Antioxidants are compounds that act as inhibitors of the oxidation process and are found to inhibit oxidant chain reactions at small concentrations and thereby eliminate the threat of pathological processes.[1] Phenolic compounds present in medicinal plants have been reported to possess powerful antioxidant activity. [2] Flavanoids are a major class of phenolic compounds present in medicinal plants and are found to have a potential role in prevention of various diseases through their antioxidant activity.[4]

Bauhinia variegata Linn. (Ceasalpiniaceae) is a medium-sized deciduous tree found throughout India. It is traditionally used in bronchitis, leprosy, and tumors. The stem bark is used as astringent, tonic, and anthelmintic. [5,6] Infusion of the leaves is used as a laxative and for piles. Dried buds are used in the treatment of worm infestations, tumors, diarrhea, and piles.[7] The stem bark is used in ayurveda for its antidiabetic activity. [8]

Introduction

Oxidation is one of the destructive processes, wherein it breaks down and damages various molecules. Oxygen via its transformation produces reactive oxygen species (ROS) such as super oxide, hydroxyl radicals, and hydrogen peroxide. They provoke uncontrolled reactions.[1] Molecular oxygen is an essential component for all living organisms, but all aerobic species suffer from injury if exposed to concentration more than 21%.[3]

Free radicals attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA.[2,3] The body possesses several defense systems comprising enzymes and radical scavengers.[1] Some of them constitute the repair systems for biomolecules that are damaged by the attack of free radicals. [3]

ABSTRACT

Objectives: To evaluate the ethanolic and aqueous extracts of Bauhinia variegata Linn. for in vitro antioxidant and antihyperlipidemic activity.

Materials and Methods: Ethanolic and aqueous extracts of the stem bark and root of B. variegata Linn. were prepared and assessed for in vitro antioxidant activity by various methods namely total reducing power, scavenging of various free radicals such as 1,2-diphenyl-2-picrylhydrazyl (DPPH), super oxide, nitric oxide, and hydrogen peroxide. The percentage scavenging of various free radicals were compared with standard antioxidants such as ascorbic acid and butylated hydroxyl anisole (BHA). The extracts were also evaluated for antihyperlipidemic activity in Triton WR-1339 (iso-octyl polyoxyethylene phenol)-induced hyperlipidemic albino rats by estimating serum triglyceride, very low density lipids (VLDL), cholesterol, low-density lipids (LDL), and high-density lipid (HDL) levels.

Result: Significant antioxidant activity was observed in all the methods, (P < 0.01) for reducing power and (P < 0.001) for scavenging DPPH, super oxide, nitric oxide, and hydrogen peroxide radicals. The extracts showed significant reduction (P < 0.01) in cholesterol at 6 and 24 h and (P < 0.05) at 48 h. There was significant reduction (P < 0.01) in triglyceride level at 6, 24, and 48 h. The VLDL level was also significantly (P < 0.05) reduced from 24 h and maximum reduction (P < 0.01) was seen at 48 h. There was significant increase (P < 0.01) in HDL at 6, 24, and 48 h.

Conclusion: From the results, it is evident that alcoholic and aqueous extracts of B. variegata Linn. can effectively decrease plasma cholesterol, triglyceride, LDL, and VLDL and increase plasma HDL levels. In addition, the alcoholic and aqueous extracts have shown significant antioxidant activity. By the virtue of its antioxidant activity, B. variegata Linn. may show antihyperlipidemic activity.

KEY WORDS: Antihyperlipidemic, antioxidant, Bauhinia variegata Linn, triton
So far, the stem bark has been investigated and reported to have antitumor,[9,10] antibacterial, antifungal, antiulcer, and hepatoprotective activity.[11] Flavonone glycoside from root is reported to have anti-inflammatory activity.[12] The stem bark is reported to contain 5,7 dihydroxy and 5,7 dimethoxy flavanone-4-O-α-L rhamnopyranyl-β-D-glycopyranosides, Kaempferol-3-glucoside, lupeol, and betasitosterol. Seeds contain protein, fatty oil-containing oleic acid, linoleic acid, palmitic acid, and stearic acid. Flowers contain cyanidin, malvidin,peonidin, and kaempferol. Root contains flavanol glycosides.[13–17]

Since polyphenolic compounds are present in the ethanolic and aqueous extracts of stem bark and root of *B. variegata* Linn., it was thought that it would be worthwhile to evaluate the plant for antioxidant activity. Lipids are one of the most susceptible targets of free radicals.[18] This oxidative destruction is known as lipid peroxidation and may induce many pathological events. Apart from antioxidant studies, the present study therefore also involves evaluation of antihyperlipidemic activity.

### Materials and Methods

**Plant material and extraction**

The stem bark and root of *B. variegata* Linn. were procured and authenticated from Regional Research Institute, Bangalore. The authenticated stem bark and root were dried in shade and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvents. Coarse powders of the root (1 kg) and stem bark (1.1 kg) were separately Soxhlet extracted with 90% ethanol. The aqueous extract was prepared using the same marc by the process of maceration. The extracts obtained were concentrated under reduced pressure to yield 5 kg of ethanolic and 1 kg of aqueous extracts of stem bark and root of *B. variegata* Linn.

**Preparation of test solution**

The various extracts such as *B. variegata* stem alcoholic (BVSA), stem water (BVSW), root alcohol (BVR), and root water (BVRW) extracts at various concentrations were prepared in water and used for *in vitro* antioxidant studies. Pilot studies were carried out for the ethanolic and aqueous extracts of stem bark and root of *B. variegata* for *in vitro* antioxidant studies and the concentrations at which the extracts gave good antioxidant activity was selected.

**Animals**

Albino male Wistar rats weighing between 150 and 200 g were procured from registered breeders. The animals were housed under standard conditions of temperature (25 ± 2°C) and relative humidity (30–70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. Approval of the Institutional Animal Ethics Committee (IAEC) of K.L.E. Society’s College of Pharmacy, Bangalore, was obtained.

**Acute toxicity studies**

Acute toxicity studies for aqueous and ethanolic extracts of *B. variegata* Linn. were conducted as per OECD guidelines 423[18] using albino Wistar rats. Each animal was administered the aqueous solution of the extract by oral route. The animals were observed for any changes continuously for the first 2 h and upto 24 h for mortality.

Antioxidant studies

The ability of the extracts to scavenge hydrogen peroxide,[19] DPPH (1,2-diphenyl-2-picrylhydrazyl) radical,[19] nitric oxide,[20,21] superoxide radical,[14] and its reducing power[19] was determined at different concentrations.

Butylated hydroxy anisole (BHA) and ascorbic acid were used as standards for the various *in vitro* antioxidant studies. The percentage scavenging of various radicals were calculated using the following formula:

\[
\% \text{ Radical scavenged} = \frac{A_0 - A_1}{A_0} \times 100
\]

where \(A_0\) is absorbance of the free radical alone and \(A_1\) is absorbance of free radical in the presence of extract/standard. All the experiments were performed in triplicate.

**Antihyperlipidemic activity**

The method of Tamasi et al.[22] was used for evaluation of antihyperlipidemic activity. Albino Wistar rats weighing between 190 and 250 g were assigned to various groups of six animals each. Animals were fasted for 16 h prior to the experiment with water *ad libitum*. The various extracts, *B. variegata* stem-water extract (BVSW), ethanolic extract (BVSA), *B. variegata* root-water extract (BVRW), and ethanolic extract (BVRA) each at doses of 200 and 400 mg/kg body weight, simvastatin at 4 mg/kg and fenofibrate at 20 mg/kg, were administered p.o. to groups II to XI, respectively. Group I served as control. On the day of the experiment, the animals of the groups II–XI received the respective drugs by oral route. Simultaneously, all the animals received Triton WR-1339 at 100 mg/kg body weight by intraperitoneal route. The control animals were given only Triton WR-1339 at 100 mg/kg body weight. Serum cholesterol, triglyceride, and HDL were estimated at 6, 24, and 48 h using AGAPP diagnostic kits. Blood samples were withdrawn by retroorbital puncture. Total cholesterol was estimated using CHOD-PAP methodology, Triglycerides by GPO-PAP methodology, and HDL by the precipitation method using phosphotungstate magnesium acetate reagent.

\[
\text{VLDL} = \frac{\text{Triglycerides}}{5}
\]

\[
\text{LDL cholesterol was calculated as}
\]

\[
\text{LDL} = \frac{\text{Total Cholesterol} - \text{HDL} - \frac{\text{Triglycerides}}{5}}{5}
\]

**Chemicals**

The chemicals DPPH (1,2-diphenyl-2-picrylhydrazyl), N-(1-Naphthyl) ethylenediamine dihydrochloride, Triton WR-1339, NADH, SNP, phenazine methosulphate, trichloro acetic acid, and potassium ferricyanide were purchased from Sigma Chemicals, St Louis, MO, USA. All other chemicals and reagents used were of analytical grade. UV-1700 Shimadzu UV-Vis spectrophotometer was used for *in vitro* anti-oxidant studies.

**Statistical analysis**

All the values are presented as mean ± SD. Data were statistically analyzed by one-way ANOVA followed by post hoc test; \(P\) values < 0.05 were considered as statistically significant. Linear regression analysis was used for calculation of IC\(_{50}\).
Results

Acute toxicity studies

There was no mortality and noticeable behavioral changes in all the groups tested. The aqueous and ethanolic extracts of stem and root of B. variegata Linn. were found to be safe up to 2000 mg/kg body weight.

Hydrogen peroxide scavenging activity

At 10 μg/ml concentration, BVSW, BVSA, and BVRA produced H₂O₂ scavenging activity comparable (P < 0.05) to that of the standards BHA and ascorbic acid, BHA, BWRV, BVRA, BVSW, and BVSA were found to have IC₅₀ (mean ± SD) of 9.917 ± 0.01, 10.95 ± 0.03, 11.74 ± 0.21, 10.78 ± 0.17, 10.23 ± 0.11, 9.85 ± 0.03, respectively [Figure 1 and Table 1].

DPPH radical scavenging activity

The various extracts produced significant DPPH radical scavenging activity from 10 μg/ml. The IC₅₀ (mean ± SD) of ascorbic acid, BHA, BVRW, BVRA, BVSW, and BVSA were found to be 30.55 ± 0.52, 29.11 ± 0.03, 37.73 ± 0.37, 36.10 ± 0.50, 45.85 ± 0.49, 30.50 ± 0.16, respectively [Table 1].

Nitric oxide radical scavenging activity

Scavenging of nitric oxide by various extracts was found to be concentration dependent. Maximum inhibition of nitric oxide formation was produced by BVSA at concentration of 500 μg/ml and had IC₅₀ of 362.90 ± 3.80 as against 368.00 ± 2.60 for BHA [Tables 1 and 2].

Figure 1: Comparison of hydrogen peroxide scavenging activity of 10 μg/ml of AA, BHA, BVRW, BVRA, BVSW and BVSA; (n = 3) AA: Ascorbic acid; BHA: Butylated hydroxy anisole; BVRW, BVRA, BVSW, BVSA: Bauhinia variegata root water, ethanolic and Bauhinia variegata stem water and ethanolic extracts, respectively

Table 1
Free radical scavenging activity of different extracts of Bauhinia variegata Linn

| Test/standard group | IC₅₀ values ± SD (μg/ml) for free radical scavenging activity |
|---------------------|-------------------------------------------------------------|
|                     | Hydrogen peroxide  | DPPH          | Nitric oxide | Super oxide |
| Ascorbic acid       | 9.91 ± 1.03       | 30.55 ± 1.12  | —            | —           |
| BHA                 | 10.95 ± 2.11      | 29.11 ± 1.03  | —            | —           |
| BVRW                | 11.74 ± 2.98      | 37.73 ± 1.37  | 478.80 ± 3.40| 435.40 ± 7.78|
| BVRA                | 10.78 ± 1.70      | 36.01 ± 1.25  | 415.20 ± 2.88| 445.30 ± 4.05|
| BVSW                | 10.23 ± 1.11      | 45.85 ± 2.49  | 405.80 ± 5.43| 481.30 ± 5.00|
| BVSA                | 9.85 ± 0.93       | 30.50 ± 1.61  | 362.90 ± 3.80| 414.60 ± 6.22|

BVRW, BVRA, BVSW, BVSA: Bauhinia variegata root water, ethanolic and Bauhinia variegata stem water and ethanolic extracts, respectively; AA: Ascorbic acid, BHA: Butylated hydroxy anisole

Total reducing power

From 50 μg/ml, all the extracts and standard-ascorbic acid and BHA showed reductive capabilities. At 500 μg/ml, the reducing power of standards and extracts showed the following order: BHA > BVRA > BVSA > Ascorbic acid > BVSW > BVRW. Reducing power of BVSA and BVRW at 500 μg/ml was comparable (P < 0.05) to that of ascorbic acid [Table 2].

Superoxide anion radical scavenging activity

Superoxide anion radical generation was inhibited by BHA (standard) and extracts from 100 μg/ml. The various extracts produced significant superoxide radical scavenging activity in a concentration-dependent manner. BVSA showed the lowest IC₅₀ value (414 ± 6.22) followed by BHA (435.40 ± 7.78).

Antihyperlipidemic activity

Administration of Triton resulted in increase in serum levels of cholesterol, triglycerides, VLDL, and LDL. A significant reversal in serum levels of cholesterol, triglycerides, VLDL, and LDL levels was noticed in the animals treated with B. variegata Linn. root and stem extracts when compared with the control group [Tables 2 and 3].

Simvastatin (standard) produced maximum cholesterol- and LDL-lowering effect at 6 h and 24 h. BVRW 400, BVRA 200 and 400 and BVSA 200 and 400 produced a significant decrease in serum cholesterol and LDL levels, which was found to be significantly greater than the effects of fenofibrate (at 6 h, 24 h and 48 h) and simvastatin (at 48 h) [Table 2].

Maximum reduction of triglyceride and VLDL levels was produced by fenofibrate at 6 h. At 24 h and 48 h, aqueous and ethanolic extracts of B. variegata root and stem produced significant triglyceride- and VLDL-lowering effect which was comparable to that of fenofibrate and significantly greater than that of Simvastatin [Table 4].

Simvastatin, fenofibrate, and various extracts except BVRW and BVRA 200 mg/kg produced significant (P < 0.01) increase in serum HDL level at 6, 24, and 48 h when compared to control. At 6 h and 24 h all the extracts except BVRA 200 and BVRW 400 produced a significant (P < 0.01) increase in HDL level, which was significantly greater than that of simvastatin and fenofibrate. At 48 h BVSW 200 and 400, BVRA 200 and BVRW 200 produced significant (P < 0.01) increase in HDL level, which was significantly greater than that of simvastatin and fenofibrate [Table 5].
The antioxidant activity than root ethanolic and aqueous extracts. Stem ethanolic extract produced significantly greater values than root ethanolic and aqueous extracts of *B. variegata* root and stem produced significant antioxidant activity. This can be attributed to the flavonoids and other phytoconstituents present in the plant material.

**Discussion**

The potentially reactive derivatives of oxygen ascribed as ROS such as superoxide radical, hydroxyl radical, and hydrogen peroxide are continuously generated inside the human body as a consequence of exposure to exogenous chemicals and/or a number of endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer. Owing to the ROS overproduction and/or inadequate antioxidant defense, there is upsurge of ROS and this culminates in oxidative stress. It is quite interesting to note that plants have good antioxidant ability and are safer than the synthetic antioxidants. The antioxidant activity can be attributed to various mechanisms like prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, reductive capacity, and radical scavenging activity.

In the present study, five different antioxidant methods for evaluation of antioxidant activity have been used. Ethanolic and aqueous extracts of *B. variegata* root and stem produced significant antioxidant activity. This can be attributed to the flavonoids and other phytoconstituents present in the plant material. Ethanolic extract produced significantly greater antioxidant activity than root ethanolic and aqueous extracts.

**Table 2**

Total reducing power, nitric oxide, and superoxide radical scavenging activity of different concentrations of AA, BHA, BVRW, BVRA, BVSW, and BVSA

| Group (mcg/ml) | Reducing power (Absorbance) | Nitric oxide scavenged (%) | Superoxide radical scavenged (%) |
|---------------|-----------------------------|----------------------------|---------------------------------|
| AA            |                             |                            |                                 |
| 100           | 0.09 ± 0.01                 | -                          | -                               |
| 200           | 0.19 ± 0.02                 | -                          | -                               |
| 300           | 0.61 ± 0.03                 | -                          | -                               |
| 400           | 0.77 ± 0.01                 | -                          | -                               |
| 500           | 1.47 ± 0.05                 | -                          | -                               |
| BHA           |                             |                            |                                 |
| 100           | 0.13 ± 0.01^a               | 13.95 ± 0.41               | 20.51 ± 0.66                    |
| 200           | 0.24 ± 0.01^a               | 20.86 ± 0.43               | 29.20 ± 0.45                    |
| 300           | 0.80 ± 0.05^a               | 34.20 ± 0.18               | 38.36 ± 0.39                    |
| 400           | 1.08 ± 0.02^a               | 40.11 ± 0.15               | 56.11 ± 0.12                    |
| 500           | 1.75 ± 0.02^a               | 60.86 ± 0.41               | 65.78 ± 0.51                    |
| BVRW          |                             |                            |                                 |
| 100           | 0.01 ± 0.00                 | 11.84 ± 0.21               | 20.40 ± 0.10^b                  |
| 200           | 0.05 ± 0.00                 | 18.78 ± 0.13               | 29.50 ± 0.16^b                  |
| 300           | 0.10 ± 0.01                 | 28.68 ± 0.26               | 35.98 ± 0.17                    |
| 400           | 0.24 ± 0.03                 | 35.01 ± 0.16               | 39.46 ± 0.19                    |
| 500           | 0.74 ± 0.03                 | 52.61 ± 0.41               | 50.67 ± 0.45                    |
| BVRA          |                             |                            |                                 |
| 100           | 0.04 ± 0.01                 | 12.44 ± 0.61               | 33.44 ± 0.62^b                  |
| 200           | 0.09 ± 0.01                 | 21.65 ± 0.14^b             | 36.48 ± 0.50^b                  |
| 300           | 0.40 ± 0.02                 | 32.18 ± 0.13               | 39.00 ± 0.24^b                  |
| 400           | 0.80 ± 0.01^a               | 39.86 ± 0.11^b             | 45.84 ± 0.20^b                  |
| 500           | 1.65 ± 0.03^a               | 59.12 ± 0.12^b             | 55.90 ± 0.25                    |
| BVSW          |                             |                            |                                 |
| 100           | 0.01 ± 0.00                 | 14.86 ± 0.44^b             | 32.64 ± 0.67^b                  |
| 200           | 0.04 ± 0.01                 | 23.84 ± 0.54^b             | 34.21 ± 0.72^b                  |
| 300           | 0.13 ± 0.02                 | 30.14 ± 0.11               | 41.97 ± 0.12^b                  |
| 400           | 0.21 ± 0.01                 | 35.64 ± 0.61               | 45.95 ± 0.67^b                  |
| 500           | 1.05 ± 0.03                 | 54.80 ± 0.31               | 56.80 ± 0.94                    |
| BVSA          |                             |                            |                                 |
| 100           | 0.05 ± 0.00                 | 10.86 ± 0.84               | 30.40 ± 0.66^b                  |
| 200           | 0.09 ± 0.00                 | 20.68 ± 0.54^b             | 35.62 ± 0.86^b                  |
| 300           | 0.47 ± 0.02                 | 31.68 ± 0.31               | 50.10 ± 0.16^b                  |
| 400           | 0.82 ± 0.01                 | 40.58 ± 0.11^b             | 54.03 ± 0.24^b                  |
| 500           | 1.60 ± 0.08^a               | 61.05 ± 0.38^b             | 56.90 ± 0.09                    |

Values are mean ± SD, (n = 3); ^a P < 0.05, ^b P < 0.01, ^c P < 0.001 as compared to *AA, ^a BHA. AA: Ascorbic acid; BHA: Butylated hydroxy anisole; BVRW, BVRA, BVSW, BVSA: *Bauhinia variegata* root water, ethanolic and *Bauhinia variegata* stem water and ethanolic extracts, respectively.
uptake of lipoprotein from the circulation by extra hepatic tissues resulting in an increase in the level of circulating lipoproteins. Triton WR-1339-induced hyperlipidemic rats treated with BVSW, BVSA, BVRW, and BVRA produced reversal of increase in serum cholesterol and triglycerides and LDL from the 6 h up to 48 h and VLDL from 24 h.

Ethanolic and aqueous extracts of B. variegata root and stem produced significant cholesterol and LDL lowering effect at 6, 24, and 48 h. This indicates that B. variegata not only reduces the synthesis of cholesterol, but may also reduces its metabolism. The extracts were found to be enriched in flavonoids and it is reported that flavonoids are found to inhibit HMG-CoA reductase activity. It may be concluded that the cholesterol-lowering effect of B. variegata stem and root extracts may be due to inhibition of HMG-CoA reductase activity. Simvastatin being a specific HMG-CoA inhibitor produces its hypocholesterolemic activity by reducing cholesterol synthesis. Increase in triglyceride level was evident in control animals due to inhibition of lipoprotein lipase (LPL) by Triton. Treatment with ethanolic and aqueous extracts of B. variegata resulted in reduction of triglyceride levels. It is likely that treatment with B. variegata might have lowered the serum triglyceride level by activating LPL. LPL is a prime enzyme related to triglyceride metabolism. Further VLDL levels were reduced significantly at 24 and 48 h.

The various extracts also showed protective action by increasing serum HDL level. The increased HDL facilitates the transport of triglyceride or cholesterol from serum to liver where it is catabolized and excreted out of the body. Significantly
greater increase in HDL levels was produced by aqueous extracts than ethanolic extracts.

**Conclusion**

The aqueous and ethanolic extracts of *B. variegata* Linn. have shown significant antioxidant activity. In the preliminary studies, it was found out that the aqueous and ethanolic extracts of *B. variegata* Linn. have shown promising antihyperlipidemic activity. *B. variegata* may partly owe its antihyperlipidemic activity to its antioxidant activity.

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