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Free radical scavenging activity of *Physalis minima* Linn. leaf extract (PMLE)

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Abstract
Antioxidant activity of ethanolic extract of *Physalis minima* leaf was 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 nitric oxide radical scavenging, DPPH scavenging, total antioxidant assay, metal chelation, and iron reducing power activity at different concentrations. Throughout the studies leaf extract showed marked antioxidant activity. The antioxidant activity of the leaf 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 bioflavonoids content in the leaf of *Physalis minima*. Overall, 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, *Physalis minima*, Radical scavenging, Reactive oxygen and Nitrogen species.

1. Introduction
The adverse effects of oxidative stress on human health have become a serious issue. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage [2, 17] and health problems [23]. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases [1, 25]. Natural and synthetic antioxidants are beneficial to free radical mediated diseases. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis [7] for this reason, interest in the use of natural antioxidants has increased.
The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components [27]. Plant and its products are rich sources of a phytochemical and have been found to possess a variety of biological activities including antioxidant potential [26]. The majority of the active antioxidant constituents are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential [19]. With this background and abundant source of unique active components harbored in plants. The chosen medicinal plant namely as *Physalis minima* leaf L belongs to the Solanaceae family. Hence, the free radical scavenging activity of *Physalis minima* leaf was not evaluated. Therefore, the present study was to investigate the free radical scavenging activity of *Physalis minima* leaf through the free radical scavenging such as DPPH scavenging, nitric oxide scavenging, metal chelation, reducing power activity and total antioxidant assay.

2. Materials and Methods
2.1 Chemicals
Nitro blue tetrazolium (NBT), Ethylenediamine tetra acetic acid (EDTA), Sodium

nitroprusside (SNP), Trichloroacetic acid (TCA), Thio barbituric acid (TBA), Potassium hexa cyano ferrate [$K_3Fe(CN)_6$], and L-Ascorbic acid was purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

2.2 Plant materials and Preparation of alcoholic extract

The mature *Physalis minima* leaf was collected in May 2014 from Vaduvur, Thiruvarur District, Tamil Nadu, India. The leaves were identified and authenticated by Botanist, Prof. Dr. Kulothugan, Department of Botany and Microbiology, A.V.V.M. Sri pushpam College (Autonomous), Poondi, Thanjavur District, Tamil Nadu. India. A Voucher specimen (No. SPCH126) has been deposited in the department. Leaves were washed several times with distilled water to remove the traces of impurities from the leaf. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Physalis minima* leaf extract (PMLE) was stored in refrigerator until used. Doses such as 20, 40, 60 and 80µg/ml were chosen for *in vitro* antioxidant activity. L ascorbic acid as standard was used as a control and prepared by dissolving 10 mg of L-ascorbic acid in 100 ml of distilled water.

Preliminary phytochemical tests were carried out on the ethanolic and aqueous extract of *Physalis minima* leaf using standardized procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) [24] and Harborne (1973, 1984) [11, 12].

2.3 DPPH Assay

The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured by the method of Shimada *et al.*, (1992) [20]. Briefly, a 2 ml aliquot of DPPH methanol solution (25 µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. L-Ascorbic acid was used as the standard.

$$\text{Radical scavenging activity (\%)} = 100 - \frac{AC - AS}{AC} \times 100$$

Where A_{C} = control is the absorbance of the control and A_{S} = sample is the absorbance of reaction mixture (in the presence of sample). All tests were run in triplicates (n = 3), and the average values were calculated.

2.4 Determination of total antioxidant capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto, Pineda, and Aguilar (1999) [18]. The assay is based on the reduction of Mo (VI)–Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 ml extract was combined with 3ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

2.5 Nitric oxide scavenging activity assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964) [5]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25 °C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated according to the following equations:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0 \times 100],$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

2.6 Fe^{2+} chelating activity assay

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al.* (1994) [3]. To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of $FeCl_2$ (2 mM) was added. After 30 s, 0.1 ml ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as

$$\text{Chelating rate (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

2.7 Reducing power assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (1986) with slight modifications. The extract (0.75 ml) at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate [$K_3Fe(CN)_6$] (1%, w/v), followed by incubating at 50 °C in a water bath for 20 min. The reaction was stopped by adding 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

2.8 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

The phytochemical characters of the *Physalis minima* leaf investigated. The qualitative phytochemical analysis of ethanolic and aqueous extract of *Physalis minima* leaf extract contains such flavonoids, saponin, terpenoids, polyphenols and tannin which are an important in disease prevention, health preservation and promotes longevity.

3.1 DPPH Assay

DPPH radical scavenging activity of plant extract of PMLE 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 (Nuutila *et al.*, 2003) [14]. Recently, the use of the DPPH reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity of 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• free radical by a scavenger (A-H) 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• is thought to be due to their hydrogen donating ability [21]. The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 63.86 µg ml^{-1} and 34.91 µg ml^{-1} respectively. 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.

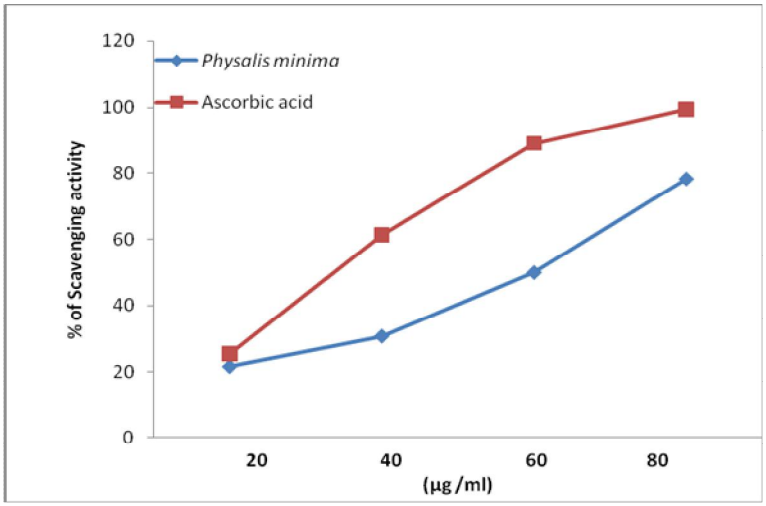

Fig 1: DPPH radical scavenging activity of *Physalis minima* leaf

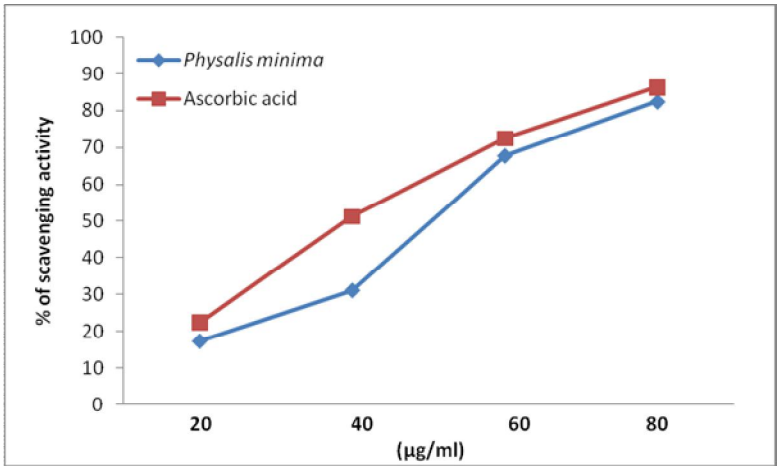

Fig 2: Total antioxidant assay of *Physalis minima* leaf

3.2 Total antioxidant activity

The yield of the ethanol extract of the plant extract and its total antioxidant capacity is given in Fig. 2. Total antioxidant capacity of PMLE is expressed as the number of equivalents of ascorbic acid. 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 extracts (Prieto *et al.*, 1999) [18]. Moreover, it is a quantitative one, since the antioxidant activity 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 13.20 µg ml^{-1} and 42.41 µg ml^{-1} respectively.

3.3 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 [13]. *Physalis minima* extract also moderately inhibited nitric oxide in dose dependent manner (Fig 3) with the IC_{50} being 46.63 µg ml^{-1} and ascorbic acid was 35.88 µg ml^{-1} respectively.

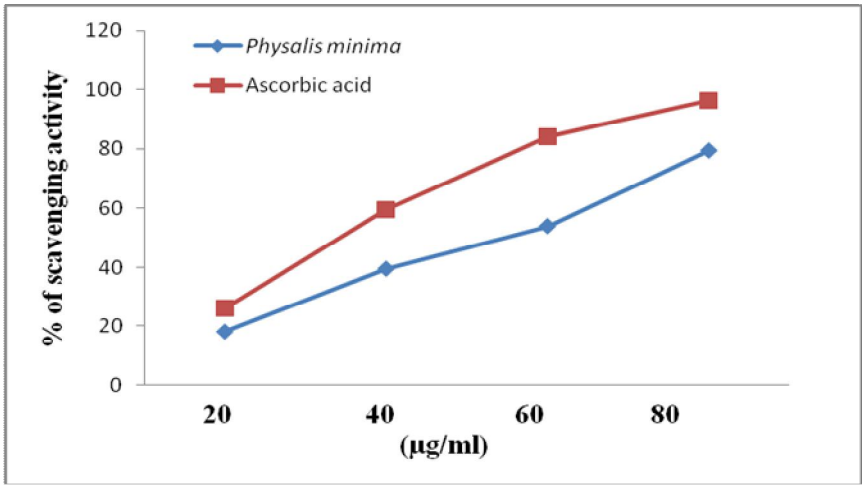

Fig 3: Nitric oxide scavenging activity of *Physalis minima* leaf

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 *Physalis minima*, indicating that have chelating activity with an IC_{50} of 45.93 µg ml^{-1} and ascorbic acid was 30.96 µg ml^{-1} respectively (Fig. 4). Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxy radicals (Halliwell, 1991; Fridovich, 1995) [9]. Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that form 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 [60]. Thus, *Physalis minima* demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

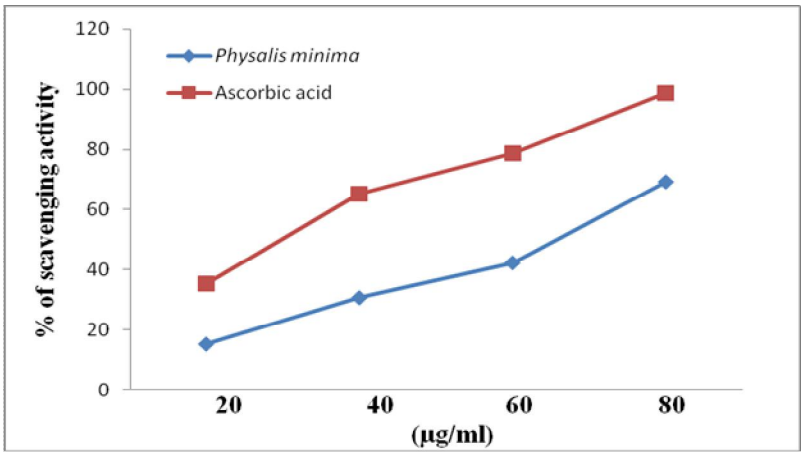

Fig 4: Ferrous iron chelating activity of *Physalis minima* leaf

3.5 Reducing power activity

For the measurements of the reducing ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of *Physalis minima*. Reducing the 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 [4, 28]. Fig. 5 depicts the reductive effect of *Physalis minima*. Similar to the antioxidant activity, the reducing power of *Physalis minima* increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Physalis minima* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

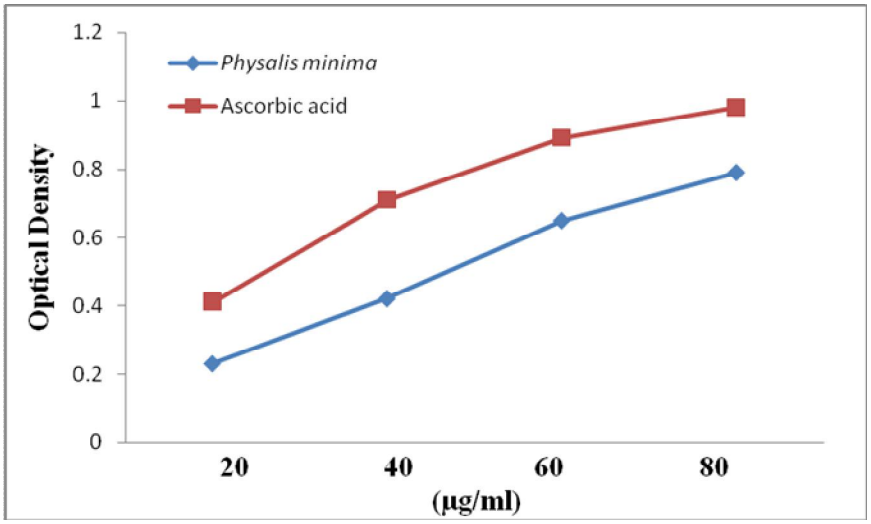

Fig 5: Reducing power assay of *Physalis minima* leaf

4. Conclusion

The results of the present study showed that the extract of *Physalis minima* leaf extract (PMLE) which contains of flavonoids and polyphenols. These phytochemicals are exhibited the greatest antioxidant activity DPPH, nitric oxide scavenging and metal chelator (iron chelator and iron reducing power) which participate in various pathophysiology of diseases including cancer, diabetic, ageing etc. Thus, it can be concluded that PMLE can be used as an accessible source of natural antioxidants with consequent health benefits.

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