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EVALUATION OF IN-VITRO ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF *PERGULARIA DAEMIA* LINN

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ABSTRACT

Pergularia daemia is a well known traditional folklore medicinal plant. The plant has been found to possess diverse number of pharmacological properties. In the present study, aqueous extract of P.daemia leaves were used to evaluate in vitro antioxidant and anti-inflammatory activities. In vitro antioxidant activity was carried out by Total antioxidant activity, ABTS assay, Hydrogen peroxide assays, and anti- inflammatory activity by membrane stabilization method and heat induced hemolysis. Aqueous extract showed an effective pharmacological activity in all assays when compared with their respective standards.

KEYWORDS: Inflammation, Traditional medicine, Antioxidant activity, Protein denaturation.

INTRODUCTION

The use of medicinal plants as a source of new drug for pharmaceutical industry has been focused since last decades. Various traditional medicinal plants are nowadays focused for development of new alternatives for allopathic drugs. According to World Health Organization, about more than 80% of the world's population including developed countries still rely on use of medicinal plants for their primary healthcare. The pharmacological action is due to various secondary metabolites present in the plant. *Pergularia daemia* (Forsk.) Chiov (Apocyanaceae), commonly known as utaran (Hindi), Dustapuchettu (Telugu), Uttamarani (Sanskrit) is a slender, hispid, fetid smelling laticiferous twiner found in the plains throughout the hot parts of India. P. daemia is said to have more magical application

than medical application as it posses diverse healing potential for a wide range of illnesses. Some of the Folklore people use this plant to treat jaundice, as laxative, anti-pyretic, expectorants and also in infantile diarrhea. The leaf latex is locally used as pain killer killer and for relief from toothache (Hebbar *et al*., 2010), the sap expressed from the leaves are held to cure sore eyes in Ghana. The plant reduces the incidence of convulsion and asthma. It is used to regulate the menstrual cycle and intestinal functions. The root is useful in treating leprosy, mental disorders, anemia and piles (Omale *et al*., 2011). Hence in this paper, the In-vitro anti-inflammatory effects of leaves of *Pergularia daemia* Linn. were investigated. Furthermore, the study also evaluated the antioxidant scavenging activities of the selected plant.

MATERIALS AND METHODS

Plant material

Fresh plant parts (*Pergularia daemia*) were collected randomly from the gardens and villages of Kovilpatti, Tamil Nadu from the natural stands. The botanical identity of these plants was confirmed by Dr. V. Nandagopalan, Associate Professor & Dean, PG & Research Department of Microbiology, National College, Tiruchirappalli, Tamil Nadu. A voucher specimen has been deposited at the Department of Botany, National College (Autonomous), Tiruchirapalli-620 001, Tamil Nadu, India.

Aqueous extraction

100 grams of dried powder were extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through What man no.1filter paper and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume.

Solvent extraction

100 grams of dried plant powdered samples were extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at 4^{o}C in airtight bottles for further studies, *viz.* antimicrobial, antioxidant, anticancer and phytochemical analysis.

Antioxidant activity

Determination of total antioxidant capacity

Total antioxidant activity of the plant from *Pergularia daemia* determined according to the method of Prieto *et al*. (1999). Briefly, 0.3 ml of sample was mixed with 3.0 ml reagent solution, (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95ºC for 90 minutes under water bath. Absorbance of all the sample mixtures was measured at 695 nm after 15 min. Ascorbic acid was used as standard.

Percentage scavenging (H_2O_2) = (A_1/ A_0) x 100)

A_o - Absorbance of control; A_1 - Absorbance of sample

Hydrogen peroxide scavenging assay

The free radical scavenging activity of the plant from *Peagularia daemia* determined by hydrogen peroxide assay (Gulcin *et al.*, 2004). Hydrogen peroxide (10mM) solution was prepared in phosphate buffered saline (0.1M, pH 7.4). 1ml of the extract containing samples of different concentration (100, 250, 500, 750 and 1000µg) was rapidly mixed with 2ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer after 10 minutes of incubation at 37°C against a blank (without hydrogen peroxide). The percentage of scavenging of hydrogen peroxide was calculated using the formula,

Percentage scavenging (H_2O_2) = (A_1/ A_0) x 100)

A_o - Absorbance of control; A_1 - Absorbance of sample

ABTS inhibition assay

The ability of the extract to scavenge ABTS (2,2 azino bis (3-etheylbenzothiazoline-6-sulphonicacid) diammonium salt) radical scavenging was determined by the method of Re *et al.* (1999). ABTS was generated by mixing 5 ml of 7 mM ABTS with 88 µl of 140 mM potassium persulfate under darkness at room temperature for 16 hours. The solution was diluted with 50% ethanol and the absorbance at 734 nm was measured. The ABTS radical cation scavenging activity was assessed by mixing 5 ml ABTS solution (absorbance of 0.7± 0.05) with 0.1ml polysaccharide (100, 250, 500, 750 and 1000 µg). The final absorbance was measured at 743 nm with spectrophotometer. The percentage of scavenging was calculated by the following formula,

% of scavenging = (A_1/ A_0) x 100)

Where A_o - Absorbance of control; A_1 - Absorbance of sample

***In vitro* Anti-inflammatory activity**

Membrane stabilization method

The SRBC membrane stabilization has been used as method to study the anti-inflammatory activity. Blood was collected from healthy volunteer who had not taken any NSAIDS for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%.pH 7.2) and a 10%(v/v) suspension was made with isosaline. The assay mixture contained the drug (concentration as mentioned in the table 2), 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5ml of HRBC suspension. Diclofenac was used as reference drug. Instead of hyposaline 2ml of distilled water was used in the control. All the assay mixtures were incubated at 370C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water as 100% (Gandhisan *et al*., 1991).

Heat induced hemolysis

A volume of 100 µL of 10% RBC was added to 100 µL of the extract. The resulting solution was heated at 56°C for 30 minutes followed by centrifugation at 2500 rpm for 10 minutes at room temperature. Supernatant was collected, and absorbance was read at 560 nm. Acetyl salicylic acid was used as a positive control. Percent membrane stabilization was calculated by the method of Saket *et al*. (2010)

% Inhibition=100− ((A1-A2)/A0)*100)

Where A1 is the absorbance of the sample, A2 is the absorbance of the product control and A0 is the absorbance of the positive control.

RESULTS AND DISCUSSION

Steroidal and non-steroidal anti-inflammatory drugs are currently the most widely used drugs in the treatment of acute inflammatory disorders, despite their renal and gastric negative secondary effects. There is a need for the new safe, potent, nontoxicor less toxic anti-inflammatory drug. Plant medicines are great importance in the primary healthcare in many developing countries. According to World Health Organization (WHO) still about 80% of the

world population rely mainly on plant-based drugs. The research is based on to evaluate for newer anti-inflammatory agents from herbal medicine with potent activity and lesser side effect substitutes for drugs. The results clearly showed that *P.daemia* extract had significant total antioxidant activity at the same concentration (1000 µl) (Table 1). The ABTS cation radical is formed by the loss of an electron by the nitrogen atom of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) which absorbs at 743nm giving a bluish-green colour in the presence of antioxidant, yielding the solution decolorization (Pisochi and Negulescu, 2011). Result of present study reveals that the aqueous extract possesses good antioxidant activity which is equal to standards, ascorbic acid as depicted in table 2. The present results is also clearly indicating the percentage inhibition of ABTS radical scavenging activity was concentration-dependent with increased in the reaction mixture for the extracts and the standards.

The scavenging ability of water extract of *P.daemia* on hydrogen peroxide is shown table 3 and compared with ascorbic acid as standards. The *P.daemia* extracts were capable of scavenging hydrogen peroxide in an amount dependent manner. The maximum hydrogen scavenging activity was noted at 1000 µl concentration (89.09%). On the other hand, using the near amounts, ascorbic acid exhibited 92.63% hydrogen peroxide scavenging activity.

Table 4 shows the results of inhibition of haemolysis and percentage of RBC membrane stabilization by the aqueous extract of *Pergularia daemia* at various concentrations.

Maximum stabilization activity was observed (65.73%) at a concentration of 500µl. The present results provide an indication for membrane stabilization and protein denaturation as an additional mechanism of *Pergularia daemia* for anti-inflammatory activity. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 78.34% at the concentration of 500 µl.

The crude extract was effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extract inhibited the heat induced hemolysis of RBCs to varying degree (Table 5). The maximum inhibitions (72.34%) were observed at 500µl concentration. The aspirin standard drug standard drug showed the maximum inhibition 84.66%.

Denaturation of proteins is a well-documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation (Mizushima and Kobayashi, 1968). The denaturation is used loosely to designate the change of proteins from a soluble to an insoluble form brought about by a large variety of chemical and physical agents, including acids, alkalies, alcohol, acetone, salts of heavy metals and dyes (Mann, 1906), and heat, light, and pressure (Robertson, 1918). Chick and Martin (1910) consider heat denaturation as a reaction between protein and water which implies in all probability a hydrolysis. Several author anti-inflammatory drugs have shown dose dependent ability to inhibit the thermally induced protein denaturation (Grant et al., 1970). Similar results were observed from many reports from plant extract (Sakat et al., 2010). The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The aqueous extract of *P. daemia* showed significantly higher anti-inflammatory and antioxidant activity at increasing concentration. Hence, *P. daemia* can be used as an anti-inflammatory agent. The investigation is based on the need for anti-inflammatory agents from natural sources with potent activity and lesser side effects as substitutes for chemical therapeutics.

Table 1: Total antioxidant activity of *Pergularia daemia* Linn.

S.No.	Concentration (µl)	% scavenging activity	
		Ascorbic acid (standard)	Aqueous extract
1.	200µl	34.43	49.33
2.	400µl	52.17	60.49
3.	600µl	64.44	70.36
4.	800µl	76.11	74.46
5.	1000µl	87.58	82.3

Table 2: ABTS assay of aqueous extract of *Pergularia daemia* Linn.

S.No.	Concentration (µl)	% scavenging activity	
		Ascorbic acid (standard)	Aqueous extract
1.	200µl	20.37	16.34
2.	400µl	47.41	51.26
3.	600µl	63.7	56.68
4.	800µl	72.13	65.59
5.	1000µl	81.26	76.54

Table 3: H_2O_2 Scavenging activity of *Pergularia daemia* Linn.

S.No.	Concentration (µl)	% scavenging activity	
		Ascorbic acid (standard)	Aqueous extract
1.	200µl	27.43	25.43
2.	400µl	52.41	45.36
3.	600µl	67.38	54.18
4.	800µl	76.63	74.41
5.	1000µl	92.63	89.06

Table 4: Membrane stabilization of aqueous extract of *Pergularia daemia* Linn.

S.No.	Concentration (µl)	% Membrane stabilization	
		Aspirin (standard)	Aqueous extract
1.	100µl	45.61	34.24
2.	200µl	51.42	38.50
3.	300µl	59.20	46.59
4.	400µl	67.48	57.52
5.	500µl	78.34	65.73

Table 5: Heat heamolysis of aqueous extract of *Pergularia daemia* Linn.

S. No.	Concentration (µl)	% heat heamolysis	
		Aspirin (standard)	Aqueous extract
1.	100µl	46.49	28.42
2.	200µl	58.41	36.39
3.	300µl	67.33	51.44
4.	400µl	75.33	63.47
5.	500µl	84.66	72.34

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