

RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY OF WHOLE PLANT OF *LEPTADENIA PYROTECHNICA*Sangh Partap², *Ujjwal Tewari¹, Kuldeep Sharma¹ and Keshari Kishore Jha²¹Department of Pharmacology, College of Pharmacy, Teerthankar Mahaveer University, Moradabad² Department of Pharmaceutical Chemistry, College of Pharmacy, Teerthankar Mahaveer University, Moradabad, Uttar Pradesh, India, 244001**Corresponding Author's E-mail: ujjwal1985in@gmail.com***ABSTRACT**

The free radical scavenging potential of methanolic extract of *Leptadenia pyrotechnica* was studied on in vitro antioxidant models. The antioxidant potential was evaluated by determining the activity of hydrogen peroxide (H₂O₂) radicals scavenging and 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay. In all these studies, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The extract was also shown to have high phenolic content i.e. 99.09±0.10 µg/mg. These results clearly indicated that MELP could be a potential source of natural antioxidant and effective against free radical mediated diseases.

Keywords: Antioxidant, In vitro, *Lagenaria siceraria*, Reactive oxygen species**INTRODUCTION**

Free radicals are natural by-products of our own metabolism. These are electrically charged molecules that attack cells, tearing through cellular membranes to react with the nucleic acids, proteins and enzymes present in the body¹. Free radicals can cause lipid peroxidation in foods which leads to their deterioration². Oxidation is well known to be major cause of foods and materials degradation³. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can produce free radicals which start chain reactions that damage cells¹. The oxidants/free radicals are species with very short half life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids⁴. Free radicals may be designated as molecular sharks that damage molecules in cell membranes, mitochondria (the cell's energy plants), DNA (the cell's intelligence) and are very unstable, tend to rob electrons from the molecules in the immediate surroundings in order to replace their own losses¹. These species may be either oxygen derived (ROS) or Nitrogen derived (RNS). The most common reactive oxygen species include superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), peroxy radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are nitric oxide (NO), peroxy nitrite anion (ONOO⁻), Nitrogen dioxide (NO₂) and Dinitrogen trioxide (N₂O₃)⁴. Chemical compounds and reactions capable of generating potential toxic oxygen species/free radicals are referred to as 'pro-oxidants'. In a normal cell there is an appropriate pro-oxidant: antioxidant balance. However, this balance can be shifted towards the pro-oxidant when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cell damage if the stress is massive or prolonged⁴. In living organisms various ROSs can form in different ways including normal

aerobic respiration, stimulated polymorph nuclear leukocytes & macrophages and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells.² These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and can eventually destroy them. They are continuously produced by our body's use of oxygen such as in respiration and some cell-mediated immune functions.¹ Free radicals attack the unsaturated fatty acid in the biomembranes resulting in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzyme, receptor activity and damage to membrane protein which lead to cell inactivation or death⁵. In general, the reactive oxygen species circulating in the body tend to react with the electron of other molecules in the body and these also effect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, aging, adult respiratory distress syndromes, rheumatoid arthritis, coronary heart disease, stroke, diabetes mellitus, rheumatic disease, liver disorders, multiple sclerosis, Parkinson's disease, autoimmune disease, Alzheimer's, AIDS and carcinogenesis³. Down's syndrome and ischemic reperfusion injury in different tissues including heart, liver, brain, kidney and gastro intestinal tract. The role played by ROS in stress induced gastric ulcer and inflammatory bowel diseases have been well established, as well as their involvement in the process of ageing¹. The exogenous sources of ROS include electromagnetic radiation, cosmic radiation, UV-light, ozone, cigarette smoke and low wavelength electromagnetic radiations and endogenous sources are mitochondrial electron transport chain, oxidation of fat.^{2,4} Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase,

superoxide dismutase and various peroxidases. Low levels of antioxidants or inhibition of the antioxidants enzymes, cause oxidative stress and may damage or kill cells¹. The harmful and pathologic action of the free radicals can be reversed or blocked by antioxidant substances. Antioxidant substances scavenge the free radicals and detoxify the physiological system. Current research in the field of free radical biology has indicated a confirmation that foods and beverages mounted with antioxidants play an essential role in the prevention of cardiovascular and neurodegenerative diseases and cancer⁵. As antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidative agents i.e. thiols, ascorbic acid or polyphenols¹. The antioxidants may be defined as “any substance, when present at low concentrations compared with that of an oxidizable substrate that significantly delays or prevents oxidations of that substrate”. The term oxidizable substrate includes every type of molecule found *in vivo*. Antioxidant defense include the antioxidant enzymes like SOD, CAT, GSH-px, etc, low molecular agents and dietary antioxidants¹.

MATERIALS AND METHODS

Chemicals and Reagents

All the drugs and chemicals used in the study were of analytical grade. 1,1-diphenyl-2-picryl hydrazyl (DPPH) reagent were obtained from Sigma Chemicals (St. Louis, MO, USA). Ascorbic acid and other chemicals used for evaluation of oxidative stress parameters were obtained from Sisco Research Laboratories (Mumbai).

Plant material

The plant of *Leptadenia pyrotechnica* was collected from “Kagore” village of Jaipur, Rajasthan, India in the month of January. It was authenticated by Dr. D. C. Saini, Sr. Scientist, Birbal Sahni Institute of Palaeobotany, Lucknow, Uttar Pradesh, India. [Voucher registration number 15531]

Preparation of extract

The successive extraction of powdered material was carried out in several batches using different solvents in increasing order of polarity in a soxhlet apparatus by hot percolation technique. The solvents used were petroleum ether, chloroform, acetone, methanol and distilled water. The powdered material of *Leptadenia pyrotechnica* was evenly packed in a soxhlet extractor for about 36 hours with different solvents. The temperature was maintained (25°C- 100°C) on an electric heating mantle with thermostat control. The extracts were then concentrated by evaporating the solvent under reduced pressure. Preliminary phytochemical studies were carried out on methanolic extract to assess the presence of various phytoconstituents⁶⁻⁷ and Antioxidant activity.

IN-VITRO ANTIOXIDANT ACTIVITY

The antioxidant properties of the *L. pyrotechnica* extract was determined with reference to hydrogen peroxide radical scavenging and DPPH assay method.

Hydrogen peroxide radical scavenging activity analysis

Hydrogen peroxide radical scavenging activity was determine by the method of **Ruch et al. (1984)**.⁸ Hydrogen peroxide (H₂O₂) is a biologically important oxidant because of its ability to generate the hydroxyl radical which is extremely potent. The ability of the hydroxyl radical to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems. It's very short half-life, however, restricts its diffusion capability and its potency.^{9, 10}

A solution of H₂O₂ (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of *L. pyrotechnica* (10-100 µg/ml) in phosphate buffer were added to a H₂O₂ solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing phosphate buffer without H₂O₂. The percentage of H₂O₂ scavenging of *L. Pyrotechnica* and Ascorbic acid as standard compound was calculated as

$$\text{H}_2\text{O}_2 \text{ radical scavenging activity (\%)} = [\{\text{A}_0 - \text{A}_1\}/\text{A}_0] \times 100$$

Where, A₀ is the absorbance of the H₂O₂, A₁ is the absorbance of the presence of the extract in H₂O₂ solution.^{11, 12}

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH[•]) free radical scavenging activity

The free radical scavenging activity of DPPH was determine by the method of **Gadow et al.**¹³ of *Leptadenia pyrotechnica* was estimated by this method, where in the bleaching rate of the stable free radical, DPPH[•] is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH[•] absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorbance decreases. Briefly, 0.1 mM solution of DPPH[•] (weigh 0.399 mg of DPPH and dissolve in 50 ml of methanol) in methanol was prepared and 1ml of this solution was added to 3 ml *L. pyrotechnica* solution (stock solution of *L. pyrotechnica* was prepared by dissolving in methanol) in methanol at different concentration (25-250 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The 1ml Dpph solution is mixed with 3 ml of methanol and the absorbance is recorded as blank.

The DPPH[•] radical scavenging activity was calculated according to the following equation.

$$\text{DPPH radical scavenging activity (\%)} = [\{\text{A}_0 - \text{A}_1\}/\text{A}_0] \times 100$$

Where, A₀ is the absorbance of the DPPH[•], A₁ is the absorbance of the presence of the extract in DPPH[•] solution.^{11, 14, 15}

RESULTS

Hydrogen peroxide radical scavenging activity analysis:-

Hydrogen peroxide radical scavenging of *L. pyrotechnica* whole plant methanolic extract was estimated by using ascorbic acid solution as standard. The inhibition data (Table no. 1 and Table no. 2) were recorded against the selected concentrations (10 - 100 $\mu\text{g/ml}$).

Standard inhibition curve for Hydrogen peroxide radical scavenging of ascorbic acid (Fig no. 1) and for methanolic extract of whole plant of *L. pyrotechnica* (Fig no. 2) were plotted. From these IC_{50} values of percentage inhibition of hydrogen peroxide radical scavenging of the Ascorbic acid and methanolic extract were calculated using regression equation (Table no. 3).

Table 1: % Inhibition data of hydrogen peroxide radical scavenging of ascorbic acid

Conc. of Ascorbic acid ($\mu\text{g/ml}$)	% Inhibition
10	7.2
20	15.2
30	24.49
40	32.3
50	45.3
60	53
70	57.9
80	64.8
90	72.5
100	82.7

Table 2: % Inhibition data of hydrogen peroxide radical scavenging by *L. pyrotechnica* methanolic extract of whole plant

Conc. of methanolic <i>L. pyrotechnica</i> extract of whole plant ($\mu\text{g/ml}$)	% Inhibition
10	14.28
20	21.42
30	25
40	25.42
50	35.71
60	39.28
70	58.57
80	59.14
90	69.85
100	78.57

Table 3: Hydrogen peroxide radical scavenging IC_{50} of Ascorbic acid and methanolic whole plant extract of *L. pyrotechnica*.

Sample	IC_{50}
Ascorbic acid	60.38 $\mu\text{g/ml}$
Methanolic whole plant extract of <i>L. pyrotechnica</i>	65.48 $\mu\text{g/ml}$

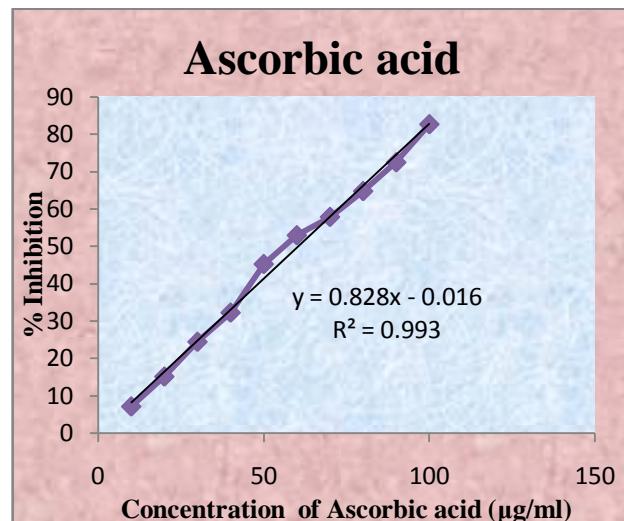


Figure 1: Standard inhibition curve of hydrogen peroxide radical scavenging of ascorbic acid

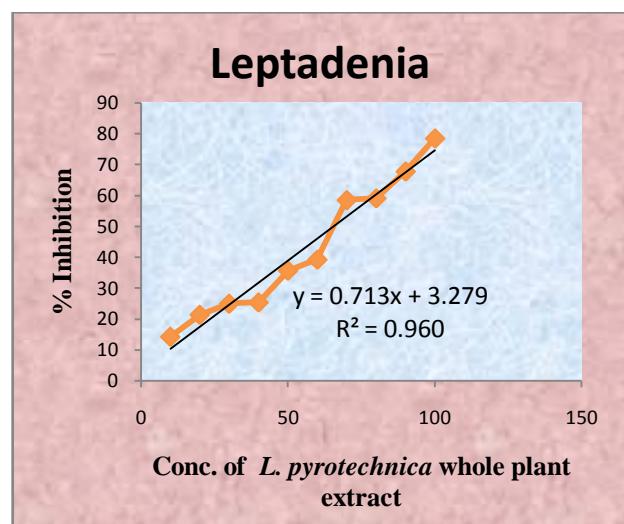


Figure 2: % Inhibition curve of methanolic *L. pyrotechnica* whole plant extract.

IC_{50} value for standard (Ascorbic acid) and the methanolic plant extract of *L. pyrotechnica* extract were 60.98 $\mu\text{g/ml}$ and 65.48 $\mu\text{g/ml}$ respectively which represent the antioxidant potential of the standard and extract samples.

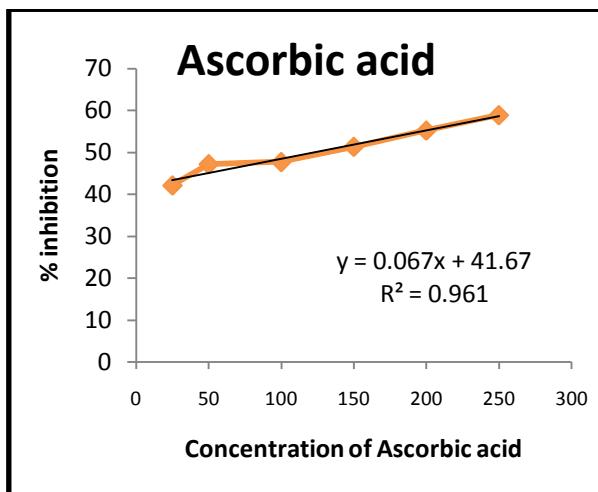
Hydrogen peroxide concentration decreased by scavenger compound by accelerating the conversion of H_2O_2 to H_2O depending on the antioxidant potential of the component present in the extract and therefore absorption value also decreased. This was due to free radical scavenging activities of ascorbic acid and methanolic plant extract of *L. pyrotechnica*.^{8,11}

DPPH Assay:-

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of methanolic *L. pyrotechnica* whole plant extract was estimated by using ascorbic acid solution as standard. The absorbance data (Table 4 and Table 5) were recorded against the selected concentrations (25, 50, 100, 150, 200, 250 $\mu\text{g/ml}$).

Table 4: % Inhibition data for DPPH assay of ascorbic acid

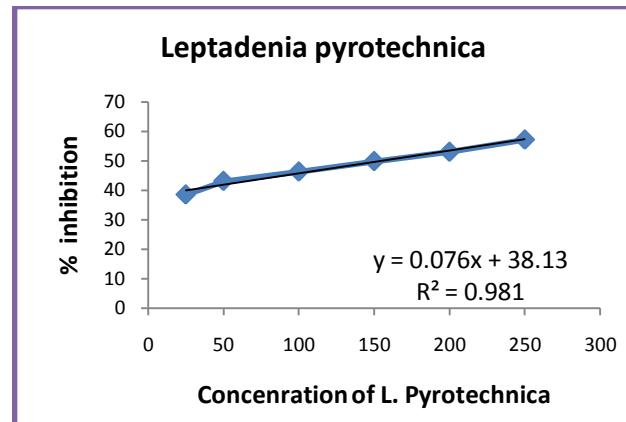
Conc. of Ascorbic acid (µg/ml)	% Inhibition
25	42.15
50	47.21
100	47.72
150	51.35
200	55.21
250	58.88

**Figure 3: % Inhibition DPPH assay curve of Ascorbic acid****Table 5: % Inhibition data for DPPH assay of *L. pyrotechnica* extract**

Conc. of <i>L. pyrotechnica</i> extract (µg/ml)	% Inhibition
25	38.59
50	43.18
100	46.36
150	49.88
200	53.04
250	57.18

Table 6: DPPH assay IC₅₀ of ascorbic acid and *L. pyrotechnica* whole plant methanolic extract

Sample	IC ₅₀ of DPPH assay
Ascorbic acid	121.69 µg/ml
Whole plant methanolic extract of <i>L. pyrotechnica</i>	152.46 µg/ml

**Figure 4: % Inhibition DPPH assay curve of *L. pyrotechnica* whole plant methanolic extract**

IC₅₀ values for standard (Ascorbic acid) and the methanolic whole plant extract of *L. pyrotechnica* were 121.69 µg/ml and 154.69 µg/ml respectively represents the antioxidant potential of the standard and extract.

The antioxidant potential of *L. pyrotechnica* was assessed by DPPH assay. The DPPH assay provided on the measurement of scavenging activity of antioxidant toward the stable radical DPPH. Antioxidant reduced the radical to the corresponding hydrazine. DPPH Radical react with suitable reducing agent, the electrons became paired off and the solution lost colour stoichiometrically depending on the number of electron taken up. The plant extract show reduction in absorption value in DPPH assay is due to radical scavenging activity of ascorbic acid and methanolic whole plant extract of *L. pyrotechnica*.

DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radical can brings about many adverse reactions leading to extensive tissue damage. Lipid proteins are all susceptible to attack by free radical. Many plant species with antioxidant activities act as protective agents against these radicals. In the present investigation potent antioxidant activity of methanolic whole plant extract of *L. pyrotechnica* was observed using different methods. However the efficacy of extract to scavenge the different radicals differed in each method depending upon the mechanism of free radical scavenging and assay methodology.

The result of DPPH scavenging activity assay in this study indicated that the plant was potently active. This suggested that the plant extract did contain compounds that could be capable of donating hydrogen to a free radical in order to remove the odd electron which is responsible for the radical's reactivity.

H₂O₂ is a weak oxidising agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly. Once inside the cell, H₂O₂ can probably react with Fe²⁺, and/or Cu²⁺ ions to form hydroxyl radical and this might be the origin of many of its toxic effects¹⁷. It is therefore biologically advantageous for cells to control the amount of H₂O₂ getting accumulated. Scavenging of H₂O₂ by the

plant extract could be attributed to its phenolics which donate electron to H_2O_2 , thus reducing it to water. The extract was capable of scavenging H_2O_2 in a concentration dependent manner.

CONCLUSION

The results obtained in the present study indicated that *L.pyrotechnica* extract exhibited free radical scavenging activity against hydrogen peroxide and DPPH \bullet . The

overall antioxidant activity of *L.pyrotechnica* might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggested that *L.pyrotechnica* could be a potential source of natural antioxidant that would have great importance as therapeutic agents in preventing or slowing the progress of reactive oxygen species and associated oxidative stress related degenerative diseases.

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