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Nutrient composition and functional properties of Bauhinia vahlii seeds

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ABSTRACT

The main aim of this study is to analyse the nutrient composition, anti-oxidant, anti-bacterial and alpha amylase inhibitory activity of *Bauhinia vahlii* seeds. AOAC standard methods were used for proximate analysis. For estimation of minerals such as calcium, iron, magnesium and zinc Atomic Absorption Spectroscopy is used. Flame photometry is used for estimation of sodium and potassium contents. The proximate composition analysis indicated that moisture, ash, energy, carbohydrate, protein, fat, fibre was found to be 47±0.6, 3.4±0.1, 385±2.7, 3.3±1.1, 23.7±0.02, 30.8±0.5, 4.3±0.2 respectively per 100g of dry sample. Mineral analysis revealed that as sodium, potassium, iron, calcium, phosphorous, zinc and magnesium were found to be 7.1±0.2, 476.2±2.6, 6.5±0.2, 303±2.4, 616±3.5, 0.8±0.1, 4.8±0.4 respectively per 100g of dry sample. Four new fatty acids were identified by GC-MS method. They were Hexadecanoic acid (15.48%), 9,12-Octadecadienoic acid (46.49%), 9-Octadecenoic acid (30.63%) and Methyl stearate (7.4%). The IC50 values of DPPH were 0.1 mg/ml and 4.1 mg/ml for aqueous and methanolic extracts respectively. The IC50 values of metal chelating activity were 3.5 mg/ml and 4.8 mg/ml for aqueous and methanolic extracts respectively. The reducing power of both aqueous and methanolic extracts increased with increasing concentration. The total phenolic content was found to be 21.6±0.07mg gallic acid equivalents and 20.9±0.4mg gallic acid equivalents in aqueous and methanolic extracts respectively. The aqueous extract of *Bauhinia vahlii* seeds, had a greater anti-bacterial potential against gram positive bacteria such as M. luteum whereas methanolic extracts exhibited greater anti-bacterial potential against to gram positive bacteria M. luteum and gram-negative bacteria. At 10mg/ml concentration, the aqueous extracts of *Bauhinia vahlii* seeds showed anti-bacterial activity against M. luteum (12mm) followed by E. coli (11mm), P. aeruginosa (10mm) and B. subtilis (10mm). At 10mg/ml concentration, the methanolic extracts of *Bauhinia vahlii* seeds showed maximum anti-bacterial activity against M. luteum (12mm), E. coli (12mm), P. aeruginosa (12mm) followed by B. subtilis (11mm). The alpha amylase inhibitory activities of *Bauhinia vahlii* seeds aqueous extracts with increasing concentrations such as 0.6, 1.25, 2.5, 5 and 10mg/ml were found to be 38.3±0.6, 68.6±0.4, 90.2±0.6, 91.7±0.2 and 93.6±0.6 percentage respectively. As well as, the alpha amylase inhibitory activities of *Bauhinia vahlii* seeds methanolic extracts with increasing concentrations such as 0.6, 1.25, 2.5, 5 and 10mg/ml were found to be 40.1±0.8, 47.3±0.5, 54.9±0.7, 74.6±0.4 and 80.5±0.4 percentage respectively. The IC50 values for the aqueous and methanolic extracts of were calculated as 0.6 and 1.6mg respectively.

Keywords: *Anti-oxidant, Total Phenolic content, DPPH, Metal chelating, IC50*

1. INTRODUCTION

Bauhinia vahlii Wight & Arn. is a very large, usually evergreen, climber. It is distributed in deciduous forests of India from Gujarat southwards to Maharashtra and Northern Andhra Pradesh, commonly on hillsides and in forest valleys (**Parrotta JA 2001**). The seeds of this legume are a rich source of crude protein (24.59%), crude lipid (23.26%), crude fibre (6.21%), carbohydrates (41.72%), minerals, and essential amino acids. The ripe seeds, when eaten raw or fried, taste like cashew-nuts (**NISC 1986**). The cooked and roasted mature seeds of *B. vahlii* are eaten by the tribes, Kondakapulu and Baagethalu of Araku valley, Visakhapatnam, Andhra Pradesh, and Mundari group of tribes in India (**Vadivel & Janardhan K 2000; Rajaram N, Janardhanan K. 1991**).

2. MATERIALS AND METHODS

Collection of plant material

Plant material was collected from Paderu division, lies between 18.0833N and 82.6667E longitude situated in Eastern Ghats Alluri Sitarama Raju District, Andhra Pradesh. Plant materials was taxonomically identified and authenticated by Prof. B.

Padal, Department of Botany, Andhra University, Visakhapatnam. Plant specimen was labelled, numbered and annotated with date of collection and locality. Herbarium was deposited in Department of Botany, Andhra University (AUV 25511). Plant materials were rinsed with tap water and then with de-ionized water. Later, plant materials were shade dried until all the water molecules evaporated. After drying, plant materials were ground well using mechanical blender into fine powder and transferred into air tight containers with proper labelling for further analysis.

Solvent extraction

Bauhinia vahlii seed samples were extracted by stirring with 100 mL of distilled water and methanol at room temperature for 24 hr and filtering through Whatman No. 4 filter paper. The solvent of the combined extract was evaporated.

Determination of nutrient content

The recommended methods of the Association of Official Analytical chemists **(AOAC, 2016)** were used for the determination of moisture, ash, crude lipid, crude fibre and nitrogen content. Carbohydrate was determined by difference method [100 - (Protein +Fats +moisture +ash)]. The nitrogen value, which is the precursor for protein of a substance, was determined by micro Kjeldahl method. The nitrogen value was converted to protein by multiplying to a factor of 6.25. The moisture and ash were determined using weight difference method while determination of crude lipid content of the samples was done using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40 - 60°C). For estimation of minerals such as calcium, iron, magnesium and zinc AAS is used. Flame photometry is used for estimation of sodium and potassium contents. Auto analyser is used for amino acid profiling.

Anti-oxidant activity

Free radical scavenging activity on DPPH

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method **of Shimada et al., 1992**. Sample extracts at various concentrations was taken and the volume was adjusted to 100 µL with methanol. Five mL of a 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27° C. The absorbance of the sample was measured at 517 nm using spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula.

% DPPH radical scavenging activity = (control OD−sample OD/ control OD) × 100

Reducing power

The antioxidant capacity of *B. vahlii* seed sample was estimated according to the procedure described by Yildrim et al. Sample solution was mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. Then the mixture was incubated at 50ºC for 20 minutes and then 2.5 ml of 10% TCA was added to the mixture and centrifuged at 1500rpm for 10 minutes. An aliquot of 2.5 ml from upper layer was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $FeCl_3$ and absorbance was read at 700 nm. Increased absorbance of the reaction mixture indicates increasing reducing power. (**Yildirim et al., 2001**)

Determination of Total phenolic content

The total phenolic content was determined according to the method described by Singleton & Rossi (1965). Aliquots of extracts (1 mL) were taken in test tubes and made up to the volume of 10 mL with distilled water. Then 1 mL of Folin-Ciocalteu phenol reagent (1:1 with water) and 10 mL of sodium carbonate solution (7%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 2 hours and the absorbance was recorded at 760 nm against the reagent blank. The concentration of total phenolics was determined by using the formula

$$T = \frac{C \times V}{M}$$

T = Total phenolic content mg/gm of plant extract as GAE

C = Concentration of gallic acid from the calibration curve in mg/ml

V = Volume of extract in ml

M = weight of pure plant extract in gm

Metal chelating activity

Metal chelating activity was determined by making slight modifications to Boyer and Mc Cleary, 1987. Test samples with different concentrations such as 0.6, 1.25, 2.5, 5 and 10 mg/ml should be mixed with 0.1 ml of 2mM $FeCl_2$ and 0.2 ml of 5mM ferrozine. The reaction mixture was allowed to stand for 20 minutes at room temperature and absorbance was read at 562nm. Blank should be prepared in the same manner with distilled water instead of sample. For the sample blank at each

concentration, $FeCl_2$ solution should be excluded and distilled water to be used instead. **(Boyer & McCleary, 1987)** The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

The chelating activity can be calculated using the formula

Chelating activity (%) = $\frac{Abs_{control} - Abs_{sample} - Abs_{blank}}{Abs_{control}} \times 100$

$Abs_{control}$: Absorbance of control; Abs_{sample}: Absorbance of sample; Abs_{blank}: Absorbance of blank

Anti bacterial assay

The bactericidal activity of *B. vahlii* seed aqueous and methanolic extracts of different concentrations (0.6, 1.25, 2.5, 5 and 10 mg/ml) was evaluated by using well diffusion method. The antibacterial activity was assessed by using gram positive bacteria such as Bacillus subtilis, Micrococcus luteus and gram-negative bacteria such as Escherichia coli, Pseudomonas aeruginosa. For antibacterial activity erythromycin was used as positive control and methanol was used as negative control. Nutrient agar medium was prepared and pour plate method was performed. 0.5 mm Wells were prepared. Wells were loaded with erythromycin, methanol, and different concentrations of seed extract. Plates were sealed with parafilm and incubated at 37^0C for 24hrs. After incubation, zone of inhibition for extracts were measured in millimeters using veneer calipers.

Alpha amylase

The antidiabetic potential of aqueous and methanolic extracts of *B. vahlii* seeds were evaluated by the quantitative estimation of alpha amylase inhibition. The α-amylase inhibitory assay was evaluated according to a previously described method by **Malik and Singh *et al.*, (1980)** with slight modification. A series of different concentrations of aqueous and methanolic extracts such as 0.6, 1.25, 2.5, 5 and 10 mg/ml were used to study the α-amylase inhibitory activity. To perform α-amylase inhibitory assay, 1 ml of extract from respective concentrations were mixed with 1 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 30 min and then amount of glucose liberated was estimated by using GOD-POD kit. To estimate glucose, 100 µl of reaction mixture from each test sample was taken in fresh test tubes and 2 ml of GOD-POD reagent was added. Then, the resulting solution was incubated for 15 minutes at room temperature and diluted with 2 ml of distilled water. The colour intensity of developed pink colour was measured at 540 nm using a spectrophotometer. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. The amount of glucose was calculated using the following equation:

Glucose (mg/dL) = Abs Control−Abs Sample×100

The inhibition of α-amylase was calculated using the following equation

Inhibition % = $\frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$

Statistical analysis

All of the statistical analyses were performed using Microsoft Excel spread sheet ver2019. The experimental results were expressed as mean ± standard deviation (SD) of three replicates.The IC50 value was calculated using the linear regression equation. **Results and Discussion**

Nutrient composition

The proximate composition analysis indicated that moisture, ash, energy, carbohydrate, protein, fat, fibre was found to be 47±0.6, 3.4±0.1, 385±2.7, 3.3±1.1, 23.7±0.02, 30.8±0.5, 4.3±0.2 respectively per 100g of dry sample. Mineral analysis revealed that as sodium, potassium, iron, calcium, phosphorous, zinc and magnesium were found to be 7.1±0.2, 476.2±2.6, 6.5±0.2, 303±2.4, 616±3.5, 0.8±0.1, 4.8±0.4 respectively per 100g of dry sample. The proximate and mineral composition are represented in table.1&2 respectively. Four new fatty acids such as Hexadecanoic acid (15.48%), 9,12-Octadecadienoic acid (46.49%), 9-Octadecenoic acid (30.63%) and Methyl stearate (7.4%) were identified by GC-MS method.

Amino acid analysis of *Bauhinia vahlii* seed revealed that it is rich in essential amino acids that are crucial for functioning of human body. These seeds are rich in Branched Chain Amino Acids (BCAAs) such as leucine, isoleucine and valine that are important regulators of mTOR signalling pathway, regulate protein synthesis as well as protein turnover, facilitate glucose uptake by liver and SK muscle and also enhance glycogen synthesis. BCAAs are also important in immunity, brain function, and other physiological aspects of well-being (**Monirujjaman & Ferdouse, 2014**). It is also a good source of non-essential amino acid arginine which have role in enhancing wound healing, regulating endocrine activity and potentiate immune activity **(Efron & Barbul, 2000)**. Amino acid content of *Bauhinia vahlii* seed is represented in table.3

Table.1. Proximate composition of *Bauhinia vahlii* seed

Parameters	Amount
Moisture	47±0.1
Ash	3.4±0.14
Energy	210.6±2.3
Carbohydrate	
Protein	23.7±0.02
Fat	30.8±0.5
Fibre	4.29±0.2

Table.2. Mineral content of *Bauhinia vahlii* seed

Mineral	Amount (mg)
Iron	6.5±0.2
Calcium	303±2.4
Phosphorous	616±3.5
Sodium	7.1±0.2
Potassium	476.2±2.6
Zinc	0.8±0.1
Magnesium	4.8±0.4

Table.3. Amino acid profile of *Bauhinia vahlii* seed

Amino acid	mg per gm N
Arginine	530
Histidine	90
Lysine	260
Tryptophan	50
Phenylalanine	200
Methionine	60
Threonine	190
Leucine	410
Isoleucine	260
Valine	260

Anti-oxidant activity

DPPH radical scavenging activity

DPPH radical is one of the few stable organic nitrogen free radicals, which has been widely used to determine the free radical scavenging ability of the various samples **(Brand-Williams et al., 1995**). The method is based on the reduction of an

alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction (**Shon MY et al., 2003**). DPPH radical scavenging activity of the tested extracts is concentration dependent and lower IC50 value reflects higher scavenging ability. The DPPH radical scavenging activity of aqueous and methanol from seeds of *B. vahlii* is presented in figure 1.

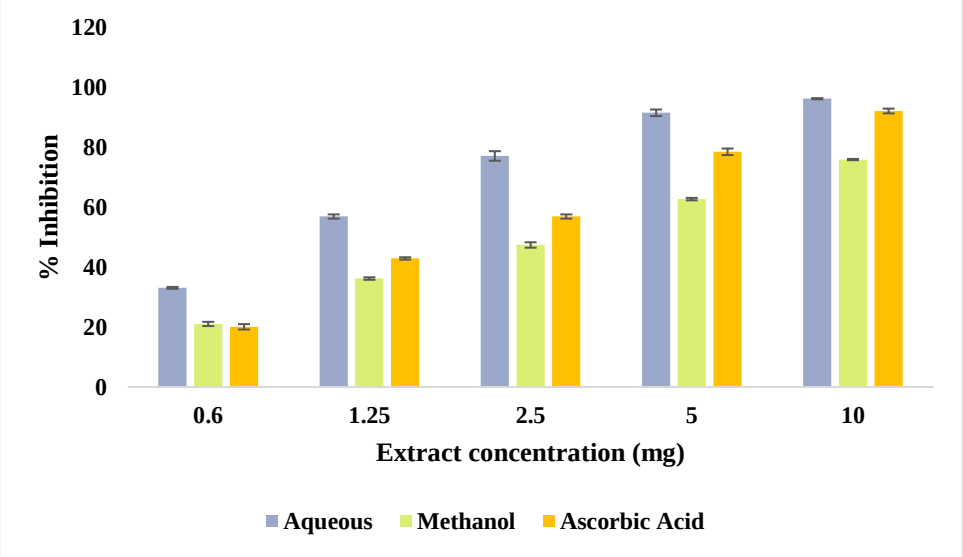

Fig.1. DPPH radical scavenging activity of *Bauhinia vahlii* seed extract

Total Phenolic content

Phenolic components are very important plant constituents with scavenging ability because of its hydroxyl group. It has been established that phenolic compounds are the major plant compounds with antioxidant activity and this activity is due to their redox properties. Phenolic compounds are a class of antioxidant agents which can adsorb and neutralize the free radicals (**Florence et al., 2011**)**.** The results indicated that aqueous extract of *B.vahlii* seeds contained higher amount (21.6±0.07 mg GAE/g of dry material) of phenolics compared to methanolic extract (20.9±0.49 mg GAE/g of dry material).

Table.4. Total Phenolic Content of *Bauhinia vahlii* seed extract

Type of extract	**Total phenolic content** (GAE mg / g of dry material) (Mean ± SD)
Aqueous	21.6±0.07
Methanolic	20.9±0.49

Reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with the presence of reductones which breaks the free radical chain by donating a hydrogen atom (**Subhasini et al., 2011**). In this assay, the ability of extracts to reduce Fe^{3+} to Fe^{2+} was determined. The presence of antioxidants in the extracts resulted into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). In reducing power assay, antioxidants cause the reduction of the Fe^{3+} into Fe^{2+}, thereby changing the solution into various shades from green to blue, depending on the reducing power of the compounds (**Ferreira et al.,2007**). Strong reducing agents, however, formed Perl's Prussian blue colour and absorbed at 700 nm. Figure 2 showed the reducing activities of various extracts of *B. vahlii seeds* in comparison with ascorbic acid as standard. The higher the absorbance of the reaction mixture, the higher would be the reducing power. Reducing power of different extracts increased with the concentration of the extract. The reducing power was found to be more in methanolic extract of seed than aqueous extract. The reducing power of reference compound (Ascorbic acid) was found to be higher than all the tested extracts.

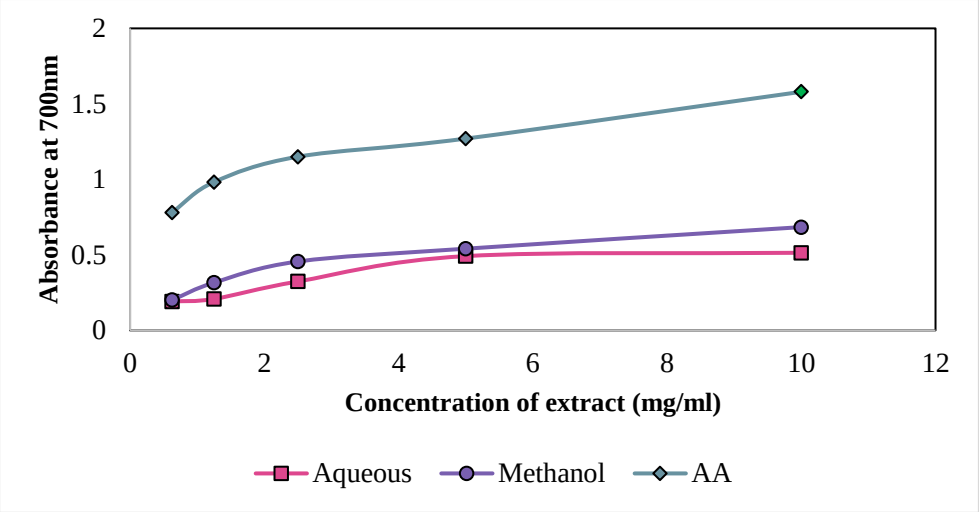

Fig.2. Graphical representation of reducing power of *Bauhinia vahlii* seed extracts

Metal chelating activity

Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress (**Hippeli S, Elstner EF. 1999**). The chelating ability of ferrous ions by the differentially processed seed extracts was estimated by the method of Boyer and Mc Cleary, 1987. Ferrozine can quantitively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that red colour of the complex is decreased. The metal chelating activity of aqueous and methanol from seeds of B. vahlii is presented in figure 3.

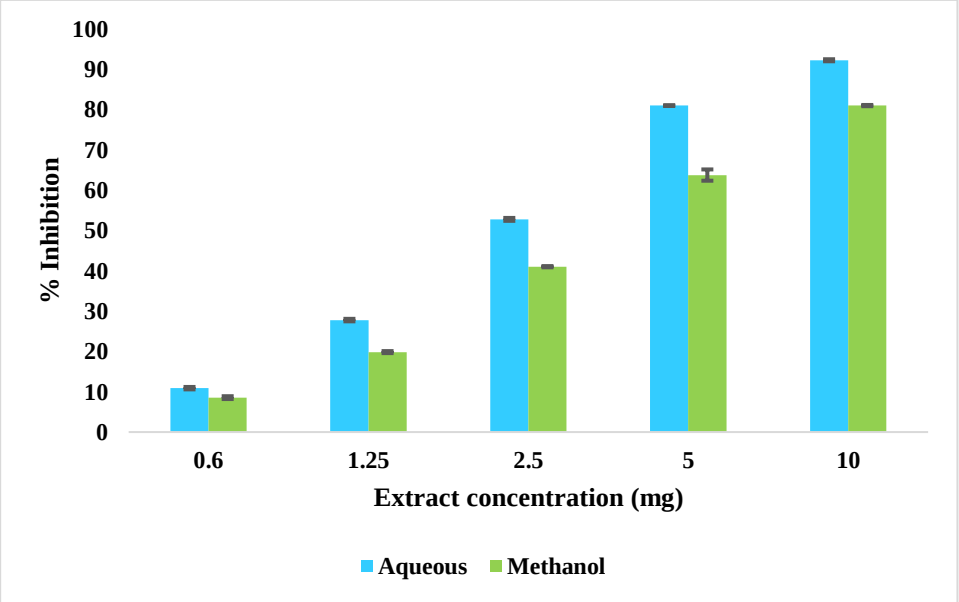

Fig.3. Metal chelating activity of *Bauhinia vahlii* seed extracts

Anti bacterial assay

The bactericidal activity of selected plant extract was evaluated against the bacterial species including *Bacillus subtilis, Micrococcus luteum, Escherichia coli, Pseudomonas aeruginosa* using agar well diffusion method. The anti-bacterial activity from the aqueous and methanolic extracts of *Bauhinia vahlii* seeds are presented in table 5. From these results, aqueous and methanolic extracts shows anti-bacterial activity with significant variations. The aqueous extract of *Bauhinia vahlii* seeds, had a greater anti-bacterial potential against gram positive bacteria such as *M. luteum* whereas methanolic extracts exhibited greater anti-bacterial potential against to gram positive bacteria *M. luteum* and gram-negative bacteria.

At 0.6mg /ml concentration, the aqueous extracts of *Bauhinia vahlii* seeds, both gram positive and gram-negative strain bacteria showed resistance and does not exhibit zone of inhibition. At 10mg/ml concentration, the aqueous extracts of *Bauhinia vahlii* seeds showed anti-bacterial activity against *M. luteum* (12mm) followed by *E. coli* (11mm), *P. aeruginosa* (10mm) and *B. subtilis* (10mm). At 0.6mg /ml concentration, the methanolic extracts of Bauhinia vahlii seeds both gram positive and gram-negative strain bacteria showed resistance and does not exhibit zone of inhibition. At 10mg/ml concentration, the methanolic extracts of Bauhinia vahlii seeds showed maximum anti-bacterial activity against M. luteum (12mm), E. coli (12mm), P. aeruginosa (12mm) followed by B. subtilis (11mm).

The anti-bacterial activity of *Bauhinia vahlii* seeds aqueous extracts with increasing concentration showed a strong positive correlations such as r=0.897, r=0.897, r=0.897 and r=0.897 against *B.subtilis*, *M.luteum*, *E.coli* and *P. aeruginosa*. The results of correlation analysis for aqueous extracts shown in table 4. In addition, the anti-bacterial activity of *Bauhinia vahlii* seeds methanolic extracts with increasing concentration showed a strong positive correlations such as r=0.897, r=0.892, r=0.897 and r=0.892 against *B.subtilis, M.luteum, E.coli* and *P. aeruginosa* respectively.

Alpha amylase inhibition activity

Diabetes Mellitus is a metabolic disorder characterized by high blood glucose level caused due to deficiency of insulin secretion or insulin action. The inhibition of carbohydrate hydrolyzing enzymes such as a-amylase can be an important strategy in the postprandial blood glucose level in patients with type 2 diabetes. Alpha amylase is an enzyme that breakdown the polysaccharides into simple sugars thereby involved in the liberation of glucose. Hence the inhibition of alpha amylase leads to the inhibition of glucose liberation.

In the present study aqueous and methanolic extracts of *B. vahlii* seeds were investigated for their potential to inhibit α-amylase activity. Five different concentrations viz., 0.6, 1.25, 2.5, 5, 10 mg/mL of aqueous and methanolic extracts were separately tested for the inhibition of α-amylase activity.

The alpha amylase inhibitory activities of aqueous and methanolic extracts *Bauhinia vahlii* seeds are shown in Fig.4. From these results, the aqueous extracts exhibited the greater alpha amylase inhibitory activity than methanolic extract. Furthermore, in both the extracts the inhibitory activity was significantly increased with increasing extract concentration. The greater activity was found in the aqueous extracts at 10mg/ml concentration and lowest activity was observed in aqueous extract at 0.6mg/ml concentration.

The alpha amylase inhibitory activities of *Bauhinia vahlii* seeds aqueous extracts with increasing concentrations such as 0.6, 1.25, 2.5, 5 and 10mg/ml were found to be 38.3±0.6, 68.6±0.4, 90.2±0.6, 91.7±0.2 and 93.6±0.6 percentage respectively. As well as, the alpha amylase inhibitory activities of *Bauhinia vahlii* seeds methanolic extracts with increasing concentrations such as 0.6, 1.25, 2.5, 5 and 10mg/ml were found to be 40.1±0.8, 47.3±0.5, 54.9±0.7, 74.6±0.4 and 80.5±0.4 percentage respectively.

The IC50 values for the aqueous and methanolic extracts of were calculated as 0.6 and 1.6mg respectively.

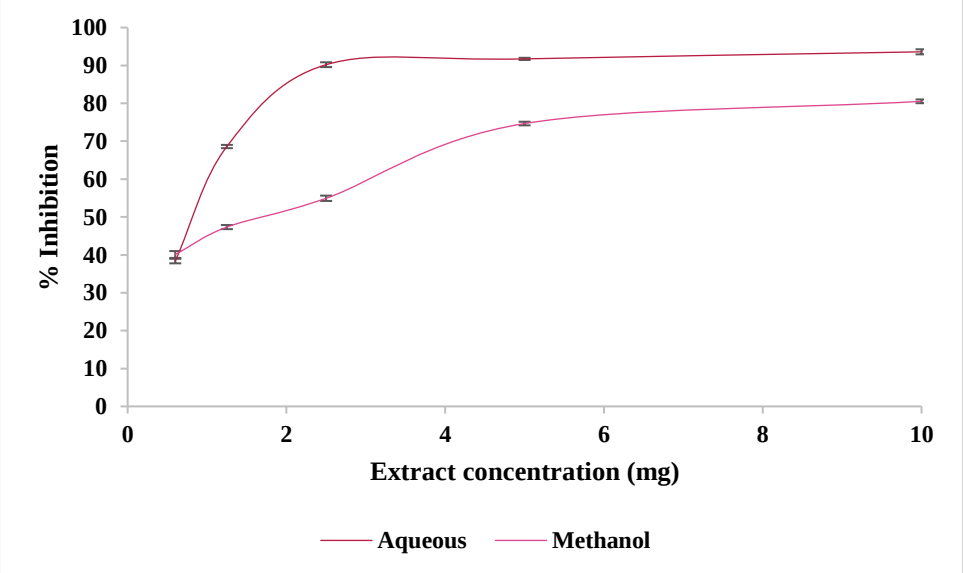

Fig.4. Alpha amylase inhibition activity of *Bauhinia vahlii* seed extracts

3. CONCLUSION

The results obtained from this study clearly indicates that *Bauhinia vahlii* seed was found to be rich in protein, energy, iron, phosphorous, good source of essential amino acids and can potentially be included in the diet to reap significant health benefits. Moreover, B. vahlii were found to possess strong antioxidant for treating radical-mediated pathological damages in human system and also as therapeutic agents as it has role in anti-diabetic activity. Further, it could be exploited as a potent antioxidant additives or as nutritional supplements.

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