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REVIEW PAPER

In-vitro Antioxidant and Anti-obesity Properties of *Bauhinia Variegata*

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ABSTRACT

Bauhinia variegata L (Synonyms: *Phanera variegata* Benth), commonly known as orchid tree or Kachnar belongs to the family of Leguminosae and found throughout India. All parts of the plants are used in the treatment of many ailments such as obesity, dyspepsia, bronchitis, leprosy, ulcer etc. The *in-vitro* antioxidant and anti-obesity properties of different solvent (hexane, chloroform, ethyl acetate, acetone, methanol and distilled water) extracts of *Bauhinia variegata* leaves is assessed. All the solvent extracts were found to contain different phytochemicals with bioactive properties at varying concentrations. The results of extraction showed that methanol was the suitable solvent for extraction of phytochemical as it recorded highest yield of 6.80 per cent. The phytochemical assay results showed the highest polyphenol content in methanol extract (243 µg GAE/mg) and flavonoids content in chloroform extract (1.87 µg CE/g). The *in-vitro* antioxidant and anti obesity property was observed more in methanolic extract than the other extracts. Suggests that *Bauhinia variegata* leaf extract could be a potential ingredient for the preparation of nutraceuticals to treat obesity and oxidative stress related complications.

Keywords: Antioxidant; Obesity; Phytochemicals; *Bauhinia*; Flavonoids

1. INTRODUCTION

Medicinal plants are valuable source of large number of secondary metabolites, which are used as potential pharmaceuticals or phytomedicines. Natural or herbal phytochemicals can be extracted from different parts such as roots, stem, leaves, flower, seeds, fruits, etc[1]. The advantages of herbal extracts are due to the combination of secondary metabolites available present in the plants. The most important constituents of these secondary products are flavonoides, phenolic compounds, alkaloids and tannins.

Many studies have reported that the oxidative stress is an important aetiology of many diseases and antioxidants will be useful in the treatment. Oxidative stress is caused due to the imbalance between free radical generation and antioxidant defence mechanism of the body. Commonly used artificial antioxidants like and butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) added in to the food products have adverse effects and are carcinogenic[2]. The use of natural antioxidants present in the plants has attracted the attention because of their safety, nutritional value and cost effectiveness. Supplementation of diets rich with antioxidant compounds such as polyphenol and flavonoides can protect the cell from damage due to free radicals and reduce the control symptoms associated with chronic or stress related illness[3]. Hence, there is more demand for the use of natural or herbal products for treating the complications caused due to reactive oxygen species.

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Obesity is a major health problem of carbohydrate and lipid and metabolism resulting due to increased fat accumulation in adipose tissue, liver and skeletal muscle, etc[4]. Obesity is one of the major risk factor for hypertension, cardiovascular diseases, type 2 diabetes and non alcoholic fatty liver metabolic syndrome[5,6]. Identifying the exact cause of obesity is difficult since many factors influence energy balance and interact at different levels. The root cause is the disturbance of homeostatic mechanisms that control energy balance. Currently Food and Drug Administration (FDA) permitted medicines like orlistat and sibutramine are being used for the treatment, are not to be used for long term use due many side effects. Epidemiological reports have suggested the use of herbal based medicines to decrease the risk of obesity and related disorders[7].

The plant *Bauhinia variegata* L (Synonyms: *Phanera variegata* Benth), commonly known as orchid tree or Kachnar is a medium sized, delicious tree found throughout India. Various parts of the plants are used in indigenous medicine for the treatment of many ailments obesity, dyspepsia, bronchitis, leprosy, ulcer, and as an astringent, tonic and anthelmintic[8-11]. Phytochemical analysis of the leaves of *Bauhinia variegata* Linn was reported to contain, alkaloids, oil, fat glycosides, phenolics, lignins, saponins, terpinoids, β -sitosterol, tannins, kaempferol-3-glucoside, rutin, quercetin, quercetin, apigenin, apigenin-7-O-glucoside, lupeol amides, carbohydrates, reducing sugars, protein, vitamin C, fibers, calcium and phosphorus[5]. The beneficial therapeutic properties of the plants are mainly due to the presence of these components. Hence, the current experiment was carried out to analyse the antioxidant and antiobesity property of leaf extracts of *Bauhinia variegata.*

2. MATERIALS AND METHODS

2.1 Plant Material Collection

The leaves of *Bauhinia variegata* were gathered from the Tirumala hills, Tirupati, Andhra Pradesh, India, and acknowledged with help of herbarium compilation, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh.

2.2 Chemicals and Reagents

Hexane, chloroform, ethyl acetate, methanol and distilled water were used for the extraction of the bioactive compounds. Orlistat, gallic acid and catechin, *p*-nitrophenyl butyrate (NPB), and lipase (Type II: from Porcine pancreas) were procured from Sigma-Aldrich, USA whereas FC (Folin–Ciocalteu) reagent and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Hi-Media (Mumbai, India) and the remaining chemicals used in the study were high quality grade and purchased from SRL (Mumbai, India).

2.3 Preparation of Plant Extracts

The plant material was washed thoroughly and dried in shade for three days and used for experiments. Fifty grams of crushed leaf material was used for sequential extraction by using non-polar to polar solvents *viz*., hexane, chloroform, ethyl acetate, acetone, methanol and water, in a shaker for 2 days. The extracts were filtered using Whatman No 1 membrane and the filtrate was concentrated using flash evaporator followed by lyophilization to remove the residual water and each extract was weighed to calculate the yield and expressed in terms of percentage. Further known quantity of the extracts were dissolved in respective solvents and a stock of 100 mg/ml was prepared and stored at 4 °C for further experiments.

2.4 Phytochemicals Analysis

In phytochemical analysis total polyphenol and total flavonoides estimation of the test samples was carried out according to standard methods.

2.4.1 Total Polyphenol Estimation

Total polyphenol content of each extract was estimated by using Folin-Ciocalteu reagent method[12]. The extracts were diluted in distilled water to get different concentrations and added to FC reagent and incubated for 10 min at room temperature. Two ml of 7 per cent Na_2CO_3 solution was added and the optical density (OD) was measured at 650 nm. Gallic acid was used as a standard and the amount of total polyphenol content was expressed as µg gallic acid equivalent per milligram (µg GAE/mg) of extract.

2.4.2 Total Flavonoids Estimation

Total flavonoids estimation of the extracts was carried as per the method described by Delcour and Varebeke[13]. The extracts/catechin (standard) were diluted to get different concentrations and mixed with chromogen reagent (0.1 per cent cinnamaldehyde of 75 ml methanol and 25 ml concentrated HCl). After 10 min of incubation, the optical density of the samples was measured at 640 nm and total flavonoid content was expressed as µg catechin equivalent per gram (µg CE/g) of extract.

2.5 *In-vitro* Antioxidant Assays

2.5.1 DPPH Radical Scavenging Activity

The DPPH (1, 1 diphenyl-2-picrylhydrazyl) radical scavenging assay was performed to evaluate the ability of antioxidants to scavenge free radicals as described by Eberhardt[14], *et al*. Individual extracts of *Bauhinia variegata* were dissolved in methanol to get test solution of 1 mg/mL. DPPH (500 µl, 0.5 mM in methanol) solution was added to different concentrations of sample and made the volume to 3.5 ml with methanol. BHA was used as standard antioxidant compound. Three ml of methanol and 0.5 ml of DPPH solution was used as positive control and samples without DPPH solution was used as blank sample. The mixtures were incubated at room temperature for 45 min in dark. The optical density was measured using spectrophotometer at 515 nm against methanol as blank and the results were expressed in terms of IC_{50} µg/ml. The DPPH radical scavenging activity was calculated using the following formula:

DPPH° scavenging activity % = [$(A_c-A_s)/ A_c$] x 100,

where A_c is the optical density of positive control solution and A_s is the optical density of test solution. IC_{50} values were calculated by linear regression equation derived from the graph of per cent DPPH scavenging activity and sample concentration.

2.6. *In-vitro* Antiobesity Assay

2.6.1 Lipase Inhibition Assay

Lipase inhibitory assay of the extracts was carried out according to the method of Han[15], *et al*. with minor modifications. Substrate solution was prepared in 9 ml of 0.1 M TES buffer (pH 7.0) by dissolving the lecithin (10 mg), sodium cholate (5 mg) and glycerol trioleate (80 mg). Individual extracts of different concentrations were prepared in 0.1 M TES buffer. To the sample (20 µl) and substrate solutions (20 µl) in microplate wells, 10 µl of lipase solution (20 µg/ml) was added and incubated for 30 min at 37 °C. The optical density was recorded at 550 nm using a microplate reader. Lipase inhibitory activity (per cent) was calculated as follows:

Lipase Inhibition (%) = {1 - $(OD_2-OD_1)/(OD_4-OD_3)$X100}

Where OD_1 is the optical density of solution containing plant extract, substrate and lipase; OD_2 is the optical density of solution containing plant extract and substrate; OD_3 is the optical density of incubated containing substrate and lipase; OD_4 is the optical density of solution containing substrate.

2.6.2 Amylase Inhibition Assay

The amylase inhibition activity was estimated according to the method reported by Xiao[16], *et al*. and Yoshikawa[17], *et al*. with minor modifications. Substrate solution was made by dissolving soluble starch (500 mg) in 25 mL of 0.4 M NaOH and heating for 5 min at 100 °C. The pH of solution was adjusted to 7.0 and the volume made to 100 ml by adding water. Different concentrations of the plant extract solutions were prepared in acetate buffer (pH 6.5). To the sample (20 µl) and substrate solutions (40 µl) in microplate wells, 20 µL

of α-amylase solution (50 µg/m) added and incubated for 15 min. 80 µL of 0.1 M HCl was added to terminate the reaction; then 200 µL of 1 mM iodine solution was added. The optical density was measured at 650 nm. Amylase inhibitory activity was calculated as follows:

$$\text{Amylase inhibitory activity (\%)} = \{1 - (OD_6-OD_5) / (OD_8-OD_7) \text{ X}100\}$$

where OD_5 is the optical density of solution containing plant extract, starch and amylase; OD_6 is the optical density of solution containing plant extract and starch; OD_7 is the optical density of solution containing starch and amylase; OD_8 is the optical density of solution containing starch.

The phytochemical analysis, *in-vitro* antioxidant and anti-obesity tests were performed in triplicate and the average values were expressed.

3. RESULTS AND DISCUSSION

3.1 Extraction Efficiency

Different solvents from non-polar (hexane) to polar (water) were used for extraction of phytochemicals from the *Bauhinia variegata* leaves and the yield is as shown in Table 1. The results of extraction experiment showed that methanol was the suitable solvent for phytochemical extraction as it recorded highest yield of 6.80 per cent and lowest yield of 0.73 per cent was observed in hexane extract. Since methanol is highly polar organic solvent compared to the other solvents used in the study, it can be inferred that the phytochemicals present in *Bauhinia variegata* leaves are more likely polar in nature. Earlier reports have shown that methanol is better solvent for extraction of compounds, since it is more efficient in cell wall degradation as compared to the other solvents[18].

3.2 Phytochemicals Analysis

3.2.1 Total Polyphenols and Flavonoids Content

Plant polyphenols and flavonoids constitute important group of compounds acting as antioxidants and offer numerous health benefits by preventing the damage to biological macromolecules[19]. Polyphenols and flavonoids posses many therapeutic applications such as antimutagenic, anticarcinogenic, prevention of low density lipoprotein and cardio protective effects[20]. In the present study highest polyphenol content was observed in methanol extract (243 µg GAE/mg) followed by water extract (214 µg GAE/mg) and chloroform extract (173 µg GAE/mg) as shown in Fig. 1. Flavonoids also contribute towards the antioxidant and anti obesity properties of the plants. Hence, flavonoid content of each solvent extract was estimated and the maximum flavonoid content was observed in chloroform extract (1.87 µg CE/g) followed by water extract (1.64 µg CE/g) and methanol extract (1.44 µg CE/g) (as shown in Fig. 2).

Table 1. Yield of *Bauhinia variegata* leaves compounds in different solvents

Name of extract	Yield (per cent)
Hexane extract	0.73 ± 0.15
Chloroform extract	1.20 ± 0.20
Ethyl acetate extract	0.40 ± 0.10
Acetone extract	0.90 ± 0.21
Methanol extract	6.80 ± 0.30
Water extract	5.40 ± 0.25

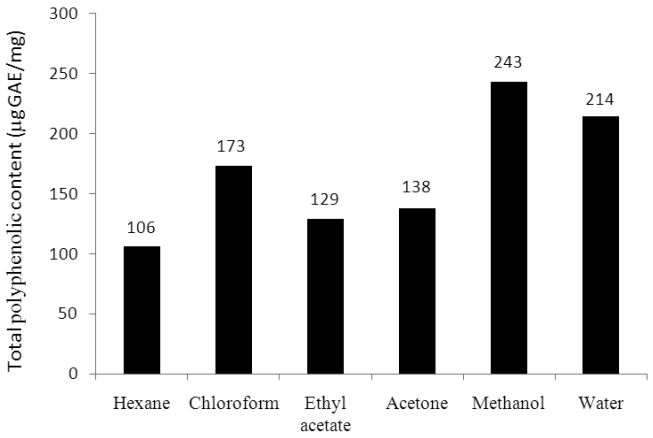

Figure 1. Total polyphenol in different extracts of *Bauhinia variegata* leaves.

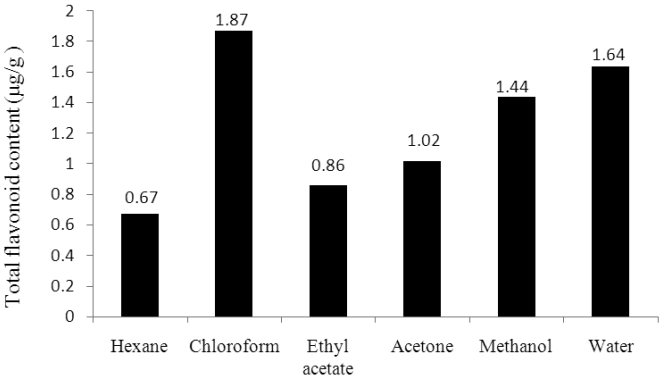

Figure 2. Total flavonoids content in different extracts of *Bauhinia variegata* leaves.

3.3 *In-vitro* Antioxidant Assays

Antioxidant property of different solvent extracts was estimated as their capacity to scavenge free radicals of DPPH. In DPPH assay the oxidation reaction is terminated by the antioxidants by changing the free radicals to their stable forms and this assay has been widely used to study the antioxidant property of natural products from plant and microbial sources[21]. DPPH is a purple colour solution that reacts with antioxidants present in the plant extract and get reduce to light yellow colour and reduction in absorbance at 515 nm. DPPH radical scavenging activity of the six extracts was carried out and IC_{50} values are as shown in the Table 2. Methanol extract (IC_{50} 12.75 µg/ml) showed maximum free radical scavenging activity while hexane extract (IC_{50} 7.85 µg/ml) is least potent. It can be inferred that highest free radical scavenging activity observed in methanol fraction since it contains highest amount of polyphenols and flavonoids when compared to other extracts.

3.4 *In-vitro* Antiobesity Assay

3.4.1 Effect of Bauhinia variegata Leaves Extract on Pancreatic α-amylase and Lipase Activity

Table 2. *In-vitro* antioxidant and anti-obesity properties of *Bauhinia variegata* leaves extracts

Name of extract	DPPH assay (IC_{50} µg/ml)	Lipase inhibition assay (IC_{50} mg/ml)	Amylase inhibition assay (IC_{50} mg/ml)
Hexane extract	12.75	47.61	2.08
Chloroform extract	13.85	41.66	4.16
Ethyl acetate extract	13.85	46.51	2.12
Acetone extract	11.23	62.50	4.38
Methanol extract	7.85	37.61	2.03
Water extract	11.11	51.28	2.27

Obesity is one of the major risk factor for increasing incidences of cardiovascular diseases, non-insulin dependent diabetes mellitus and non alcoholic fatty liver metabolic syndrome. Many plant extracts with anti-obesity property have been identified and are commonly used in Ayurveda since many years.

In the present study the inhibitory effect of *Bauhinia variegata* leaves on amylase and lipase was studied. The α-amylase from the pancreas and salivary glands is involved in the digestion of carbohydrates. α-amylase inhibitors from natural sources found to be useful in decreasing post-prandial hyperglycemia by reduction in carbohydrates digestion and glucose absorption, which in turn reduces glucose uptake into adipose tissue and reduces synthesis and accumulation of triacylglycerol[22].

Dietary lipid has to be hydrolysed into fatty acid and 2-monoacylglycerol by pancreatic lipase for its absorption by intestine. Hence, inhibition of these digestive enzymes α-amylase and pancreatic lipase could be beneficial in treatment of diet induced obesity.

The inhibitory activity of *Bauhinia variegata* leaves extracts against pancreatic and lipase was determined using different concentrations of the extracts. Different solvent extracts showed dose dependent inhibitory activity on α-amylase and pancreatic lipase.

Methanol extract (IC_{50}- 2.03 mg/ml) showed highest α-amylase inhibitory activity while acetone extract (IC_{50}- 4.38 mg/ml) is the least potent. Similarly the inhibition of lipase activity was maximum by methanol extract (IC_{50}-37.61 mg/ml) and lowest by acetone extract (IC_{50}- 62.50 mg/ml).

4. CONCLUSION

The phytochemicals from *Bauhinia variegata* leaves were extracted by using various solvents from non-polar to polar. The results of the assays conducted in this study proved that the methanolic extract contains phytochemical possessing in-*vitro* antioxidant and anti obesity property. However further studies are necessary to understand the antioxidant and anti obesity property of *Bauhinia variegata* at molecular level.

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