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In Vitro Anti Oxidant and Anti Lipid studies on Ethanolic Extract of Pergularia Daemia Leaves

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Abstract: ***The presents study aims to determine the total antioxidant capacityand anti-lipid activity of the ethanolicleaf extract of the Pergularia daemia. The total antioxidant capacity of the ethanolic extract was found by using the formation of phosphomolybdenum complex. The total antioxidant capacity of the leaf extract of Pergularia daemia was found to be 287.5mg/g AAE (Ascorbic acid equivalent). The anti-lipid activity of the plant was determined by using anti lipid peroxidation assay. The anti-lipid activity of the extract has an*** IC_{50} ***of 57. 14. Thus the plant has both antioxidant and anti-lipid activity which makes it useful in the therapeutics for various ailments including cancer.***
Keywords: Pergularia daemia, total antioxidant activity, lipid peroxidation, AAE and medicinal plants.

I. INTRODUCTION

Oxidative stress is found to be the major cause for the development of various diseases. To combat the adverse effects of the reactive oxygen species (ROS) which induces oxidative damages, addendum of exogenous antioxidants or increasing endogenous antioxidant defences the body in a potential way. Naturally plants have the ability to synthesize an inclusive range of non-enzymatic antioxidants which is capable of attenuating ROS- induced oxidative damage. Antioxidants can be synthesized in vivo (e.g., reduced glutathione, superoxide dismutase, etc.) or from the dietary intake[1],[2]. Plants are the chief source of exogenous antioxidants. The two- thirds of the world's plant species have medicinal significance and they have an excellent antioxidant potentials [3]. The discovery and subsequent isolation of ascorbic acid from plants paved a way for the interest in exogenous antioxidants in plants [4]. This lead to a tremendous consideration because increased oxidative stress has been found to be the most important causative factor in the development and progression of numerous life threatening diseases which includes diseases like neurodegenerative, diabetes mellitus, cancer and cardiovascular disease [5], [6].

Pergularia daemia is a lactiferous twiner found in the hotter parts of India. It belongs to the family Asclepidaceae. The common name is Dog's bane white low plant [7].

This plant has been used for its therapeutic effects for various ailments traditionally. It is used to treat asthma, snake bite, rheumatic swelling, amenorrhea, and dysmenorrhea. It is also used as an anti- helminthic and expectorant [8]. Many phytochemical compounds have been analysed in this plants among all, the plant has a higher amount of flavonoid content [9]. So, for further investigation, ethanolic leaf extract of the plant was subjected to *in vitro* antioxidant studies. There are number of in vitro methods to screen the antioxidant activity.

II. METHODOLOGY

A. In VitroTotal Antioxidant Capacity (TAC) Assay

Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. Total antioxidant capacity can be calculated by the method described by Prieto et al. (1999). About 0.1 ml of sample (200 µg) solution is combined with 1 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molyb date). The tube is capped and incubated in a boiling water bath at 95 °C for 90 minutes. After cooling the sample to room temperature, the absorbance of the aqueous solution is measured at 680 nm against blank in UV spectrophotometer. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it is incubated under same conditions as rest of the sample. For samples of unknown composition, antioxidant capacity can be expressed as equivalents of ascorbic acid [10].

$$TAC = C \times V / M$$

TAC = total antioxidant capacity, milligram per gram of sample extract, in AAE(Ascorbic Acid Equivalent)
C = the concentration of ascorbic acid established from the calibration curve, mg/ml
V =the volume of extract, millilitre
M = the weight of sample extract (g)

B. In vitro anti-lipid peroxidation assay

The effect of each extract on lipid peroxidation in liver homogenate was determined by the method of Ohkawa *et al*., 1979 with minor modifications (Kumar *etal*., 2000). Freshly excised goat liver was processed to get 10% homogenate in cold phosphate buffered saline (pH 7.4) using a glass Teflon homogenizer and filtered to get a clear homogenate. The degree of lipid peroxidation was assessed by estimating the thiobarbituric acid reactive substances (TBARS). Different concentrations (10-500 µg/ml) of the extracts were added to the liver homogenate.

Lipid peroxidation was initiated by adding 100 µl of 15 mM ferrous sulphate solution to 3 ml of tissue homogenate. After 30 min, 100 µl of this reaction mixture was taken in a tube containing1.5 ml of TCA. After 10 min, tubes were centrifuged and supernatant was separated and mixed with 1.5 ml of 0.67% TBA in 50% acetic acid. The mixture was heated for 30 minutes in a boiling water bath. The intensity of the pink colored complex formed was measured at 535 nm [11],[12]. Anti-lipid peroxidation activity of the extracts was calculated by the following formula: % Inhibition = [(Absorbance of the control group- Absorbance of the sample)/Absorbance of the control] × 100

Vitamin E was used as a standard antioxidant compound.

III. RESULTS AND DISCUSSION

Various studies has shown that the indigenous antioxidants may be useful in the prevention of the adverse effect of oxidative stress. This study also aimed to determine the invitro antioxidantactivity and anti-lipid activity. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Anti-oxidants such as thiols or ascorbic acid (Vitamin C) terminate these chain reactions. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases.Antioxidant based drugs and formulations are being used for the prevention and treatment of complex diseases like Alzheimer's disease and cancer for the past three decades [13].The antioxidant activity of the ethanolic leaf extract of Pergularia daemia is determined as 287.5mg/g AAE. The antioxidant activity of the plant is higher when compared to the Chandigarh yellow variety of Lantana camara and Salvia of ficinalis, the antioxidant activities are 222.20/g AAE [14] and 67.26-138.44 mg AAE/g respectively [15]. This shows that the plant has a good antioxidant activity (e.g. Fig. 1). Thus it can be used for treating many diseases especially cancer.

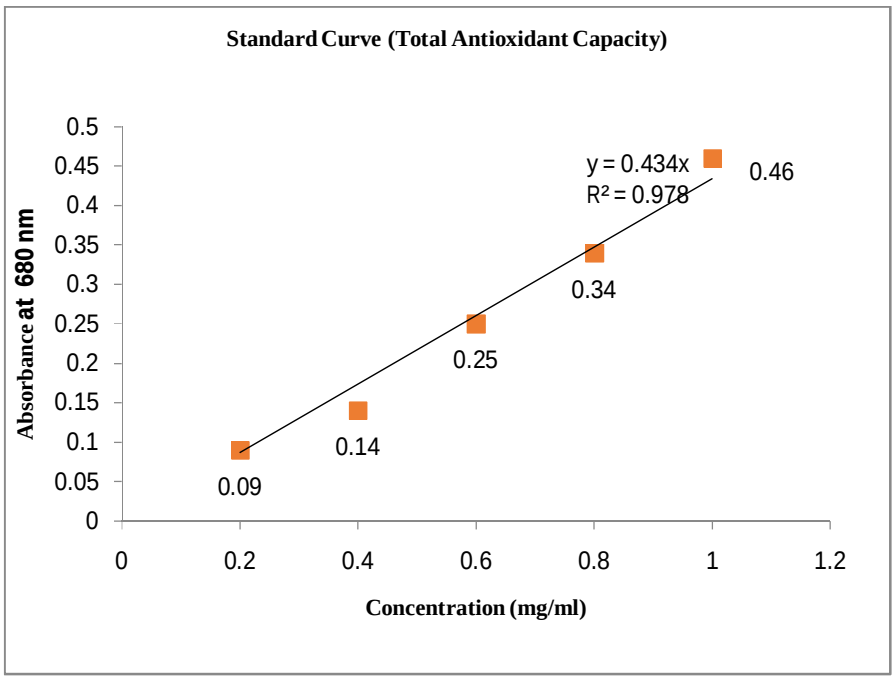

Fig.13. shows the total antioxidant capacity of the plant extract of *Pergularia daemia*

The plant also shows anti-lipid activity where the IC_{50} is 57.14 which is higher (e.g. Tab. 1). At a concentration of 500, there is an inhibitory concentration (e.g. Fig. 2).A similar effect was found in the *Mucunapruriens* [16]. Since it has anti-lipid activity, this plant can be used as a treatment for diseases caused by hyperlipidaemia. Hyperlipidaemia is an abnormal level of lipids or lipoproteins in the blood including triglycerides and cholesterol. This causes an increase in fatty deposits in arteries and risk of blockages finally leading to coronary artery disease, cerebrovascular disease and peripheral vascular disease. So this plant has a significant role in treating the life threatening disease.

TABLE I
ANTI-LIPID ACTIVITY OF PERGULARIA DAEMIA

Concentration (µg/ml)	Absorbance at 535 nm		Percentage inhibition (%)	
	Vitamin E (Standard)	Sample (Plant extract)	Vitamin E (Standard)	Sample (Plant extract)
Control	0.14	0.14	-	-
10	0.08	0.12	42.86	12.29
50	0.07	0.11	50.00	21.43
100	0.05	0.09	64.29	35.71
250	0.04	0.08	71.43	42.86
500	0.02	0.06	85.71	57.14 (IC_{50})

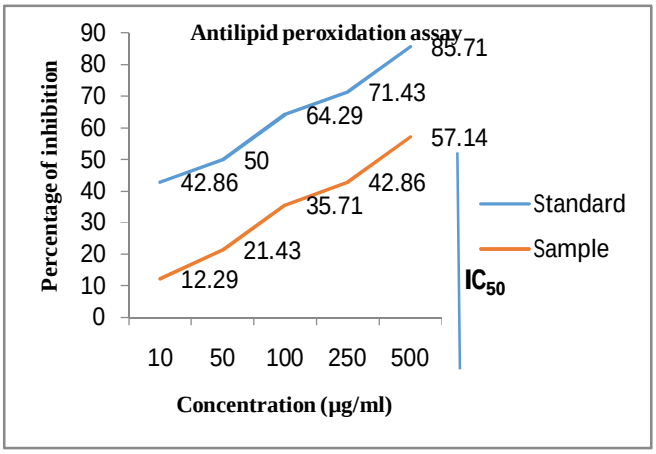

Fig.2. Shows the percentage of anti-lipidactivity of the plant extract.

IV. CONCLUSION

Phenolic compounds play an important role as an antioxidant and also contribute efficiently in stabilizing lipid peroxidation process [17].Currently there is a great interest in natural antioxidants and their role in human health and nutrition [18]. Extensive amount of data have been generated on antioxidant properties of plants around the world [19], [20]. From the above study it is evident that the plant *Pergularia daemia* have both antioxidant activity and anti-lipid activity. Since they have these properties, they can be useful in the field of medicine for treating many diseases. Antioxidants play a vital role in our health. The free radicals occur naturally in our body but they accelerate ageing process and cause diseases by attacking the fats, protein and DNA of the cells. Antioxidants neutralize the free radicals either by giving an extra electron or by breaking the molecule to make it harmless. Cardiovascular diseases are getting into concern today because of the unhealthy foods and lack of exercise. Hyperlipidaemia is also found to induce oxidative stress in many organs like liver, heart and kidney. Plant therapy is one of the therapies in which there is no or less side effects and alsoit increases the immune system. So further research can be done to find out more potential activities of the plant.

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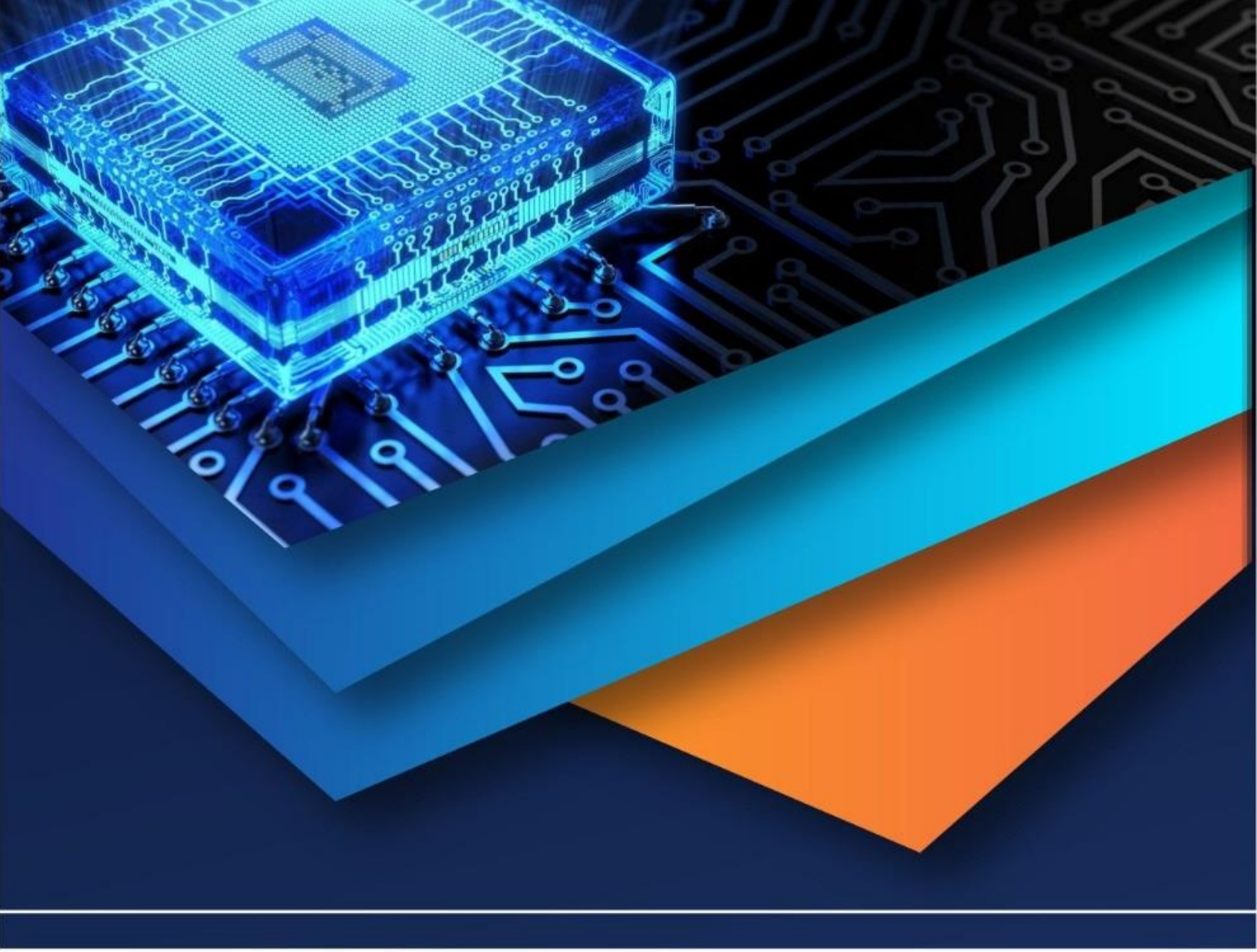

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