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Research article

FREE RADICAL SCAVENGING ACTIVITY OF *TERMINALIA ARJUNA* LEAVES

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ABSTRACT

Antioxidant activity of chloroform extract of *Terminalia arjuna* leaves was investigated for its free radical scavenging activity by determining the nitric oxide and superoxide radical scavenging activity. Maximum scavenging of nitricoxide and superoxide radical found were 27.71% and 48.55% respectively at 250 µg/ml concentration.

Keywords: *Terminalia arjuna*, antioxidant, free radical, nitric oxide

INTRODUCTION

Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases. Thus the present investigation was aimed at evaluating the antioxidant activity of *Terminalia Arjuna* leaves (**Badoni A.K. 2000**).

Terminalia arjuna belonging to the family Combretaceae is an herbaceous, The *Terminalia arjuna* is usually found growing on river banks or near dry river beds in West Bengal and south and central India.. Generally it is called as Arjuna Tree (**Rana T. S. and Datt B 1997**).

It is generally known for its medicinal properties. Seeds are used as astringent, nutritious, while the oil is used in the treatment of skin disease and rheumatism. Fruit are used in colic disorders; roots are used in leucorrhoea treatment. As a medicine, it is especially used for the complaints of the veins, such as phlebitis, haemorrhoids, vari-cose veins; in

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ulcers; to prevent thrombosis; in some cases of migraine, effusions of blood; for limb complaints and forstbite.The plant is reported to contain a mixture of saponins,one of which is described as aescine, which easily crystallizes. In addition it also contains flavonoid glycosides, aesculine, albumin and fatty oils. Aesculin issued medically and extracts from the seeds are used industrially. The hydrosycoumarin glycoside aesculin from the bark of the branch absorbs ultra-violet rays' andis an ingredient for suntan oil. (**Perianayagam J.B. *et al.*, 2004)**

MATERIALS AND METHODS

Plant material: Fresh leaves of the plant *Terminalia Arjuna* were obtained from the Bangalore region. The collected leaves were dried inshade, crushed to coarse powder and used for further studies.

Preparation of extract: The dried plant material leaves (500 gm) were subjected to continuou shot extraction with chloroform for 48 h in a soxhlet apparatus. The chloroform extract was filtered and partitioned by using petroleum ether to remove the fixed oils, fats and other non polar constituents present in it. The solvent was evaporated under reduced pressure anddried in a vacuum desiccator and was used when required. The dried extract thus obtained (280gm) was used for the assessment of antioxidant activity for 36 hours (**Kokate C. K. 2005)**. The extracts were subjected to preliminary qualitativetests to identify the various phytoconstituents present in leaves (**Harbone J.B. 1998**). The qualitative chemical tests performed were Shinoda test, ammonia fuming test, lead acetate, boricacid for flavonoid containing compounds and ferricchloride test, nitric acid test, ammonia hydroxide –potassium ferricyanide test, lead acetate test for the presence of tannins. All these test gave positive results when they were compared with Rutin and Quercetin, standard drugs of the class.

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition was estimated by the use of Griess I llosvoy reaction (**Hyoung Lee S. 1990**). In this investigation, Griess I llosvovy reagent was generally modified by using naphthyl ethylene diamined ihydrochloride (0.1 % w/v) instead of the use of 1-naphthylamine (5 %). The reaction mixture (3 ml) containing sodium nitroprusside(10 mM, 2 ml), phosphate buffer saline (0.5 ml) and the extract (20-250 µg/ml) standard solution (rutin, 0.5 ml) was incubated at 25^{o}C for 150 minutes. A control testcompound equivalent amount of methanol was taken. After incubation, 0.5 ml of the reaction mixture mixed with 1ml sulfanilic acid reagent (0.33 % in 20 % glacialacetic acid) and allowed to stand for 5 min for completion of the reaction process of diazotization.Further, 1ml of the naphthyl ethylene diamine dihydrochoride was added, mixed and was allowed to stand for 30 min at 25^{o}C. The concentration of nitrite was assayed at 540 nm and was calculated with the referenceto the absorbance of the standard nitrite solutions. Rutin was taken as a standard. The percent inhibition was calculated using the formula:

$$\% \text{ inhibition} = \frac{(\text{Acont-Atest}) \times 100}{\text{Acont}}$$

Where Acont is the absorbance of the control reaction and Atest is the absorbance in the presence of samples with the extracts.

Superoxide radical scavenging activity

Superoxide radical scavenging activity is generally based on the anion radical which is associated with PMS NADH system. The measurement of superoxide scavenging activity of this is based on method as described by (**Liu F *et al.*, 1997**). They are generated within PMSNADH systems by the oxidation of NADH and are assayed by the reduction of nitroblue tetrazolium (NBT). Tris HCl buffer (3ml, 16mM, pH 8.0) containing 1ml NBT (50µM) solution, 1ml NADH (78µM) solution and a sample solution of extract (20-250µg/ml) in water were mixed. The reaction was started when 1ml of phenazine methosulfate (PMS) solution (10µM) was added to the mixture. The reaction mixture was incubatedat 25^{0}C for 5 min, and the absorbance was read at 560nm against the corresponding blank samples. Quercetin was used as a reference drug. Decreased absorbance of thereaction mixture indicated increased superoxide anionscavenging activity. The percentage inhibition was calculated by using the same formula **(Perianayagam J.B *et al.*, 2004).** Chloroform extract was dried under reduced vacuum pressure and finally it was made soluble in the corresponding solvents (phosphate-buffer). The tests were performed with the filtrate soluble portion.

RESULTS AND DISCUSSION

The preliminary qualitative tests indicated the presence of flavonids, phenols and tannins. Table 1 and Table 2 shows that the percentage inhibition of nitric oxide and superoxide radical by CSCE. Oxidative stress in large quantities of reactive oxygenspecies (ROS) are generated is one of the earliestresponses to stress. The antioxidant system protects the pathogens against the ROS-induced oxidative damage. The percentage inhibition for the superoxide radical was

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found to be moderate andnitric oxide radical is less significant when compared to the reference standard. Values are expressed as mean + SEM of five measurements. Statistical analysis was performed by Dunnett's Test by ANOVA.

IC 50 values for all the above experiments were determined by linear regression analysis. The activity is increasing with the concentration and difference were statistically significant ($p<0.01$). After 250µg/ml there was decrease in the activity with anegative effect.

Table 1: Antioxidant activity of chloroform extract of *Terminalia Arjuna* specious Linn. Leaves

Nitric oxide inhibition Assay

Concentration (µg/ml)	Rutin (Std. %)	AICE (%)
20	31.0 ± 0.42	6.67 ± 0.21
40	44.2± 1.3	11.1 ± 1.00
100	54.3 ± 0.77	19.64 ± 1.12
125	68.9 ± 1.15	22.13 ±2.31
250	76.4 ± 0.43	26.82 ± 1.66
IC 50 (µg/ml)	67.7	512.94

Values are presented as the mean±SEM (n=10)

AICE- Terminaliaarjuna chloroform extract;Std.- Standard

Table2.Antioxidant activity of chloroform extract of *Terminalia Arjuna* Linn. Leaves

Superoxide Scavenging Assay

Concentration (µg/ml)	Rutin (Std. %)	AICE (%)
20	34.0 ± 0.40	15.85 ± 0.43
40	51.1± 0.50	28.2 ± 1.01
100	56.4 ± 0.85	35.5 ± 1.13
125	58.5 ± 1.15	42.7±2.84
250	60.5 ± 1.10	47.85± 1.71
IC 50 (µg/ml)	61.7	236.4

* Values are presented as the mean±SEM (n=10)

AICE- Terminaliaarjuna chloroform extract;Std.- Standard

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CONCLUSION

Nitric oxide radical generated from the sodium nitropruside and measured by the Greiss reduction. Sodium nitropruside at physiological pH spontaneously generates nitric oxide, which thereby interacts with oxygen to produce nitrate ions that can be estimated by use of Greiss reagents. Thus the scavengers of nitricoxide compete with the oxygen, leading to reduced production of nitric oxide. In the PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Thus, CSCE fraction, possessed good antioxidant property so, workshould be carried out with more antioxidant models. The results were positive at the laboratory level and further work can be carried out to find out the exact constituent responsible for these activities by the process of modern analytical tools. Thus the plant can be a potential sourcefor antioxidant property.

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