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# Reactive oxygen and nitrogen species scavenging activity of *Cayratia pedata* (lam) leaves – an *in vitro* study

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**ABSTRACT**
Free radical scavenging activity of ethanolic extract of *Cayratia pedata* leaves were carried out for proving its utility in free radical mediated diseases including diabetic, cardiovascular, cancer etc. The ethanolic extract was screened for *in vitro* antioxidant activity by oxygen and nitrogen radical scavenging such as DPPH scavenging, nitric oxide, superoxide anion radical scavenging, metal chelation, reducing power activity and total antioxidant assay at different concentrations. Throughout the studies leaves extract showed marked antioxidant activity. The antioxidant activity of the leaves extract may be due to the phytochemicals present in it. The antioxidant activity was found to be concentration dependent and may be attributed to the presence of bioactive compounds in the leaves of *Cayratia pedata.* The results of the present study concluded that the plant extract is a source of natural antioxidants which might be helpful in preventing the progress of various oxidative stress mediated diseases including aging.

**Keywords:** Antioxidant activity, *Cayratia pedata*, radical scavenging, Reactive oxygen species.

## 1. Introduction
In living organisms, various reactive oxygen and nitrogen species (ROS/RNS) e.g., superoxide anions ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$), nitric oxide radicals ($NO^{\bullet}$) and non-radical compounds, can be formed by different mechanisms. It is unavoidable one because of they are continuously produced by the body's normal use of oxygen. Such species are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer, and cardiovascular diseases and the aging process [1]. This effect was significantly reversed by prior administration of antioxidant providing a close relationship between free radical scavenging activity (FRSA) and the involvement of endocrinological responses [2].

The recent abundant evidence suggesting the involvement of oxidative stress in the pathogenesis of various disorders and diseases has attracted much attention of the scientists and general public to the role of natural antioxidants in the maintenance of human health and prevention and treatment of diseases [3]. Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential [4]. The majority of the active antioxidant constituents are flavonoids, isoflavones, flavones, anthocyanin, coumarins, lignans, catechins, and iso catechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potentia [5]. With this background and abundant source of unique active components harbored in plants. The chosen medicinal plant namely as *Cayratia pedata* leaves L belongs to the Vitaceae family. Therefore, the present study were to investigate the free radical scavenging activity of *Cayratia pedata* leaves through the free radical scavenging such as DPPH scavenging, nitric oxide, superoxide anion radical scavenging, metal chelation, reducing power activity and total antioxidant assay.

## 2. Materials and Methods
### 2.1 Chemicals
Nitroblue tetrazolium (NBT), ethylenediaminetetraacetic acid (EDTA), Sodium nitroprusside (SNP), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Potassium hexacyanoferrate [$K_3Fe(CN)_6$], and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

**2.2 Plant materials**

The fully mature *Cayratia pedata* (Lam) leaves were collected in April 2013 from Vandayar Iruppu, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Botanist, Dr. S John Britto, Department of Botany, St. Josephs College, Tiruchirappalli, Tamil Nadu, India. A Voucher specimen (SR 001) has been deposited at the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil nadu, India.

**2.3 Preparation of plant extract**

The collected leaves of *Cayratia pedata* were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. The powder material of *Cayratia pedata* leaves were macerated with 70% ethanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45 $^{o}$C. A semi solid extract was obtained after complete elimination of alcohol. The *Cayratia pedata* leaves extract (CPLE) was stored in refrigerator until used. Doses such as 20, 40, 60 and 80 µg/ml were chosen for *in vitro* antioxidant activity.

**2.4 *In Vitro* Antioxidant Activity**

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.,* [6]. The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*., [7]. The superoxide anion radicals scavenging activity was measured by the method of Liu *et al*., [8]. The chelating activity of the extracts for ferrous ions Fe2+ was measured according to the method of Dinis *et al*., [9]. The $Fe^{3+}$ reducing power of the extract was determined by the method of Oyaizu [10].

**2.5 Statistical analysis:** Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, $IC_{50}$, was graphically estimated using a nonlinear regression algorithm.

**3. Results and Discussion**

**3.1 DPPH Assay**

Recently, the use of the DPPH reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH$^{\bullet}$ free radical by a scavenger causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH$^{\bullet}$ is thought to be due to their hydrogen donating ability [11]. DPPH radical scavenging activity of plant extract of CPLE and standard as ascorbic acid are presented in Fig 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants [12]. The half inhibition concentration ($IC_{50}$) of plant extract and ascorbic acid were 47.55 µg $ml^{-1}$ and 34.91 µg $ml^{-1}$ respectively (Fig 7). The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

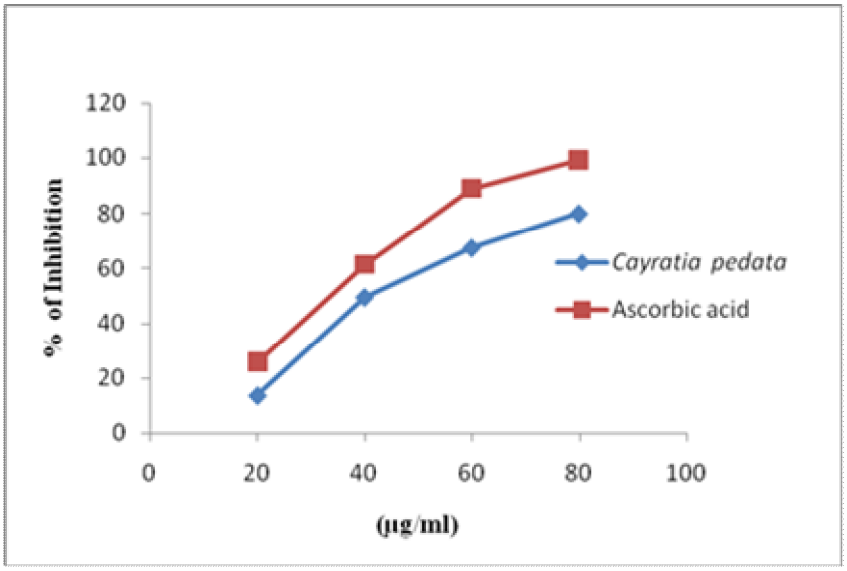


**Fig 1:** DPPH radical scavenging activity of *Cayratia pedata*

**3.2 Total antioxidant activity**

The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract [7]. Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The yield of the ethanol extract of the plant extract and its total antioxidant capacity are given in Fig. 2. Total antioxidant capacity of CPLE is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing

concentration of the plant extract. The half inhibition concentration ($IC_{50}$) of plant extract and ascorbic acid were 48.08 µg $ml^{-1}$ and 42.41 µg $ml^{-1}$ respectively (Fig 7).

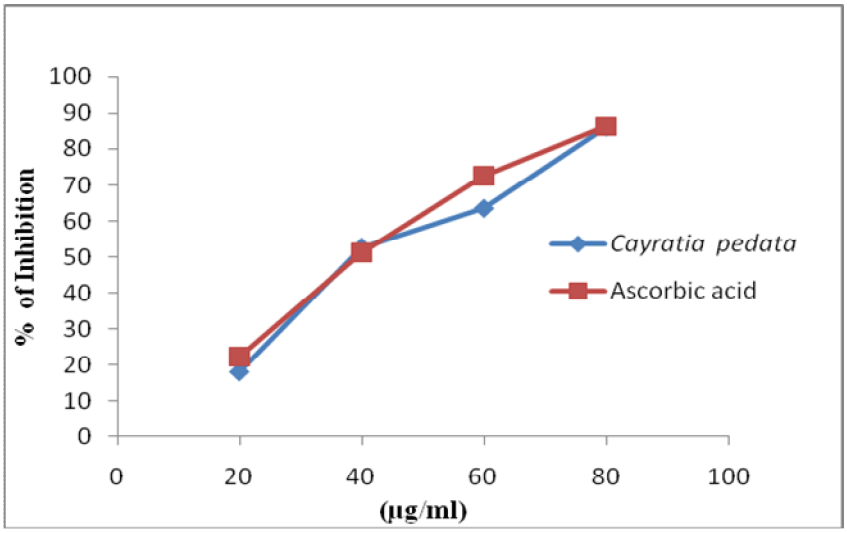


**Fig 2:** Total antioxidant assay of *Cayratia pedata*

### 3.3 Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system [13]. The superoxide anion radical scavenging activities of the extract from *Cayratia pedata* assayed by the PMS-NADH system were shown in Fig 3. The superoxide scavenging activity of *Cayratia pedata* was increased markedly with the increase of concentrations. The half inhibition concentration ($IC_{50}$) of *Cayratia pedata* was 45.55 µg $ml^{-1}$ and ascorbic acid were 31.62 µg $ml^{-1}$ respectively (Fig 7). These results suggested that *Cayratia pedata* had notably superior superoxide radical scavenging effects.

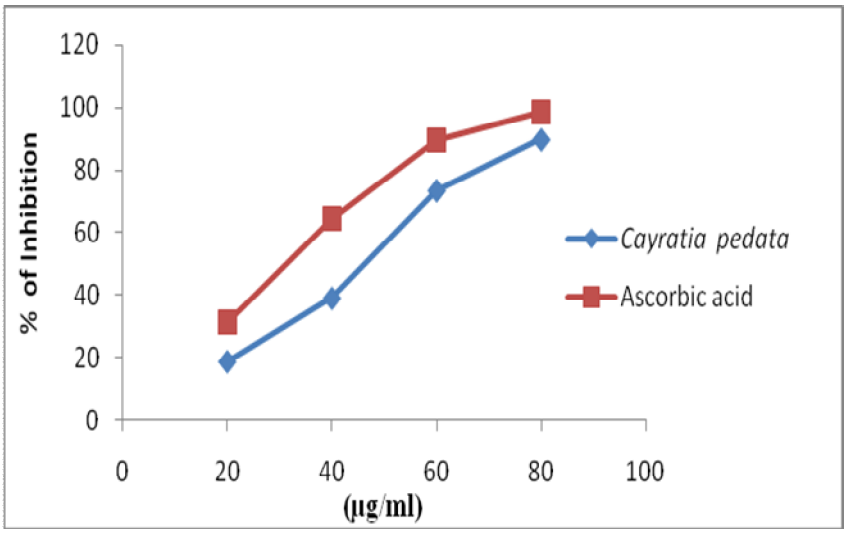


**Fig 3:** Super oxide scavenging activity of *Cayratia pedata*

### 3.4 The ferrous ion chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The formation of the ferrozine– $Fe^{2+}$ complex is interrupted in the presence of aqueous extract of *Cayratia pedata* , indicating that have chelating activity with an $IC_{50}$ of 45.12 µg $ml^{-1}$ and ascorbic acid was 30.96 µg $ml^{-1}$ respectively (Fig. 4; (Fig 7). Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxy radicals [14]. Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that forms bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion [15]. Thus, *Cayratia pedata* demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

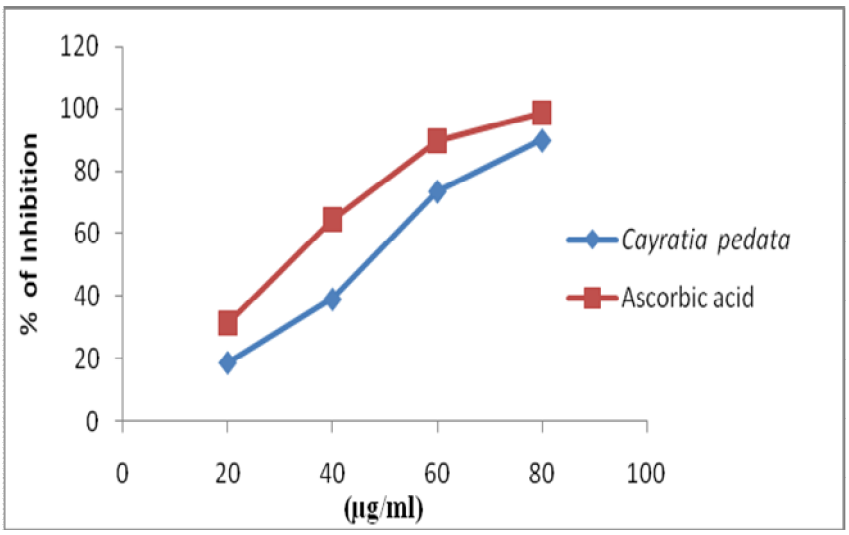


**Fig 4:** Ferrous iron chelating activity of *Cayratia pedata*

### 3.5 Reducing power activity

The measurements of the reducing ability, the $Fe^{3+}$–$Fe^{2+}$ transformation was investigated in the presence of *Cayratia pedata*. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [16]. Fig. 5 depicts the reductive effect of *Cayratia pedata* Similar to the antioxidant activity, the reducing power of *Cayratia pedata increased* with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Cayratia pedata* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

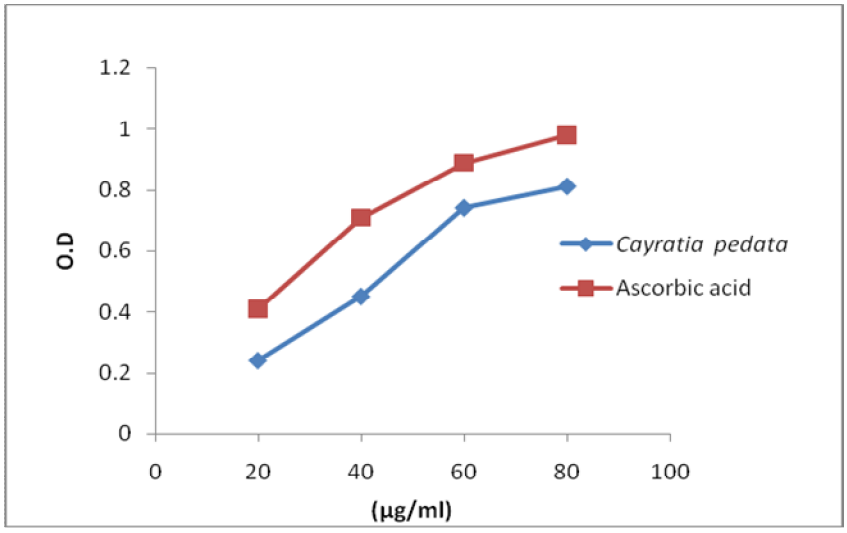


**Fig 5:** Reducing power assay of *Cayratia pedata*

### 3.6 Nitric oxide radical scavenging activity

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities [17]. *Cayratia pedata* extract also moderately inhibited nitric oxide in dose dependent manner (Fig 6) with the IC50 being 45.12 µg $ml^{-1}$ and ascorbic acid is 46 µg $ml^{-1}$ respectively (Fig 7).

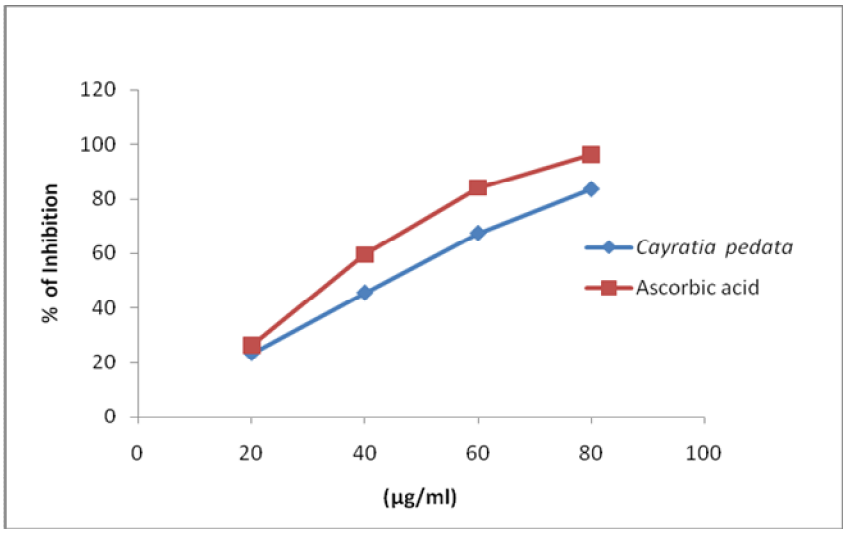


**Fig 6:** Nitric oxide scavenging assay of *Cayratia pedata*

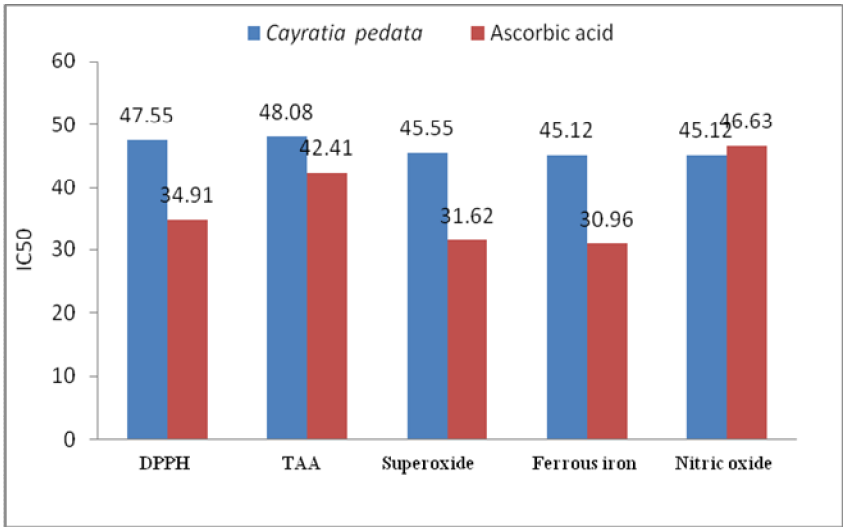


**Fig 7:** IC50 Values of Cayratia pedata and standard (Ascorbic acid)

### 3.7 Conclusion

On the basis of the results of this study, it clearly indicates that *Cayratia pedata* leaves had powerful *in vitro* antioxidant capacity against various antioxidant systems as DPPH, nitric oxide, superoxide anion scavenging and metal chelator. From our results, the antioxidant activity of *Cayratia pedata* leaves was concentration dependent. The extracts could exhibit antioxidant properties approximately comparable to commercial synthetic antioxidants as ascorbic acid. From the above assays, the possible mechanism of antioxidant activity of *Cayratia pedata* leaves includes reductive ability, metal chelator, hydrogen donating ability and scavengers of superoxide and free radicals.

### 4. Acknowledgments

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