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ANTIOXIDANT AND DIURETIC ACTIVITY OF LEAVES OF *BLEPHARIS BOERHAAVIAEFOLIA*

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ABSTRACT

Blepharis boerhaaviaefolia is used in the traditional medicine as diuretic. In the present study, the diuretic activity of Benzene, Chloroform, Alcohol and Aqueous extract of leaves of *Blepharis boerhaaviaefolia* was studied and the activity was compared with furosemide as standard. All the four extract exhibited significant diuretic activity as evidenced by increased total urine volume and the urine concentration of Na^+, K. The Aqueous extract was studied for antioxidant activity by three methods, Super oxide radical scavenging activity, Hydroxyl radical scavenging activity and Nitric oxide radical scavenging activity. These results clearly indicate that *Blepharis boerhaaviaefolia* is effective against free radical mediated diseases.

Key words: Diuretic activity, Antioxidant activity, *Blepharis boerhaaviaefolia*, Flavonoids.

INTRODUCTION

Highly reactive free radicals and oxygen species are present in biological systems from wide variety of sources. These free radicals may oxidize nucleic acid, proteins, lipids or DNA and can initiate a variety of disease process such as cancer, cardiovascular diseases, cataracts, diabetes, asthma and macular degeneration and inflammatory diseases (Yohanrashiman TN, 2000; The Wealth of India, 1998; Kiritikar and Basu, 1987). Antioxidant compounds like Phenolic acids, Poly phenols and Flavonoids scavenge free radicals and that inhibit the oxidative mechanisms that lead to degenerative diseases (Pulok K.Mukherjee, 2002). Antioxidant effect of a plant is mainly due to phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Trease GE, 1989). Further studies have revealed that some phenolic molecules have anti cancer and antimutagenic activites (Harbone JB, 1984). Herbs are used as medicine since time immemorial. Many of the natural products in plants of medicinal value offer us new sources of drugs which have been used effectively in traditional medicine. There is an increased consciousness regionally and globally in production and use of plants with healing property.

Blepharis boerhaaviaefolia (*Acanthaceae*) (Yohanrashiman TN, 2000; The Wealth of India, 1998; Kiritikar and Basu, 1987; Pulok K.Mukherjee, 2002) is a prostate herb rooting at nodes, internodes elongate creeping, flowering and fruiting time is September and January. Propagation by seeds. Leaves commonly sold in Indian market, are reported to be useful in wounds, ulcers, nasal hemorrhage, asthma, throat inflammation, ascitis, liver and spleen disorders. Root is considered dysmenorrhoea. Seeds are considered to be expect deobstruent and useful in strangury and conjunctivitis. Seeds are used for dysuria, diseases of nervous system and aphrodisiac in ayurveda . A perusal of literature

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revealed that its diuretic effects remain to be studied. Here in we report the diuretic effect of the Benzene, Chloroform, Alcohol and Aqueous extract of leaves of *Blepharis boerhaaviaefolia* in albino rats.

The *Blepharis boerhaaviaefolia* has been claimed to have diuretic activity, but no detailed scientific investigations have been carried out to define the diuretic activity of *Blepharis boerhaaviaefolia* extract. The effect produced by *Blepharis boerhaaviaefolia* was compared to that of furosemide, a standard drug.

MATERIALS AND METHODS

Plant material

The plant materials were collected from Tirunelveli District, Tamilnadu, India and authenticated by Dr.Chelladurai, survey of medicinal plants unit, Palayamkottai. The voucher specimen was kept at Dept .of. Pharmacognosy in our laboratory for future reference.

Preparation of the Extract

The air dried leaves were pulverized in to coarse particles and extracted exhaustively with Benzene, Chloroform, Alcohol and Aqueous by cold maceration for 20 days. These extracts were concentrated under reduced pressure and preserved in dessicator for further use.

Experimental animals

In bred colony strains of Wistar rats of either sex weighing 150-250 g procured from the animal house were used for the study. The animals were maintained in polypropylene cages of standard dimensions at a temperature of 28±1° C and standard 12 hour : 12 hour day / night rhythm. The animals were fed with standard rodent pellet diet (Hindustan Lever Ltd) and water *ad libitum*. Prior to the experiment the animals were acclimatized to the laboratory conditions.

Preliminary phyto chemical analysis

The preliminary phytochemical analysis (Trease GE, 1989; Harbone JB, 1984) were carried out to find out the phytoconsituents present in the crude extracts.

Antioxidant Activity

Superoxide anion scavenging activity assay

The scavenging activity of the *Blepharis boerhaaviaefolia* towards superoxide anion radicals was measured by the method of Liu, Ooi and Chang (Liu F *et al*., 1997) .Superoxide anions were generated in a non enzymic phenazine methosulphate –nicotinamide adenine dinuleotide (PMS-NADH) system through the reaction of PMS, NADH and oxygen. It was assayed by the reduction of nitroblue tetrazoline (NBT). In these experiments the superoxide anion was generated in 3 ml of Tris- HCl buffer (100 Mm, pH 7.4)containing 0.75 ml of NBT (300µM) solution ,0.75 ml of NADH (936 µM) solution and 0.3 ml of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation:

$$\% \text{ inhibition} = A_0\text{-}A_1 / A_0 \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radicals was measured with Fenton reaction (Yu W *et al*., 2004) . Reaction mixture contained 60µl of 1mM $FeCl_3$,90 µl of 1mM 1,10 phenanthroline,2.4 ml of 0.2M phosphate buffer (pH 7.8) ,150 µl of 0.17 M H_2O_2 and 1.5 ml of extract at various concentrations .Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with a spectrophotometer. The hydroxyl radical scavenging activity was calculated according to the following equation:

$$\% \text{ inhibition} = A_0\text{-}A_1 / A_0 \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Nitric oxide scavenging activity assay

Nitric oxide scavenging activity was determined according to the method reported by Garrat (Garrat DC, 1964). Sodium nitroprusside in aqueous solutions at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of Griess illosovy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml of phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25° C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1 ml of sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1 ml napthyl ethylene diaminedihydrochloride (0.1% W/V) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated according to the following equation:

$$\% \text{ inhibition} = A_0\text{-}A_1 / A_0 \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Diuretic Activity

Male rats (wister albino strain) weighing 150 to 180gm were maintained under standard condition of temperature and humidity. The method of Lipschitz *et al* (Lipschitz WL *et al*., 1943; Murugesan T *et al*., 2000) was employed for the assessment of diuretic activity. The experimental protocols have been approved by the

Institutional Animal Ethical Committee. Four groups of six rats in each and were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control, received normal saline(25ml/Kg,p.o.); the second group received furosemide (20mg/Kg,i.p.) in saline; the third, fourth, fifth and sixth groups received the Benzene, Chloroform, Alcohol and Aqueous extract at the doses of 250 mg/Kg, respectively, in normal saline. Immediately after administration the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and feaces, kept at room temperature of 25± 0.5ºC through out the experiment. The urine was collected in measuring cylinders up to 3 hrs after dosing. During this period, no food or water was made available to animals. The parameters taken for individual rat were body weight before and after test period, total concentration of Na^+, K^+, and Cl^- in the urine. Na^+, K^+ concentrations were measured by Flame photometry (Jeffery GH *et al*., 1989) and Cl^- concentration was estimated by titration (Beckette AK and Stenlake JB, *et al*., 1997) with silver nitrate solution(N/50)using three drop of 5% potassium chromate solution as indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20mg/Kg of furosemide per kg body weight in series of supportive experiments. Results are reported as mean ± SD, the test of significance ($p<0.01$ and $p<0.05$) was stastically.

Statistical analysis

All the results are expressed as mean ± standard error. The data was analyzed statistically using ANOVA followed by student 't' test at a probability level of $P < 0.001$.

RESULTS

Antioxidant activity

Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological systems (Okhawa H *et al*., 1979). The super oxide anion radical scavenging activity of the extract from *Blepharis boerhaaviaefolia* assayed by the PMS-NADH system is shown in (Table 1 and Fig 1) .The super oxide scavenging activity of *Blepharis boerhaaviaefolia* was increased markedly with the increase of concentrations. The half inhibition concentration of *Blepharis boerhaaviaefolia* was 0.058 mg/ml. These results suggested that *Blepharis boerhaaviaefolia* had important superoxide radical scavenging effect.

Hydroxyl radical scavenging activity

Hydroxyl radical is very active and can be generated in biological cells through the Fenton reaction. (Table 1 and Fig 2) showed the *Blepharis boerhaaviaefolia* exhibited concentrations dependent scavenging activities against hydroxyl radicals generated in a Fenton reaction system. The IC_{50} of *Blepharis boerhaaviaefolia* was 0.107 mg/ml. The potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution. Similarly, high molecular weight and the promity of many aromatic rings and hydroxyl groups are more important for the free radical scavenging by specific functional groups (Korycka M and Richardson M, 1978).

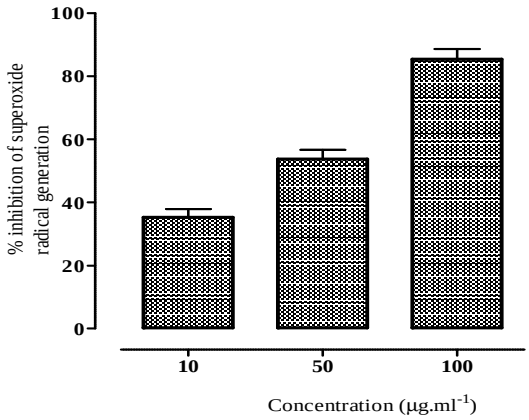
Nitric oxide radical scavenging activity

Nitric oxide is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of plat let aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, antimicrobial and antitumor activities (Hagerman AE *et al*., 1998). *Blepharis boerhaaviaefolia* extract moderately inhibited nitric oxide in dose dependent manner (Table 1 and Fig 3) with the IC_{50} was 0.856 mg/ml.

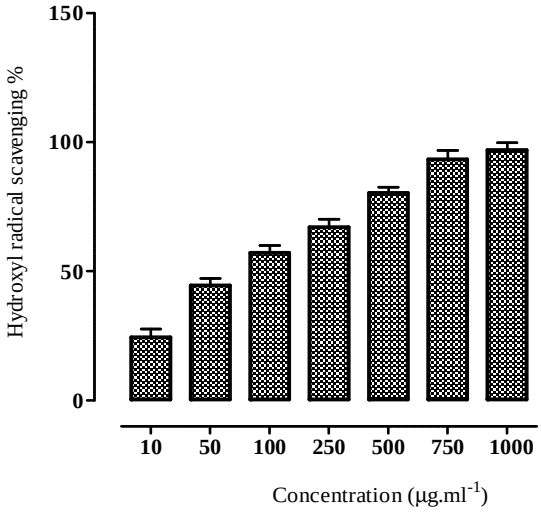
Table 1 – Radical Scavenging activity of Aqueous extract of *Blepharis Boerhaaviaefolia* at different concentrations

Concentration (µg/ml)	Superoxide radical scavenging %	Hydroxyl radical scacenging %	Nitric oxide radical scavenging %
10	32.24±2.65	24.43±3.24	11.24±0.65
50	53.78±2.89	44.54±2.65	16.34±2.13
100	85.45±3.21	57.18±2.78	20.54±2.78
250	-	67.06±3.14	28.13±2.18
500	-	80.28±2.31	37.78±2.86
750	-	93.45±3.45	43.47±2.34
1000	-	97.01±2.87	54.01±2.48
IC50 (mg/ml)	0.058	0.107	0.856

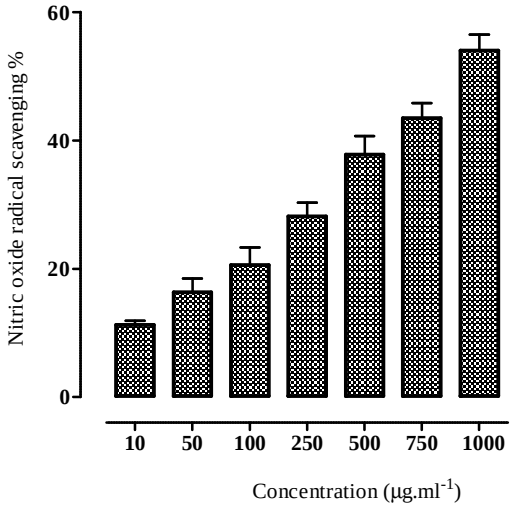
"Table 1 (continued)"
Values are means ±SD (n=3)



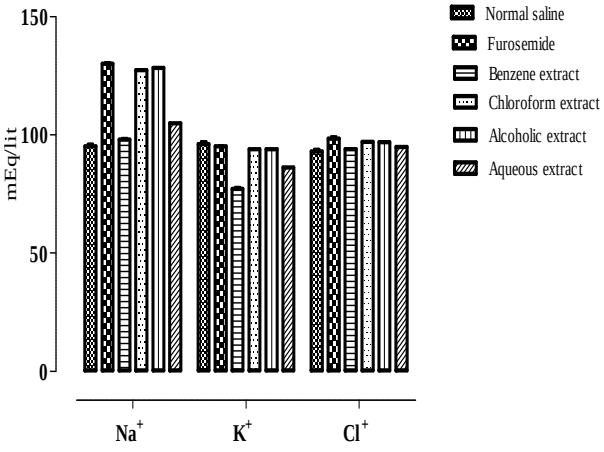
Superoxide anion radical scavenging activity percentage
Fig.1



Hydroxyl radical scavenging activity percentage
Fig.2



Nitric oxide radical scavenging activity percentage
Fig.3



Diuretic activity of benzene, chloroform, alcohol an aqueous extracts of Leaves of *Blepharis Boerhaaviaefolia.*

Fig.4

Table 2: Diuretic activity of Benzene, Chloroform, Alcohol and Aqueous extracts of leaves of *Blepharis Boerhaaviaefolia*.

Treatment	Dose	Total Urine(ml)	Na^+(mEq/lit)	K^+(mEq/lit)
Normal saline	25ml/kg p.o	1.9±0.17	95.2±0.95	96.2±1.10
Furosemide	20mg/kg i.p	4.5±0.18	130.1±0.53	95.2±0.32
Benzene extract	250mg/kgi.p	3.3±0.11	98.1±0.52	77.2±0.73
Chloroform extract	250mg/kgi.p	4.4±0.32	127.4±0.63	94.0±0.17
Alcohol extract	250mg/kgi.p	4 .5±0.02	128.4±0.18	94.0±0.23
Aqueous extract	250mg/kgi.p	3.6±0.20	105.0±0.36	86.2±0.38

Mean ± S.E.M, n=6, Students "t" test, P< 0.001(compared to control) was considered significant.

Diuretic activity

The preliminary phyto chemical analysis showed the presence of flavanoids, saponins, carbohydrates, terpenoids and alkaloids in all the extracts. The Chloroform and Alcohol extract 250mg/kg p.o. showed significant increase in excretion of sodium, potassium and chloride ions in the urine in a dose dependent manner. The obtained effect was comparable to that of furosemide (20mg/kg). The study supported the presence of effective diuretic constituents in the Chloroform and Alcohol extract of *Blepharis Boerhaaviaefolia*. The results are shown in (Table 2 and Fig 4).

DISCUSSION AND CONCLUSION

Oxygen is vital for aerobic life. But the cellular biochemistry of dioxygen is Janius faced that comprise of both bright and dark side. The bright side includes numerous enzymes catalyzed reactions of dioxygen that occur in respiration and normal metabolism while the dark side encompasses deleterious reactions of reactive species derived from dioxygen that inflict damages to the cellular components. Reactive oxygen species are ubiquitous and occur naturally in all aerobic organisms arising from both endogenous and exogenous sources (Shackelford RE *et al*., 2000). They are normally produce as a by product of cellular metabolism. They are capable of damaging biomolecules, provoking immune response, activating oncogenes and enhancing aging process (Devasakayam TPA and Kamat JP, 2000). Reactive oxygen species metabolites can be generated by the stepwise reduction of oxygen leads to the production of series of oxidant molecules such as super oxide (O_2) and other reactive nitrogen species like nitric oxides (NO) (Jenner P, 1996) . Compounds with antioxidant activity categorized into three groups namely excellent, good and moderate. Excellent ones are those that perfectly quench excited state as well as ground state radicals. Good antioxidants strongly inhibit the peroxide formation but are less effective in quenching excited states. Moderate antioxidants fail to excel in both reactivates (Beautner B *et al*., 2001). Antioxidants from plant products may fall under any of three categories of antioxidants .The antioxidant activity may also depend on the type and polarity of the solvent that the plant is extracted. There are some synthetic antioxidants such as butylated hydroxy anisole and butylated hydroxy toluene. Reaction with the α,α di phenyl picryl hydrazyl (DPPH) will serve as a method for the direct detection of radical scavenging as screening methods also reliable for bio assay guided Lactination. ROS contribute to a great variety of diseases. ROS including H_2O_2, super oxide radical anion, Nitric oxide, singlet oxygen react biological molecules leading to cell and tissue injury (Ilhami Gulcin *et al*., 2005). In our work we have performed some popular methods that were easy to perform in our laboratory condition.

Diuretics relive pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure (Hoeland RD and Mycek MJ, 2000). Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that ethanol, aqueous and chloroform extract may produce diuretic effect by increasing the excretion of Sodium, Potassium and Chloride. The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles (Guyton AC and Hall JE, 1998). The regulation of Sodium , Potassium balance is also intimately related to renal control of acid-base balance. The Potassium loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended (Sturat IF, 1987). In present study chloroform and alcohol extracts showed elevated levels of Potassium in urine, which may increase risk of hypokalemia and hence its potassium sparing capacity has to be investigated. Active principles such as flavanoids, saponins and terpenoids are known to be responsible for diuretic activity (Chodera A *et al*., 1991; Sood AR *et al*., 1985; Rizvi SH *et al*., 1980). Results of present investigation showed that ethanol is most effective in increasing urinary electrolyte concentration of all the ions i.e Sodium, Potassium and Chloride followed by chloroform and aqueous extracts while other extracts did not show significant increase in urinary electrolyte concentration.

In the present study *Blepharis Boerhaaviaefolia* showed good diuretic effect in the experimental models compared to control. And also it has well free radical scavenging activity.

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