



IN-VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF *ERAGROSTIS PILOSA

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ABSTRACT

In this study, the antioxidant anti-inflammatory activities of methanolic extract of the whole plant of *Eragrostis pilosa* were evaluated by different in vitro methods. The whole plant of *Eragrostis pilosa* was extracted with methanol employing a maceration process. These extracts were screened for antioxidant activity by different *in vitro* assay methods including Reducing power assay, Iron chelating assay, and Nitric oxide scavenging activity. The screening was carried out at different concentrations including 100-500µg/ml in reducing power assay and Nitric oxide scavenging activity while in the iron-chelating assay, the extract was used in a concentration of 50, 100, 150, 200, and 250µg/ml. In vitro anti-inflammatory activity was evaluated by the membrane stabilization method at a concentration range of 100-500µg/ml. The extract of *Eragrostis pilosa* exhibits antioxidant potential with increasing concentration. The antioxidant and anti-inflammatory activity of the whole plant of *Eragrostis pilosa* might be attributed to its flavonoids, tannins, and other phenolic constituents. Our study concluded that the methanolic extract of the whole plant of *Eragrostis pilosa* may contain antioxidants components, which might help prevent the progress of various oxidative stresses. Besides, the extract was found to possess considerable anti-inflammatory properties and could have a significant effect against chronic inflammation.

Keywords: *Eragrostis pilosa*, Reducing power assay, Iron chelating assay, scavenging assay, Membrane stabilization.

INTRODUCTION

Cells of our body Produce the oxidants in normal and pathological conditions. Such Oxidants are useful to our body to destroy microbes. Sometimes the uncontrolled production of oxygen-derived free radicals such as Reactive Oxygen Species (ROS). This ROS mediated the oxidative damage to micro molecules and it causes various diseases such as Cardiovascular disease, Cancer, Aging, Diabetes, Rheumatoid arthritis, Cirrhosis, etc [1]. However, antioxidants have evolved with protective roles against such damage. Many medicinal plants have an antioxidant value that can prevent the destructive/ degenerative effects caused by oxidative stress [2]. Oxidative stress indicates a serious imbalance between the production of free radicals and the antioxidant defense system, resulting in tissue damage [3]. Chronic inflammation is usually associated with an increase in reactive nitrogen and oxygen species production bringing about oxidative stress initiated by an imbalance between reactive oxygen species and the biological system's defense able to eliminate these free radicals [4]. This oxidative stress has been observed in several diseases including cancer, neurodegenerative diseases, atherosclerosis, malaria, chronic fatigue syndrome, and rheumatoid arthritis [5]. Recently there has been extensive interest in the therapeutic potential of medicinal plants as antioxidants in scavenging such free radical-induced tissue injury [6]. *Eragrostis pilosa* is a weedy species that occurs throughout the world in tropical and temperate regions belongs to the family Poaceae [7]. The scientific study indicated that the presence of flavonoid, tannins, and phenolics constituents in the seeds of *Eragrostis pilosa* [8] and medicinal plants containing such constituents can express antioxidant potential [9]. In this study, the in-vitro antioxidant activity of methanolic extract of the complete plant of *Eragrostis pilosa* was evaluated by various methods including Reducing power assay, Iron chelating assay, and Nitric oxide scavenging activity. It has been used as one of the antioxidant capability indicators of medicinal plants [10] while in vitro anti-inflammatory activity was evaluated by membrane stabilization method. Reducing properties are generally associated with the presence of reductones, which are believed to break the radical chain and the donation of H atom indicating the antioxidant properties of plants concomitant with the development of reducing power. The higher the absorbance, the stronger the reducing power [11]. Iron chelating activity assay of ortho-substituted phenolic compounds may exert pro-oxidant effects by interacting with iron. o-phenanthroline quantitatively forms complexes with Fe²⁺, which get disrupted in the presence of chelating agents [12]. Nitric oxide is an essential bio-regulatory molecule required for several physiological processes like neural signal transmission, immune response, control of vasodilatation, and control of blood pressure, etc [13]. However the elevation of NO results in several pathological conditions including cancer. Moreover, in the pathological conditions nitric oxide react with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspects of inflammation and tissue damage seen in inflammatory diseases [14]. The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drug act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since HRBC (human red blood cell) membrane is similar to the lysosomal membrane, the study was undertaken to check the stability of the HRBC membrane by the extracts to predict the anti-inflammatory

activity in vitro [15].

MATERIAL AND METHODS

Collection of Plant material and chemicals

The plant material of *Eragrostis pilosa* was collected from the Shivnibandh region in Sakoli taluka of Maharashtra. It was identified and authenticated in the Department of Botany, MB Patel college Sakoli. All chemicals and reagents used in the study were of analytical grade and were procured from the Department of Pharmaceutical Chemistry, Bajiraoji Karanjekar College of Pharmacy Sakoli, Bhandara, Maharashtra, India.

Preparation of Extract

The coarsely grounded plant material of *Eragrostis pilosa* was macerated with methanol. The maceration process allowed for seven days in tightly sealed vessels at room temperature and stirred several times daily. After seven days, the mixtures were filtered through muslin cloth and thereafter concentrated by evaporation at room temperature.

Determination of Antioxidant Potential

Reducing Power Assay:

The reducing Power of methanolic extract of plant material was determined by the method prescribed by Oyaizu [16]. The various concentration of extract in methanol was mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50°C in the water bath for 20 min. After cooling, 2.5 ml of 10% trichloroacetic acid was added and centrifuge at 3000 rpm for 10 min whenever necessary. The 2.5 ml of an upper layer of solution was mixed with 2.5ml of distilled water and 0.5 ml of freshly prepared ferric chloride solution. The blank was prepared similarly excluding the sample. The absorbance was measured at 700 nm. Ascorbic acid at various concentrations was used as standard. The increased absorbance of the reactions mixture indicates an increase in reducing power.

Iron Chelating Assay

The reaction mixture containing 1ml o-phenanthroline, 2ml Ferric chloride solution, and 2ml extract at various concentrations (50-200 ug/ml) in the final volume of 5ml was incubated for 10 min at ambient temperature and the absorbance was recorded at 510 nm. Ascorbic acid as a standard drug was added instead of extract and absorbance was taken as equivalent to 100% reduction of all ferric ions. Blank was carried out without drug [17].

$$\% \text{ chelating activity} = \{1 - \text{absorbance (T)} / \text{Absorbance (B)}\} \times 100$$

Where, Absorbance (T): Absorbance of test

Absorbance (B): absorbance of Control

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity was measured by using Griess' reagent. The 2 ml of 10 mM sodium nitroprusside in standard phosphate buffer (pH 7.4) was mixed with 0.5 ml of extract at various concentrations (100-400 ug/ml) and the mixture was incubated at 25°C for 150 minutes. The 0.5 ml of incubated solution was mixed with 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and incubated at room temperature 25°C for 5 min. Finally, 1ml of Naphthylethylene diamine dihydrochloride (0.1% v/v) was added and incubated at room temperature for 30 minutes. The absorbance was measured at 540 nm by using a UV-visible spectrophotometer. In this method, ascorbic acid was used as the standard for comparison purposes. [18]

$$\% \text{ Nitric oxide inhibitor activity} = (A_o - A_s / A_o) \times 100$$

Where, A_o = Absorbance of control

A_s = absorbance in the presence of the extract.

Determination of in vitro Anti-inflammatory activity

Membrane stabilization method

Preparation of red blood cell (RBCs) suspension

The blood was collected from the healthy human volunteer who has not taken any NSAIDs (Non-Steroidal Anti-Inflammatory) for 2 weeks before the experiment and transferred to centrifuge tubes. The tubes were centrifuged at 3000 RPM for 10 min and were washed three times with an equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat-induced hemolysis

The reaction mixture (2ml) consists of a 1 ml test sample of different concentrations (100-500 µg/ml) and 1 ml of 10% RBCs suspension. The only saline was added instead of a test sample. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction. Mixtures were incubated in the water bath at 56°C for 30 min. at the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 RPM for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for the entire test sample.

$$\% \text{ inhibition} = (Abs \text{ control} - Abs \text{ sample}) \times 100 / Abs \text{ control}$$

RESULT AND DISCUSSION

The antioxidant activity of methanolic extract of the whole plant of *Eragrostis pilosa* was evaluated by reducing power assay, o-phenanthroline method, and nitric oxide radical scavenging method while in vitro anti-inflammatory activity was evaluated by membrane stabilization method. Reducing power assay of the

methanolic extract of the whole plant of *Eragrostis pilosa* showed an almost similar increasing trend in reducing power with the increased extract concentration. In this assay, the presence of reducers as an antioxidant causes the reduction of the ferric to the ferrous form. The color of the test solution change to various shades of green and blue depending upon the reducing power of extract. The data present in the figure indicate the antioxidant activity of different concentrations of extract which is due to the presence of reductones by donating the electrons and reacting with free radicals to convert them to a more stable product and terminate free radical chain reaction. In Iron chelating assay, the extract interferes with the formation of ferrous-o-phenanthroline complex, thereby suggesting that the extract has metal chelating activity. [22] Iron stimulates lipid peroxidation by Fenton reaction and accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals which themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation.[23] The metal chelating capacity is important since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. It has been reported that the chelating agents, that form bonds with metal are effective as a secondary antioxidant because they reduce the redox potential, thereby stimulating the oxidized form of the metal ion. The observed results demonstrate a marked capacity of the extract for iron-binding, suggesting that their action as a peroxidation protector may be related to its iron-binding capacity. Nitric oxide (NO) is a reactive free radical produced by phagocytes and endothelial cells to yield more reactive species such as peroxynitrite which can be decomposed to form OH radicals. In this study, the level of nitric oxide was significantly reduced by extract, explaining its role as an antioxidant. NO scavenging capacity of the extract may help to arrest the chain of reactions initiated by excess generation of NO that lead to various pathogenic pathways underlying a large group of disorders including muscle diseases, inflammatory bowel disease, sepsis, and septic shock, primary headaches, and stroke [19, 20]. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the drug may well stabilize the lysosomal membrane. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents. The lysosomal enzymes released during inflammation produce various disorders.[21] The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. The methanolic extract of the whole plant of *Eragrostis pilosa* shows a high percentage of protection against hemolysis [15]. It is stated that flavonoids, as well as tannins, possess anti-inflammatory effects.

CONCLUSION

All performed studies indicate that methanolic extract of the whole plant of *Eragrostis pilosa* may contain antioxidants components, which might help prevent the progress of various oxidative stresses. Besides, the extract was found to possess considerable anti-inflammatory properties and could have a significant effect against chronic inflammation. The methanolic extract of the whole plant of *Eragrostis pilosa* needed more investigation to identify the major active compounds and to demonstrate their mode of action.

Table

Table.1 Reducing Power Assay

Concentration ($\mu\text{g}/\text{ml}$)	Abs (Standard)	Abs (Test)
100	1.13	0.599
200	1.525	0.668
300	1.817	0.729
400	1.859	0.794
500	1.961	0.847

Table.2 Iron Chelating Method

Concentration ($\mu\text{g}/\text{ml}$)	% inhibition (Standard)	% inhibition (Sample)
50	0.67%	0.34%
100	1.12%	0.48%
150	1.26%	0.59%
200	1.29%	0.72%
250	1.33%	0.87%

Table.3 Nitric Oxide Scavenging Assay

Concentration ($\mu\text{g}/\text{ml}$)	% inhibition (Standard)	% inhibition (Sample)
100	61.08%	15.02%
200	65.56%	21.26%
300	71.85%	24.53%
400	78.52%	33.58%
500	80.36%	45.64%

Table.4 Membrane Stabilization Method

Concentration ($\mu\text{g}/\text{ml}$)	% Inhibition (Standard)	% inhibition (Sample)
100	54.44%	45.56%
200	65.25%	49.23%
300	73.87%	54.67%
400	77.95%	59.83%
500	84.59%	63.71%

Figure

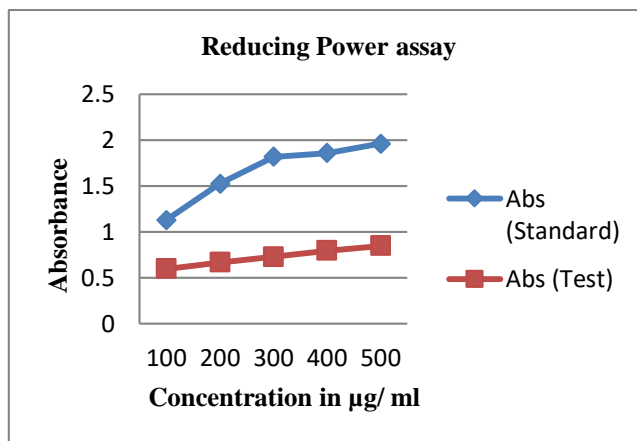


Fig.no 1 Reducing power assay

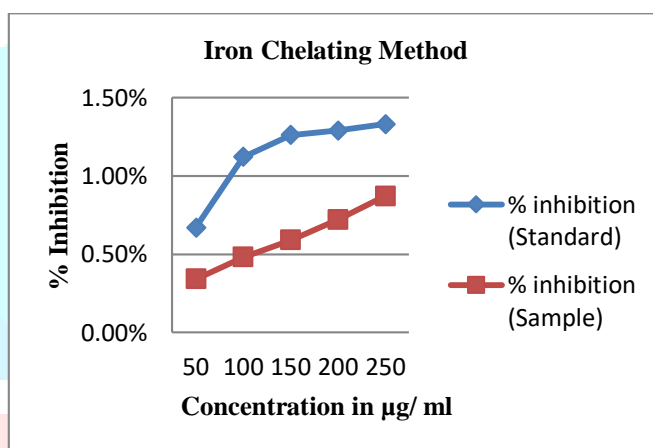


Fig.no 2. Iron Chelating Method

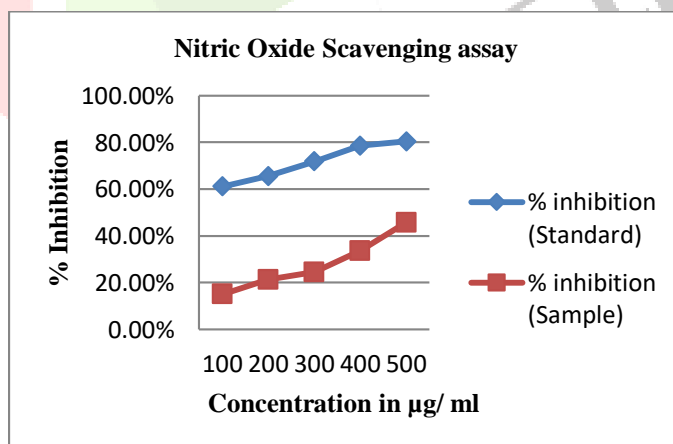


Fig.no 3 Nitric oxide Scavenging Assay

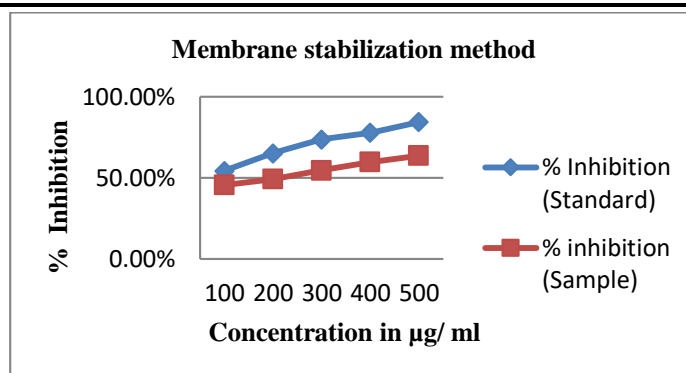


Fig.no4. Membrane Stabilization Method

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