REPORT

In-vitro antioxidant and anti-hyperlipidemic activities of *Blumea eriantha* DC

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Abstract: Alcoholic and water extracts of the stem and root of *Blumea eriantha* DC were prepared and evaluated for *in-vitro* antioxidant activity by methods like total reducing power, scavenging of us free radicals like as 1,2-diphenyl-2-picrylhydrazyl (DPPH), super oxide, nitric oxide, and hydrogen peroxide. The percentage scavenging effect of free radicals was compared with standard antioxidants like ascorbic acid and Butylated- hydroxyl anisole (BHA). Different extracts were also tested for anti-hyperlipidemic activity in triton WR-1339 (iso-octyl polyoxyethylene phenol)-induced hyperlipidemia in albino rats by determination of serum triglyceride like VLDL, LDL, HDL levels. Significant antioxidant activity was estimated in different methods, ($P<0.01$) for reducing power and ($P<0.001$) for scavenging DPPH, super oxide, nitric oxide, and hydrogen peroxide radicals. The different extracts having 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. There was significant increase ($P<0.01$) in HDL at 6, 24 and 48 h. From the VLDL was also significantly ($P<0.05$) reduced from 24 h and maximum reduction ($P<0.01$) results, it is clear that alcoholic and water extracts of *Blumea eriantha* DC can remarkably decrease plasma cholesterol, triglyceride, LDL, and VLDL and increase plasma HDL levels. In addition, the alcoholic and aqueous extracts have shown significant antioxidant activity.

Keywords: Anti-hyperlipidemic, antioxidant, *Blumea eriantha* DC, triton.

INTRODUCTION

Oxygen is a mandatory component for each and every living organisms, but all aerobic species can affect from injury if exposed to concentration more than 21%. All free radicals attack and induce oxidative damage to various biochemical including proteins, lipids, lipoproteins, and DNA (Shetgiri, 2003). The body having several protective systems including enzymes and radical scavengers (Nasik, 2003). Antioxidants are compounds that act by inhibition of the oxidation process and are found to inhibit oxidantion chain reactions at low concentrations and thereby inhibits the threat of pathological processes. Phenolic content present in plants have been reported to having powerful antioxidant activity. Flavanoids are main class of phenolic compounds present in plants and are found to have a potential role in prevention of different ailments through their antioxidant activity (Gurpreet, et al, 2006). The *Blumea eriantha* DC is known as 'Nimurdi' (Marathi), Kukronda in Hindi. The essential oil extracted from leaves and stem showing potent antibacterial activity, anti-fungal, insecticidal.

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MATERIAL AND METHODS

Extraction of Plant material

The shade dried stem and root powder of *Blumea eriantha* DC. were stored and authenticated. Extraction was done as per standard procedures by taking analytical grade solvents. Coarse powders of the root (1kg) and stem (1.1kg) were separately extracted with 90% alcohol in Sox let apparatus. The water extract was prepared by using the same marc by the process of cold maceration. The extracts obtained were concentrated by using Rotary Vacuum Evaporator .The yield of the alcoholic extract of stem and root was found (7.3 and 7.2%, respectively) and the water extract of stem and root was (3.4% each).

Acute toxicity studies

Water and alcoholic extracts of *Blumea eriantha* DC were conducted as per OECD guidelines 423 using albino Wistar rats. Each animal was administered the aqueous solution of the extract by oral route. The animals observed for any changes in behavior for the first 2h and upto 24h for causality.

Study of Antioxidant studies

Scavenging hydrogen peroxide (Ilhami, *et al.*, 2005) DPPH radical, nitric oxide (Yaowared, *et al.*, 2005) super-

Table 1: IC values± SD (µg/ml) for free radical scavenging activity

Test/standard group	Hydrogen peroxide	DPPH	Nitric oxide	Superoxide
Ascorbic acid	9.91±1.03	30.35±1.12	-	-
BHA	10.96±2.11	29.11±1.03	368.00±2.60	435.40±2.60
BERW	11.74±2.98	37.73±1.73	478.80±3.40	502.10±8.00
BERE	10.78±1.70	36.01±1.25	415.12±2.88	445.30±4.04
BESW	10.23±1.11	48.25±2.49	405.80±5.43	481.30±5.00
BESE	9.85±0.93	30.5±1.61	362.90±3.80	414.60±6.12

BHA= Butylated Hydroxy Anisole, BERW=Blumea eriantha root aqueous extract. , BERE= Blumea eriantha root ethanolic extract, ESW= Blumea eriantha stem aqueous extract, ESE= Blumea eriantha stem ethanolic extract.

Table 2: Result for antioxidant activity.

Group (µg/ml)	Reducing power (Absorbance)	Nitric acid scavenged (%)	Superoxide radical scavenged (%)
ASCORBIC ACID			
100	0.09±0.01	-	-
200	0.19±0.03	-	-
300	0.61±0.03	-	-
400	0.77±0.01	-	-
500	1.47±0.05	-	-
BHA			
100	0.13±0.01*t2	13.95±0.41	20.51±0.60
200	0.24±0.01*t2	20.86±0.43	29.20±0.45
300	0.80±0.05*t2	34.20±0.18	38.36±0.39
400	1.08±0.02*t2	40.11±0.15	56.11±0.12
500	1.75±0.02*t2	60.86±0.41	65.78±0.51
BERW			
100	0.01±0,00	12.44±0.21	20.40±0.62*t1
200	0.04±0.01	18.78±0.13	29.50±0.16*t1
300	0.10±0.02	26.68±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	50.67V0.45
BERE			
100	0.04±0.01	12.44±0.61	33.44±0.62*t3
200	0.09±0.01	21.65±0.14*t3	36.48±0.50*t2
300	0.40±0.02	32.18±0.13	39.00±0.24*t2
400	0.80±0.01*t3	39.86±0.11*t3	45.84±0.20*t3
500	1.65±0.03*t3	59.12±0.12*t3	55.9. ±0.25
BESW			
100	0.01±0.00	14.86±0.44*t3	32.64±0.67*t2
200	0.04±0.01	23.84±0.54*t3	35.21±0.72
300	0.40±0.02	30.14±0.11	40.97±0.12*t1
400	0.80±0.01	35.64±0.61	46.95±0.65*t1
500	1.05±0.01	54.84±0.31	55.80±0.44
BESE			
100	0.05±0.00	10.86±0.44*t3	31.44±0.66*t3
200	0.09±0.00	20.68±0.54*t2	35.00±0.84*t3
300	0.47±0.02	31.68±0.31	50.12±0.14
400	0.82±0.01	40.58±0.11*t3	54.30±0.22*t3
500	1.60±0.08*t3	61.05±0.38*t3	57.90±0.09

Values are mean ±SD. (n=6) *t1<0.05, *t2<0.01, *t3<0.001 as compared to BHA= Butylated Hydroxy Anisole, BERW=Blumea eriantha root aqueous extract. , BERE= Blumea eriantha root ethanolic extract. , BESW=Blumea eriantha stem aqueous extract, BESE= Blumea eriantha stem ethanolic extract respectively.

Table 3: Result for antihyperlipidemic activity.

Group	Serum cholesterol (mg/dl) after 6h	Serum LDL (mg/dl) after 6h	Serum cholesterol (mg/dl) after 24h	Serum LDL (mg/dl) after 24h	Serum cholesterol (mg/dl) after 48h	Serum LDL (mg/dl) after 48h
Control	99.70±1.86**	88.27±0.73	81.54±0.99	59.06±4.17	60.44±2.71**	45.53±0.71**
BESW 200	88.32±1.35	58.88±0.79***	84.51±1.38	65.04±0.40***	59.39±3.26	29.75±0.76
BESW 400	76.26±0.97**	43.71±0.79	57.57±1.17**	23.56±0.76**	55.25±3.09**	23.65±0.72***
BERW200	93.54±0.90*	73.35±0.86**	81.62V1.17	57.54±0.74	68.34±3.24	49.23±0.69*
BERW 400	65.58±0.56**	33.55±0.85	60.33±0.93	25.42±1.42**	57.98±1.41**	29.32±0.70
BESE 200	66.53±0.60	38.74±0.95**	67.44±0.70***	36.74±0.80***	55.38±1.30***	31.33±0.72**
BESE 400	63.48±0.70**	39.13v0.89	59.52±0.67	26.60±0.84	53.44±2.34	28.22±0.67***
BERE 200	61.35±0.80	39.49±0.70***	59.69±0.58*	33.47±1.31**	54.34±2.34**	30.64±0.72***
BERE 400	59.61±0.74***	32.19±0.89**	60.27±1.43***	26.41±0.97*	51.52±1.41**	27.61±0.80*
simvastatin	52.38±0.93**	22.54±0.89	51.20±1.01**	22.75±0.96**	62.29±1.47***	47.21±0.80**
fenofibrate	61.16±0.67	29.73±0.90***	62.28±1.43	33.32±0.94**	62.38±1.37*	44.73±0.47**

Values are expressed as mean ± SD. (n==6). Cholesterol and LDL concentration are estimated by the standard method and the values are expressed as *P1<0.05, **P2<0.01, ***P3<0.001 when compared with standard groups, a-Simvastatin, b-fenofibrate, BERW=Blumea eriantha root aqueous extract. , BERE= Blumea eriantha root ethanolic extract. , BESW= Blumea eriantha stem aqueous extract, BESE= Blumea eriantha stem ethanolic extract respectively at 200 and 400 mg/kg body weight.

Table 4: Result for antihyperlipidemic activity.

Group	Serum triglyceride (mg/dl) after 6h	Serum VLDL (mg/dl) after 6h	Serum triglyceride (mg/dl) after 24h	Serum VLDL (mg/dl) after 24h	Serum triglyceride (mg/dl) after 48h	Serum LDL (mg/dl) after 48h
Control	67.70±0.71	13.72±0.95	61.17±0.84*	12.10±0.89	79.40±1.34	15.81±1.01
BESW 200	63.72±1.36	12.66±0.88**	60.22±0.83**	20.17±1.01	54.47±0.66**	10.84±0.80***
BESW 400	60.77±1.39**	12.10±0.94	57.65±0.70	11.50±0.88	54.56±0.74*	11.82±0.86***
BERW200	68.32±0.52	13.66±0.73**	54.17±0.66	10.71±1.00	59.68±1.73***	10.18±0.81***
BERW 400	63.43v0.79*	12.60±0.87	54.55±0.82**	10.81±0.82**	50.29±0.69	9.75±0.88***
BESE 200	62.50±0.87*	12.46±0.86*	51.61±0.60	10.43±0.76	49.45±0.66**	8.84±0.88***
BESE 400	60.18±1.01	11.90±0.78	52.31±0.67	10.42±0.76**	43.67v0.73	12.13±0.81**
BERE 200	67.48±1.52**	13.48±0.85	58.46±4.16	12.20±0.89***	48.26±0.71*	9.60±0.77**
BERE 400	60.55±0.70*	12.23±0.83*	50.89±1.06**	10.56±0.75**	48.45±0.68***	10.82±0.71**
Simvastatin	63.73±1.71*	12.66±0.86	63.24±0.91	12.50±0.86	65.34±0.75	13.19±0.95*
fenofibrate	55.20±0.98**	11.34±0.32**	54.1±0.71***	10.84±0.64	58.58±1.27**	11.91±0.75

Values are expressed as mean ± SD. (n==6) triglyceride and VLDL concentration are estimated by the standard method and the values are Expressed as *P1<0.05, **P2<0.01, ***P3<0.001 when compared with standard groups, a-simvastatin, b-fenofibrate, BERW=Blumea eriantha root aqueous extract., BERE= Blumea eriantha root ethanolic extract., BESW= Blumea eriantha stem aqueous extract, BESE= Blumea eriantha stem ethanolic extract respectively at 200 and 400 mg/kg body weight.

oxide radical, and its reducing power was find out at various concentrations.

Butylated hydroxy anisole and ascorbic acid were taken as standards in *in-vitro* antioxidant studies. The percentage scavenging calculated by the following formula: % Radical scavenged= Ao-A1/Ao
where A_0 is absorbance of the free radical only and A_1 = absorbance of free radical in the presence of extract/standard. Experiments were performed in triplicate.

Study of Antihyperlipidemic activity

As per Tamasi *et al* was used for evaluation of anti-hyperlipidemic activity. Animals fasted for 16 h prior to the experiment with water *ad libitum*. *Blumea eriantha* DC stem-water extract (BESW), ethanolic extract (BESA), *Blumea eriantha* DC root-water extract (BERW), and ethanolic extract (BERA) each at doses of 200 and 400mg/kg body weight, fenofibrate at 20 mg/kg and Simvastatin at 4 mg/kg were administered per oral. Group 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 100mg/kg body weight by intraperitoneal route. The control animals were given only triton WR-1339 at 100mg/kg body weight. Serum cholesterol, triglyceride, and HDL were estimated at 6, 24 and 48h using AGAPPE diagnostic kits. Blood samples were withdrawn by retroorbital puncture. Total cholesterol was estimated by CHOD-PAP methodology, triglycerides by GPO-PAP methodology, and HDL by the precipitation method using phosphotungstate magnesium acetate reagent.

Chemical required

The chemicals DPPH, Triton WR-1339, NADH, SNP, phenazine methosulphate, trichloro acetic acid and

Table 5: Result for change of serum HDL

Group	Serum HDL(mg/dl) after 6 hrs	Serum HDL(mg/dl) after 24 hrs	Serum HDL(mg/dl) after 48 hrs
Control	34.20±1.39	32.72±1.00	31.6 ±0.88
BESW 200	42.12±0.87***	40.73±0.94***	40.76 ±0.89***
BESW 400	44.53±1.58	45.54±0.94***	42.61 ±0.88***
BERW200	34.41±1.84***	34.48±0.78	31.28 ±1.01
BERW 400	44.79±0.73**	45.41±0.92***	39.01 ±0.34***
BESE 200	40.20±0.75	42.2±0.83	35.35 ±0.56**
BESE 400	40.40±1.20***	44.56±0.97**	34.42 ±0.14**
BERE 200	35.38±1.50	37.54±0.69	36.30 ±045*
BERE 400	39.41±0.57**	44.08±0.85***	38.35 ±045**
simvastatin	42.65±0.76*	40.89±0.87*	38.35 ±0.98*
fenofibrate	42.09±0.74	39.73±0.81**	39.45 ±044**

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.

RESULTS

Acute toxicity studies

There was no mortality and noticeable behavioral changes in all the groups tested.

The Hydrogen peroxide scavenging activity

*(*At 10µg/ml concentration,) BESW, BESA, and BERA produced H_2O_2 scavenging activity comparable ($P<0.05$) to that of the standards BHA and ascorbic acid, BHA, BERW, BERA, BESW, and BESA were found to have IC_{50} (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 (table 1).

The DPPH radical scavenging activity

*(*At 10µg/ml concentration,) The IC_{50} (mean ±SD) of ascorbic acid, BHA, BERW, BERA, BESW, and BESA 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

The Nitric oxide radical scavenging activity

Maximum inhibition of nitric oxide formation was produced by BESA at concentration of 500µg/ml and had IC_{50} of 362.90±3.80 as against 368.00±2.60 for BHA.

Total reducing power

*(*50 µg /ml) At 50 µg/ ml, the reducing power of standards and extracts showed the following order: BHA >BERA >BESA >Ascorbic acid >BESW >BERW. Reducing power of BESA and BERA at 500µg/ml was comparable ($P<0.05$) to that of ascorbic acid

Superoxide anion radical scavenging activity

(from 100µg/ml.) The extracts produced significant Superoxide radical scavenging activity. BESA showed the lowest IC_{50} value (414±6.22) followed by BHA (435.40± 7.78).

Antihyperlipidemic activity

A significant reversal in serum levels of cholesterol, triglycerides, VLDL and LDL levels was remarked in the animals treated with *Blumea eriantha* DC root and stem extracts when compared with the control group

DISCUSSION

Alcoholic and water extracts of *Blumea eriantha* DC root and stem produced significant antioxidant activity. This may be due to the flavanoids and other phytoconstituents present in the extracts. Stem ethanolic extract giving significantly greater antioxidant activity than root ethanolic and aqueous extracts. Triton WR-1339-induced hyperlipidemic rats treated with BESW, BESA, BERW, and BERA produced increase in serum cholesterol and triglycerides and LDL from the 6h upto 48h and VLDL from 24h. Ethanolic and aqueous extracts of *Blumea eriantha* DC root and stem produced significant cholesterol and LDL lowering activity at 6, 24 and 48h. This indicates that *Blumea eriantha* DC not only reduces the synthesis of cholesterol, but may also inhibit cholesterol metabolism. It may be concluded that the cholesterol-lowering effect of *Blumea eriantha* DC 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. Treatment with ethanolic and aqueous extracts of *Blumea eriantha* DC resulted in reduction of triglyceride levels. It is likely that treatment with *Blumea eriantha* DC root may lowered the serum triglyceride level by activating LPL. LPL is a prime enzyme related to triglyceride metabolism.

CONCLUSION

Alcoholic and water extracts of *Blumea eriantha* DC root and stem have shown significant antioxidant activity.

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