methanol) were purchased from Thermo Fischer Scientific (India). The reagents and chemicals such as ethanol, sodium carbonate, sodium acetate, aluminium chloride, Folin-Ciocalteu phenol reagent, acarbose, quercetin, gallic acid, dimethyl sulphoxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), soluble starch, disodium hydrogen phosphate, monosodium hydrogen phosphate, sodium potassium tartarate, porcine pancreatic amylase (PPA) were purchased from Sigma-Aldrich Company. All reagents and solvents were of analytical reagent grade.

2.2. Sample collection

The aerial parts (i.e., leaf, stem, and seed) of *Bauhinia vahlii* were manually collected from their natural habitat in June 2021 from the Dhading district (27º40’ to 28º14' N latitude and 84º 0’ to 85º 1’ E longitude) of Nepal based on its ethnobotanical uses by the traditional health practitioners. The plant was identified by the Taxonomist, at the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal. A voucher specimen no. 220079 was deposited at the same department for future reference. The photographs of the collected plant samples are shown in Fig. 1.



Fig. 1. Aerial parts of *Bauhinia vahlii* leaf, stem, and seed collected from the study area.

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2.3. Sample preparation

The different parts of the plant were thoroughly washed with tap water to remove dust particles and then chopped into small pieces; air dried at room temperature (25 ± 5 °C) under shade. After completely drying, plant parts were ground to a coarse powder. During this process, plant parts were broken down into smaller pieces for exposing internal tissues and cells to the solvents. The powdered samples were stored in a clean closed glass container until the extraction of plants' secondary metabolites.

2.4. Extraction

The 100 g of fine powder of aerial parts of the plant was kept in an Erlenmeyer flask and dipped in methanol for 72 hours with frequent agitation. The cap of the flask was closed properly to resist the entrance of air into the flask. The dipped plant samples were filtered with Whatman filter paper No 1. The filtrate was kept in a rotatory evaporator to evaporate the solvent. The partially concentrated plant extracts were kept in the water bath at 35 °C for complete evaporation of the solvent. After running this procedure, gummy concentrated extracts were obtained which were preserved in the refrigerator at 4 °C for further use.

2.4.1. Solvent-solvent extraction

The solvent-solvent fractionation of the crude methanolic extract of leaves was fractionated using solvents of increasing polarity, such as n-hexane, dichloromethane, ethyl acetate, and water. All four fractions were evaporated to concentrate and made dry by using a water bath at low temperature and then kept in beakers for further analysis.

2.5. Total phenolic content (TPC)

The total phenolic content of all selected plant extracts was estimated using Folin-Ciocalteu phenol reagent using gallic acid as standard in 96 well plate reader which was modified from the colorimetric method [17]. Briefly, 20 µl of the sample (0.5 mg/ml) standard gallic acid /plant extracts were followed by the addition of 100 µl Folin-Ciocalteu phenol reagent in a 96-well plate, and initial absorbance was taken. Afterward, 80 µl 1M Na_2CO_3 (sodium carbonate) solution was added to make a final volume of 200 µl. The content was incubated for 30 minutes after that the absorbance was measured at 765 nm with the help of a synergy LX microplate reader. The TPC was quantified using a calibration curve of gallic acid, the outcome data were expressed in milligrams of gallic acid equivalent per gram (mg GAE/g) of dry extract. Triplicates of each measurement were carried out for validation of the experimental results.

2.6. Total flavonoid content (TFC)

TFC was determined by the aluminium chloride colorimetric method in which the maximum absorbance was shown at 415 nm [18]. The 20 µl of each extract (0.5 mg/ml) was loaded on 96 well plates in triplicate. In each well 110 µl deionized water was added to maintain a final volume of 130 µl. Then, 60 µl ethanol, 5 µl $AlCl_3$, and 5 µl sodium acetate were added to each well and the reaction mixture was allowed to stand for 30 minutes. The TFC value was expressed as milligrams of quercetin equivalent per gram (mg.QE/g) of the dry weight of extracts using the quercetin standard calibration curve.

2.7. DPPH radical scavenging

The free radical scavenging activities of the plant extracts were evaluated in a rapid, simple, and inexpensive method by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Antioxidant compounds donate the electron to DPPH thus causing its reduction and in the reduced form its color changes from deep violet to yellow. The decolorization indicates the antioxidant potential

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of the sample. The ability of different plant extracts to scavenge DPPH free radicals was performed by adopting the standard protocol of Jamuna et al. 2012 [19]. Briefly, solution (0.1M) of DPPH was freshly prepared in methanol and kept at 4 °C until use. The 100 µl plant extract and 100 µl DPPH solutions were loaded sequentially on a 96-well plate. After that, it was incubated for 30 minutes in the dark, and absorbance was noted at 517 nm. The percentage of the free radical scavenging activity was calculated by using the following equation.

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where Abs control is the absorbance of DPPH radical with 50 % DMSO. The 50 % inhibitory concentration (IC_{50}) value is indicated as the effective concentration of the sample that is required to scavenge 50 % of the DPPH radicals. The inhibition curve was established by plotting extract concentration versus the corresponding scavenging effect. The IC_{50} value was calculated using GraphPad Prism 9.

2.8. In vitro α-amylase inhibition

The *α*-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method with slight modification [20]. The different extracts of *Bauhinia vahlii* were dissolved in the minimum amount of 50 % DMSO and were further dissolved in buffer (Na_2HPO_4/NaH_2PO_4 (0.02M) and NaCl (0.006M) at pH 6.9 to give the concentrations of varying range. A volume of 200 µl α- amylase solution was mixed with 200 µl of the extract and was incubated for 10 minutes at 30 °C. Thereafter, 200 µl of the starch solution was added (1 % in water $^{w}/_{v}$) to each tube and was incubated for 3 minutes. The reaction was terminated by the addition of 200 µl DNSA reagent (1 g 3,5-dinitrosalicylic acid, 20 ml 2M NaOH, and 30 g of potassium sodium tartarate). The tubes were kept in boiling water for 10 min and cooled to ambient temperature then, diluted with 5 ml distilled water, and absorbance was measured at 540 nm using a Microplate reader. The blank with 100 % enzyme activity was prepared by replacing the plant extract with 200 µl of the buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive sample was prepared using acarbose and the reaction was performed similarly to the reaction with plant extract as mentioned above. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation. The % α-amylase inhibition was plotted against the extract concentration and the IC_{50} values were calculated using the Graph Pad Prism 9.

2.9. Antimicrobial assay

2.9.1. Collection of test organisms

The antimicrobial properties of plant extracts were tested against four different general lab pathogenic test organisms i.e. gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 10031) and gram-positive bacteria (*Staphylococcus aureus* KCTC 1916 and *Bacillus subtilis* ATCC 66333) which were isolated from Research Institute for Bioscience and Biotechnology (RIBB) Saptakhel-9 Balkumari, Chyasal, Lalitpur while *Fusarium spp* was provided by National Academy of Science and Technology (NAST) Lalitpur, Kathmandu, Nepal.

2.10. In vitro antibacterial screening

The bacterial susceptibility of the plant extracts was assessed by the agar well diffusion method in Mueller Hinton agar plates [21]. Briefly, an overnight incubated broth culture of entire test organisms was prepared in nutrient broth and diluted with sterile nutrient broth media to maintain the turbidity at 0.5 McFarland

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standards (10^{6-8} CFU/ml). Then, about 100 µl of inoculum was taken and spread on MHA agar plates. After that, wells were bored aseptically into the agar surface by using a sterile gel puncture of 7 mm diameter and filled with 20 µl of samples (concentration of 50 mg/ml). Finally, the plates were incubated at 37 °C for 24 hours and after incubation, the plates were checked to see if a clear zone had formed around each well which would indicate the antibacterial activity of the plant samples. The zone of inhibition (ZOI) for each sample was measured using a ruler in mm. In this bioassay, ampicillin (1 mg/ml) was taken as positive control and DMSO as a negative control.

2.11. Brine shrimp assay

This assay has been successfully employed as an important tool for toxicity screening of plant extracts using *Artemia salina* brine shrimp nauplii [22]. In this experiment, 20 mg of each of the extracts was dissolved in methanol, and solutions of varying concentrations (1000 µg/ml, 100 µg/ml, 10 µg/ml) were prepared by serial dilution of the stock solution. The 2 ml of extracts solution from each concentration (1000 µg/ml, 100 µg/ml, 10 µg/ml by following the protocol) was transferred to nine different test tubes labeled 1-9, three for each concentration. Similarly, 2 ml of methanol was taken in three test tubes as a blank. After these, the test tubes were kept for 24 hours to evaporate the whole solvent. After complete evaporation of the solvent, 5 ml of simulated seawater was added to each test tube and the solution was gently shaken so that the dry compounds diffused adequately in the aqueous solution. The ten matured shrimps were transferred to each test tube. All the test tubes were maintained under illumination. The number of surviving nauplii was counted after 24 hours. From this data, the percent mortality of the brine shrimp nauplii for each concentration was calculated by using the following formula.

$$\% \text{ Mortality} = \frac{\text{No of nauplii taken} - \text{no of nauplii alive}}{\text{No of nauplii taken}} \times 100$$

The LC_{50} values were statistically examined using Finney's probit analysis method.

3. Results

3.1. Total phenolic content (TPC)

The quantitative determination of total phenolic contents in leaf, stem, and seed extracts of *Bauhinia vahlii* was performed using the Folin-Ciocalteu phenol reagent taking gallic acid as standard (The obtained standard calibration curve equation: $y = 0.03436x - 0.07942$, $R^2 = 0.9890$). The results of TPC are shown in Fig. 2. The total phenolic content of the various extracts and the partially purified fraction was found to vary from 81.78 ± 1.25 to 168.47 ± 1.94 mg GAE/g of dry extract. The aqueous extract of the leaf possessed the highest total phenols of 168.47 ± 1.94 mg GAE/g as compared to the methanol extract of a leaf of 154.13 ± 5.19 mg GAE/g, stem extract 166.14 ± 2.36 mg GAE/g and seed extract 145.29 ± 1.34 mg GAE/g.

3.2. Total flavonoid content (TFC)

The standard calibration curve was constructed between the concentration of quercetin and the absorbance with regression equation $y = 0.0169x - 0.0591$, $R^2 = 0.9917$. The flavonoid content among the different crude extracts and semi-purified fractions is presented in Fig. 3. The results showed that the aqueous extract of the leaf possessed the highest flavonoid content of 158.51 ± 2.99 mg.QE/g followed by the ethyl acetate fraction.

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3.3. Antioxidant activity

The antioxidant activity of the crude methanol extracts and the semi-purified fractions are expressed in IC_{50} values. The antioxidant potential of the plant extracts showed the inverse relation to the IC_{50} values, the plant extract showed the lower value of IC_{50} indicates a high antioxidant potential. The graphical representation of the results of the DPPH radical scavenging assay is displayed in Fig. 4.

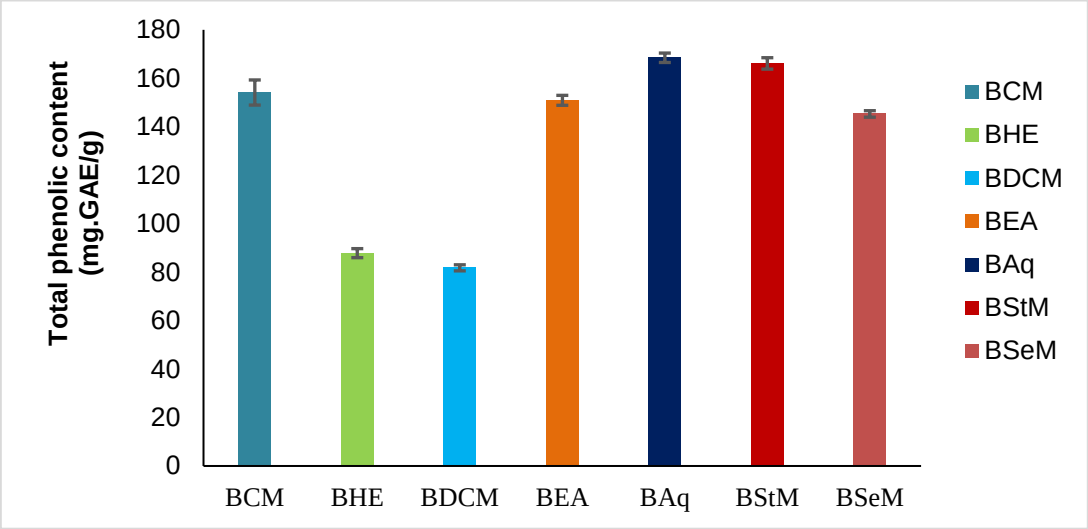


Fig. 2. Comparative TPC values of crude extracts and semi-purified fractions *(BCM- (crude methanolic extract of B. vahlii leaf), BHE- (hexane fraction of B. vahlii leaf), BDCM- (dichloromethane fraction of B. vahlii leaf), BEA- (ethyl acetate fraction of B. vahlii leaf), BAq- (aqueous fraction of B. vahlii leaf), BStM- (crude methanolic stem extract of B. vahlii) BSeM- (Crude methanolic seed extract of B. vahlii)*

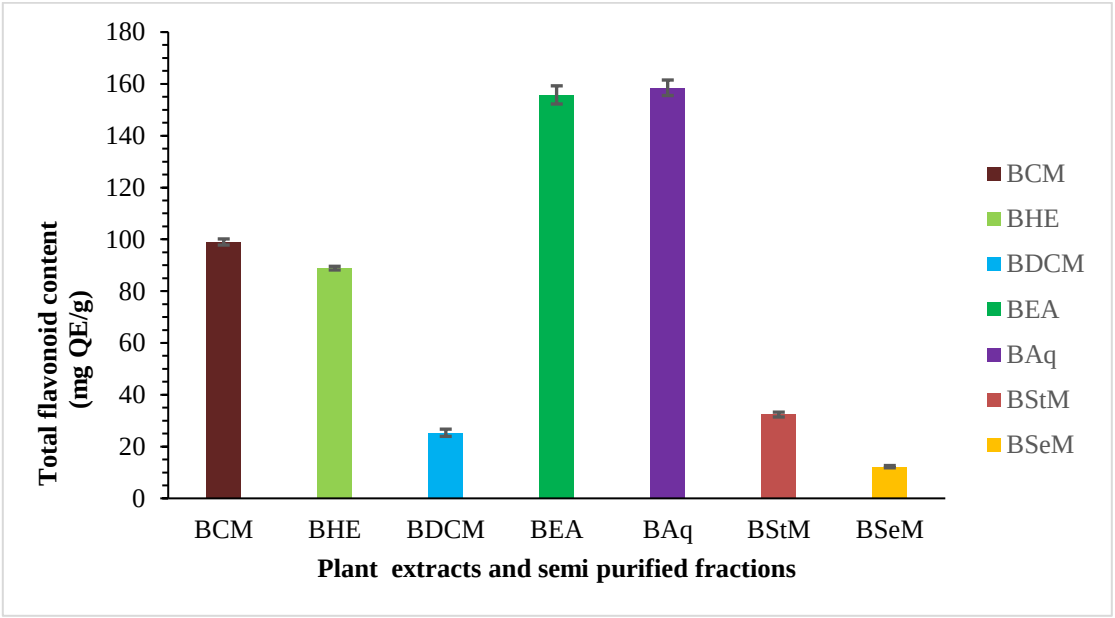


Fig. 3. Comparative TFC of the crude methanol extracts and semi-purified fractions (*BCM- (crude methanolic extract of B. vahlii leaf), BHE- (hexane fraction of B. vahlii leaf), BDCM- (dichloromethane fraction of B. vahlii leaf), BEA- (ethyl acetate fraction of B. vahlii leaf), BAq- (aqueous fraction of B. vahlii leaf), BStM- (crude methanolic stem extract of B. vahlii) BSeM- (Crude methanolic seed extract of B. vahlii)*

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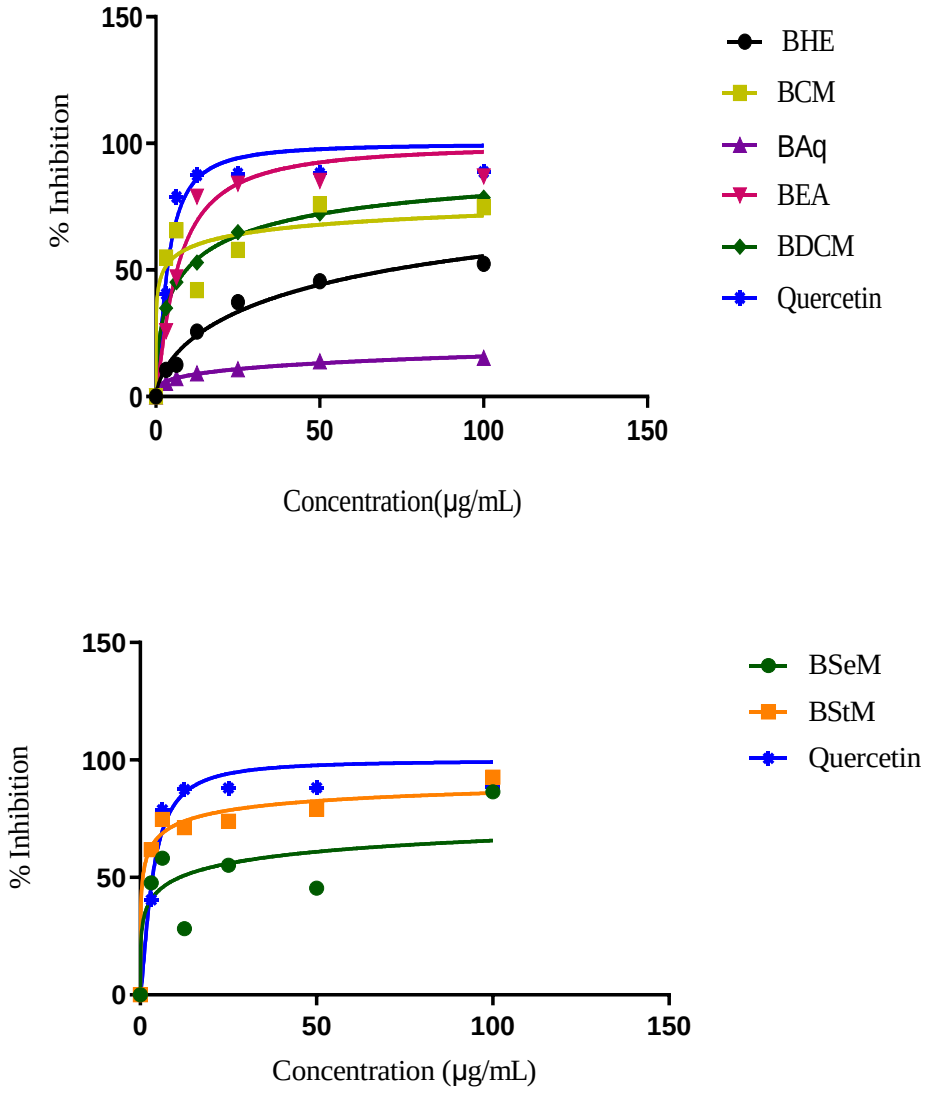


Fig. 4. Percentage inhibition against the concentration of plant extracts and semi-purified fractions as compared to the standard quercetin (*BCM- (crude methanolic extract of B. vahlii leaf), BHE- (hexane fraction of B. vahlii leaf), BDCM- (dichloromethane fraction of B. vahlii leaf), BEA- (ethyl acetate fraction of B. vahlii leaf), BAq- (aqueous fraction of B. vahlii leaf), BStM- (crude methanolic stem extract of B. vahlii) BSeM- (Crude methanolic seed extract of B. vahlii)*

The linear regression of the percentage of radical scavenging versus concentration was used for the calculation of the concentration of each plant extract and the semi-purified fractions required for 50 % inhibition of DPPH activity. The IC_{50} values of the plant extract and the semi-purified fraction along with the standard quercetin are presented in Fig. 5.

As a result, methanolic extracts of leaf, stem, and seed as well as ethyl acetate and DCM fractions of leaf exhibited the strongest radical scavenging activity with IC_{50} near the standard quercetin. Their IC_{50} were found to be increased in the following order; methanolic extract of the stem (1.91 ± 0.33 µg/ml) > quercetin (3.46 ± 0.40 µg/ml) > methanolic extract of seed (5.79 ± 3.05

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µg/ml) > DCM fraction of a leaf (6.30 ± 0.16 µg/ml) > ethyl acetate fraction of a leaf (6.61 ± 0.54 µg/ml) > hexane fraction of a leaf (61.64 ± 13.22 µg/ml) > aqueous fraction of a leaf (83.57 ± 3.62 µg/ml). The IC_{50} obtained from the crude extracts and the semi-purified fractions showed potential antioxidant activity concerning the standard quercetin. The IC_{50} of hexane and aqueous extract were found higher than those of the other fractions of the crude leaf extract. A crude methanolic stem extract of *Bauhinia vahlii* showed significantly higher antioxidant activity than the standard quercetin.

3.4. α-amylase inhibition

The graphical presentation for the inhibition of crude extracts, and semi-purified fractions as compared to the standard acarbose is shown in Fig. 6.

The strength of α-amylase enzyme inhibition activity of the crude extracts and semi-purified fractions is expressed in IC_{50}, which is displayed in Fig. 7.

The α-amylase enzyme inhibition property of plant extracts showed an IC_{50} ranging from 62.84 ± 2 to 578.0 ± 2.0 µg/ml, representing an approximate nine-fold variation. Among the tested fractions, the DCM fraction of leaf and methanolic crude extract of stem showed moderate α-amylase inhibition properties with an IC_{50} of 62.84 ± 2.0 µg/ml and 112.7 ± 2.0 µg/ml respectively as compared to standard acarbose of IC_{50} 9.34 ± 2.0 µg/ml.

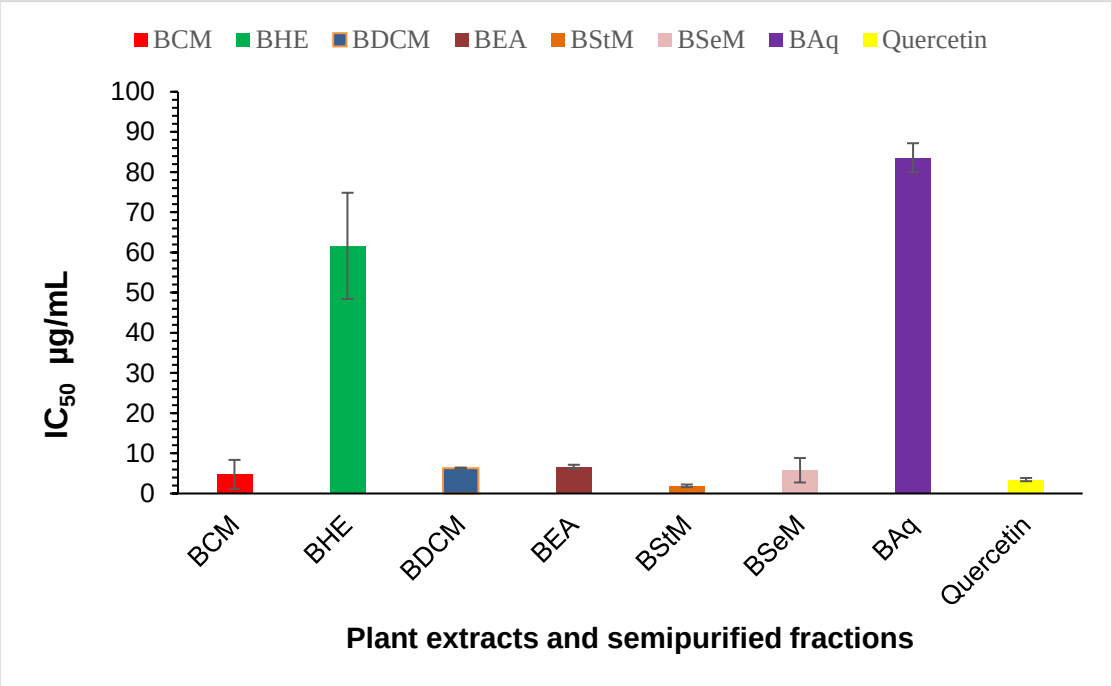


Fig. 5. IC_{50} of different crude extracts and semi-purified fractions showing the antioxidant activity. (*BCM- (crude methanolic extract of B. vahlii leaf), BHE- (hexane fraction of B. vahlii leaf), BDCM- (dichloromethane fraction of B. vahlii leaf), BEA- (ethyl acetate fraction of B. vahlii leaf), BAq- (aqueous fraction of B. vahlii leaf), BStM- (crude methanolic stem extract of B. vahlii) BSeM- (Crude methanolic seed extract of B. vahlii)*

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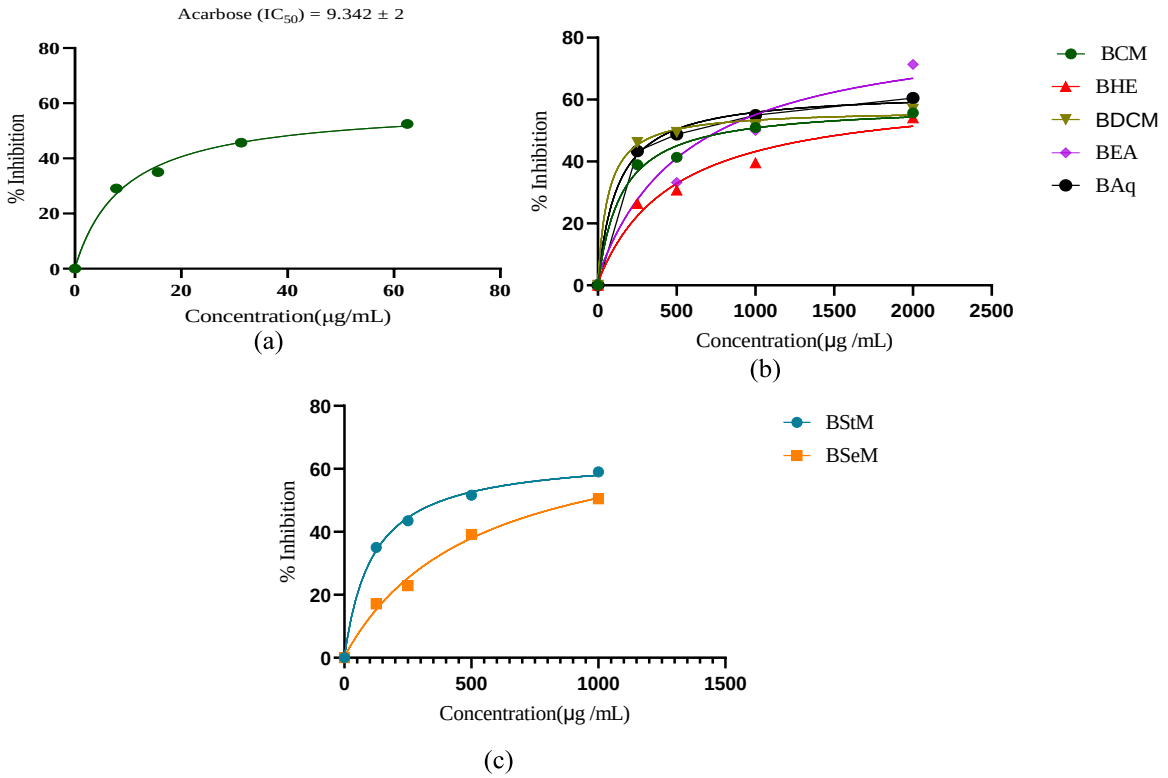


Fig. 6. (a) *α*-Amylase inhibition shown by standard acarbose against the different concentrations, **(b)** *α*-Amylase inhibition shown by the methanolic extracts and semi-purified fractions, and **(c)** α-amylase inhibition shown by crude methanolic extracts of stem and seed (*BCM- (crude methanolic extract of B. vahlii leaf), BHE- (hexane fraction of B. vahlii leaf), BDCM- (dichloromethane fraction of B. vahlii leaf), BEA- (ethyl acetate fraction of B. vahlii leaf), BAq- (aqueous fraction of B. vahlii leaf), BStM- (crude methanolic stem extract of B. vahlii) BSeM- (Crude methanolic seed extract of B. vahlii)*

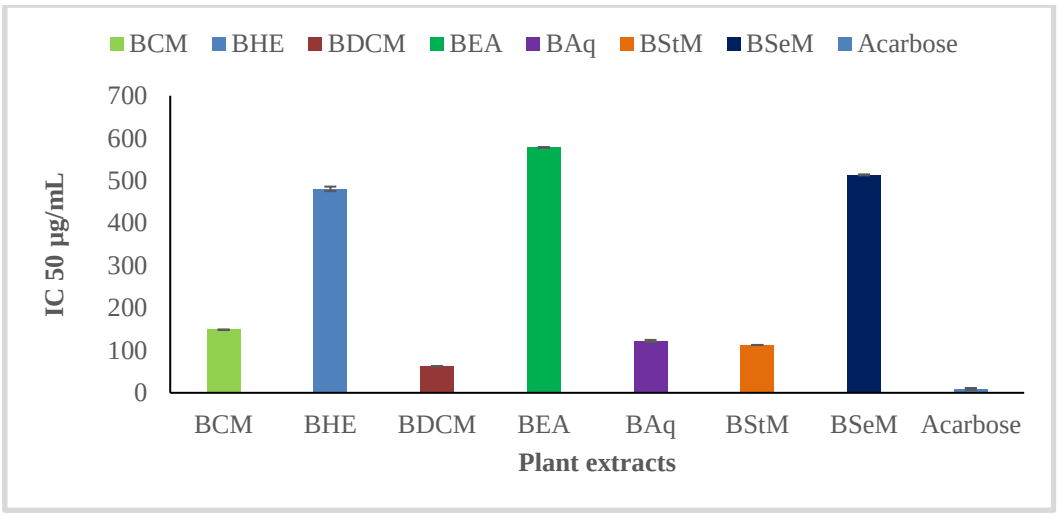


Fig.7. Comparative IC_{50} shown by crude extracts and semi-purified fractions against the α-amylase inhibition (*BCM- (crude methanolic extract of B. vahlii leaf), BHE- (hexane fraction of B. vahlii leaf), BDCM- (dichloromethane fraction of B. vahlii leaf), BEA- (ethyl acetate fraction of B. vahlii leaf), BAq- (aqueous fraction of B. vahlii leaf), BStM- (crude methanolic stem extract of B. vahlii) BSeM- (Crude methanolic seed extract of B. vahlii)*

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3.5. Antibacterial activity

The antibacterial activity of plant extracts against *Staphylococcus aureus* KCTC 1916, *Bacillus subtilis* ATCC 66333, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 10031 were measured as ZOI (Zone of inhibition in mm). The ZOI shown by the methanolic crude extract of seed was found to be 8 mm against *Klebsiella pneumoniae*, 8 mm against *Staphylococcus aureus* and 6.6 mm against and *Bacillus subtilis*. Similarly, the ZOI shown by the stem extract against the *Klebsiella pneumoniae* was found to be 7 mm, 11.3 mm against *Staphylococcus aureus* and 10.0 mm *Bacillus subtilis.* The crude methanol extracts and semi-purified fractions of the leaf (n-hexane and DCM) were found to be ineffective against all the test strains whereas, the ethyl acetate fractions of the leaf showed the ZOI as 10.3 mm against *Bacillus subtilis*.

3.6. Toxicity

The toxicity shown by the crude extracts and semi-purified fractions against the brine shrimp nauplii is shown in Table 1.

The calculation of the lethal concentration (LC_{50}) of plant extracts and fractions against the brine shrimp nauplii is shown in Table 1. For easy presentation, the regression equation obtained by plotting the log concentration versus probit (Finney's table for the transformation of the percentage of mortality to probit values) is shown in Table 1 from which the LC_{50} is calculated.

The seven different extracts and semi-purified fractions of the plant were tested for their cytotoxicity against brine shrimp nauplii. The lethal concentration (LC_{50}) that kills 50 % of the exposed population of A. salina values in µg/ml obtained from the brine shrimp lethality assay for different concentrations of plant extracts and semi-purified fractions was determined. The degree of lethality was found to be proportional to the concentration of the plant extracts. The results showed that maximum mortalities of the brine shrimp larvae took place at the concentration of 1000 µg/ml and the least mortalities at 10 µg/ml. The methanolic and aqueous extract of the leaf showed high toxicity against brine shrimp nauplii with LC_{50} 257.63 µg/ml and 100 µg/ml respectively.

Table 1. Calculation of LC_{50} for crude extracts and semi-purified fractions

Plant extracts/ Fractions	Regression equation obtained by plotting the log C against probit values of percentage mortality	LC_{50} (µg/ml)
BCM	Y= 0.68x + 3.36 R^2 = 0.9146	**257.63**
BHE	Y = 0.38x + 3.36 R^2 = 0.9918	20653.80
BDCM	Y = 2.08x – 2.77 R^2 = 0.75	5432.50
BEA	Y = 2.08x – 1.533 R^2 = 0.82	1380.38
BAq	Y = 0.25x + 4.5 R^2 = 1	**100.00**
BStM	Y = 2.08x – 1.36 R^2 = 0.75	1142.92
BSeM	Y = 1.86x – 2.48 R^2 = 0.75	10495.42

(*BCM- (crude methanolic extract of B. vahlii leaf), BHE- (hexane fraction of B. vahlii leaf), BDCM- (dichloromethane fraction of B. vahlii leaf), BEA- (ethyl acetate fraction of B. vahlii leaf), BAq- (aqueous fraction of B. vahlii leaf), BStM- (crude methanolic stem extract of B. vahlii) BSeM- (Crude methanolic seed extract of B. vahlii).*

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3.7. Statistical analysis

Each experiment was conducted three times and data were expressed as mean ± standard deviation. The plots were constructed using Microsoft Excel, GraphPad Prism 9, and sigma plot.

4. Discussion

The different parts of *Bauhinia vahlii* were found to have most of the secondary metabolites as listed in Table 1. But, few of the secondary metabolites previously reported in the same genus are found absent in this plant. This could be due to the variation in altitudes of plant grown, different environmental conditions, method and time of sample collection, extraction procedure, and also due to laboratory setup and chemical grade.

The results of this study showed that *Bauhinia vahlii* grown in Dhading Nepal is found rich in total phenolic content as compared to the stem bark of the same genus reported of 15.91 ± 0.20 µg/mg in petroleum ether fraction, 41.42 ± 1.63 µg/mg in ethyl acetate fraction, and 88.71 ± 1.04 µg/mg in methanol fraction [23]. The phenolic compounds are potent antioxidants that work in a structurally dependent manner. They can scavenge the ROS (Reactive Oxygen Species) and chelate transition metals which play vital roles in the initiation of free radical reactions in the human body which causes oxidative damage to the cell [24]. The greater amount of polyphenol content present in the plant extracts reflects the higher antioxidant activity.

The phenolic content found in this plant is slightly different from those reported in the literature. The obtained TPC value could be different or altered by their structural features, variations in plant samples, presence of interfering substances, geographical variation, different analytical assay methods, and presence of different amounts of sugars, ascorbic acid, or carotenoids.

Negi et al. 2012, has reported the total flavonoid content in the same genus of this plant was 132.89 ± 3.34 µg/mg in methanol fraction and 120.06 ± 1.91 µg/mg in ethyl acetate fraction of stem bark [23]. The results of the present study showed that the plant *Bauhinia vahlii* is found rich in flavonoid content and it could be biologically more potent due to the presence of varieties of phytochemicals. Farag et al. 2015, reported that *Bauhinia vahlii* contained the highest amount of flavonoid content than *Bauhinia variegata*, *Bauhinia forficata*, and *Bauhinia racemose* [25]. It has been reported that the flavonoid content in the plant samples was significantly affected by the factors such as genetic diversity, environment, and year-to-year seasonal variation [26]. The rich in phenolic and flavonoid content as secondary metabolites, particularly *Bauhinia vahlii* makes an important source of phytotherapeutic and medicinal agents.

The results of antioxidant activity showed that *Bauhinia vahlii* is a source of natural powerful free radical scavenger that may prevent oxidative damage in the human body which ultimately leads to oxidative stress and finally to cancer. The antioxidant activity reported in the same genus, *Bauhinia scandens* bark and leaf extracts of IC_{50} 34.77 µg/ml and 113.5 µg/ml respectively. This supports that the *Bauhinia vahlii* growing in this area is found potent source of antioxidants as compared to the other species [27].

The extracts and semi-purified fractions of *Bauhinia vahlii* growing in Nepal were found poor against the few bacterial strains used. The extraction of bioactive substances is influenced by the solvent polarity which ultimately affects the antibacterial activity of the extracts and the semi-purified fractions [22]. These facts and

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observations might have been attributed to the different structural features and permeability of the cell wall of bacteria, the season in which the plant is collected, the age of the plant, intraspecies variation, ambient circumstances, and the plant section collected [26]. Some of the plant extracts and the semi-purified fractions were found toxic against the Brine shrimp nauplii showing an LC_{50} less than 1000. It is common practice to compare the LC_{50} value of herbal extracts to either the Meyers or the Clarkson Toxicology Index to determine their level of toxicity. As defined by Clarkson's toxicity criterion for assessing plant extract toxicity, extracts with LC_{50} values above 1000 are nontoxic, extracts with LC_{50} values 500-1000 are less toxic, extracts with LC_{50} values 100-500 are moderately toxic, while extracts with LC_{50} values 0-100 are highly toxic [28]. Further studies can be performed based on the bioassay-guided isolation and identification of target compounds as promising leads for therapeutic applications.

5. Conclusion

In conclusion, the evaluation of the antioxidant activity of *Bauhinia vahlii* may be a substantial source of potential natural antioxidants. The findings of this study showed that the plant is rich in total phenolic and flavonoid content as secondary metabolites. The high antioxidant activity of the plant extracts may be suggested for the prevention of harmful oxidation that takes place in the human body and to treat cardiovascular disorders. Our findings open the door to the plant becoming a good source of natural compounds for more effective control of diabetes and microbial infections in human beings. This report may provide scientific validation for the traditional uses of this plant against diabetes and infectious diseases. The findings of the present study may provide a valuable idea for further work to isolate, purify, characterize, and standardize the bioactive constituents from the bioactive extracts and semi-purified fractions of *Bauhinia vahlii* for the development of new therapeutic agents. This study also revealed that the plant *Bauhinia vahlii* is a good source of natural antioxidant, antidiabetic and antibacterial compounds. The findings of this study provide a basis for developing valuable food additives to enhance human nutrition through the phenolic, and flavonoid composition regarding antidiabetic and antioxidant activity.

Conflicts of interest

All the authors have no conflict of interest in publishing this manuscript.

Author contribution

IL: Experimental analysis and preparation of the manuscript draft. KRS: Supervision of the research, preparation of the final manuscript, original idea presentation, study supervision, and final approval of the version to be published.

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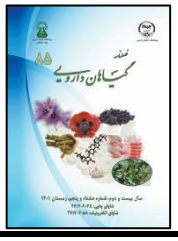
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مقاله تحقیقاتی

آنالیز کمی و بررسی فعالیت‌های بیولوژیکی *Bauhinia vahlii* Wight & Arn. رویش یافته در نپال

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اطلاعات مقاله

گل‌واژگان:
Bauhinia vahlii
Phanera vahlii
اثرات فارماکولوژیکی
آنتی‌اکسیدان
آلفا- آمیلاز
ضددیابتی

چکیده

مقدمه: جنس *Bauhinia* از حدود ۳۰۰ گونه تشکیل شده است و به طور گسترده در اکثر کشورهای گرمسیری جهان پراکنده است. **هدف:** اثرات آنتی‌اکسیدانی، آنتی‌باکتریایی، ضددیابتی و آنالیز کمی عصاره‌های ساقه، برگ و دانه *Bauhinia vahlii* (نام مترادف *Phanera vahlii*) مورد بررسی قرار گرفت. **روش بررسی:** فعالیت آنتی‌اکسیدانی عصاره‌های خام و فراکسیون‌ها با استفاده از روش مهار رایکال آزاد ۲،۲- دی فنیل-۱- پیکریل هیدرازیل (DPPH) مورد ارزیابی قرار گرفت. فعالیت ضددیابتی با روش مهار آلفا- آمیلاز مورد بررسی قرار گرفت. سمیت عصاره‌های گیاهی با استفاده از روش کشندگی میگوی آب شور (BSLA) با کمک آرتمیا سالینا به عنوان ارگانیسم آزمایش بیولوژیکی ارزیابی شد. **نتایج:** فراکسیون آبی برگ دارای محتوای فنلی تام بالایی برابر با ۱۶۸/۴۷ ± ۱/۹۴ میلی‌گرم گالیک اسید در گرم بود. محتوای تام فلاونوئیدی حداکثر در فراکسیون آبی برگ با میزان ۱۵۸/۵۱ ± ۲/۹۹ میلی‌گرم کوئرستین در گرم یافت شد. عصاره ساقه فعالیت آنتی‌اکسیدانی قوی با IC_{50} برابر با ۱/۹۱ ± ۰/۳۳ میکروگرم در میلی‌لیتر در مقایسه با استاندارد کوئرستین با IC_{50} برابر با ۳/۴۶ ± ۰/۴۰میکروگرم در میلی‌لیتر نشان داد. فراکسیون دی کلرومتانی برگ (DCM) دارای خواص مهاری قابل توجهی در مهار آلفا- آمیلاز با IC_{50} برابر با ۱۱۲/۷۰ ± ۲/۰ میکروگرم بر میلی‌لیتر در مقایسه با استاندارد آکاربوز ۹/۳۴ ± ۲/۰ میکروگرم بر میلی‌لیتر نشان داد. نتایج بررسی اثرات ضد میکروبی عصاره متانولی ساقه و دانه هاله عدم رشد در برابر استافیلوکوکوس اورئوس، باسیلوس سوبتیلیس و کلبسیلا پنومونیه نشان داد اما در برابر اشریشیاکلی بی‌اثر بود. عصاره متانولی و آبی برگ به ترتیب با LC_{50} برابر با ۲۵۷/۶۳ و ۱۰۰ میکروگرم در میلی‌لی‌تر بر ناپلی میگوی آب شور سمیت نشان دادند. **نتیجه‌گیری:** این مطالعه نشان داد که *Bauhinia vahlii* غنی از محتواهای فلاونوئیدی و فنولی است و می‌تواند به عنوان منبع غنی از آنتی‌اکسیدان‌های طبیعی و عوامل ضددیابت، کاندید جداسازی دارو شود.

مخففها: DPPH، ۲،۲- دی فنیل-۱- پیکریل هیدرازیل؛ IC_{50}، میانگین غلظت مهارکنندگی؛ ROS، گونه‌های اکسیژن فعال؛ NTFP، تولیدات جنگلی غیر چوبی؛ FCR، معرف فولین سیوکاتیو؛ DMSO، دی متیل سولفوکسید؛ LC_{50}، میانگین غلظت کشندگی؛ ZOI، هاله عدم رشد. GAE: معادل اسید گالیک؛ QE، معادل کوئرستین؛ PPA، آمیلاز پانکراس خوک؛ SD، انحراف استاندارد؛ CKD، بیماری مزمن کلیه

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