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ANTIOXIDANT ACTIVITY OF LEAF, UNRIPE AND RIPE FRUITS OF *LANTANA CAMARA* L

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ABSTRACT

The antioxidant activity of leaf, unripe and ripe fruits of *Lantana camara* L. were determined using H_2O_2 radical scavenging activity and Ferric Reducing antioxidant power (FRAP) assay. Ethanol, methanol, ethyl acetate and water extracts prepared from dry leaf powder and fresh fruits of both unripe and ripe fruits were used to find out the antioxidant activity. All the four extracts of leaf, unripe and ripe fruits showed considerable antioxidant effect. Water extract of powdered leaf showed a maximum activity of 94.81% where as aqueous extract of unripe fruit showed the least of 0.05% in FRAP activity . In H_2O_2 radical scavenging activity water extract of unripe showed the highest activity of 14.53% and methanol, ethyl acetate and water extracts of leaf powder showed the least activity of 3.05%. As the leaf, unripe and ripe fruits of Lantana *camara* have antioxidant potential they can be studied from pharmaceutical and nutraceutical aspects.

KEY WORDS: **Antioxidant activity, FRAP, H_2O_2, Lantana *camara***

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INTRODUCTION

The Verbenaceae species *Lantana camara* L. is a native to tropical and subtropical America and has been dispersed throughout the world as a popular ornamental plant, becoming one of the World's worst weeds[1]. However, it is listed as one of the important medicinal plants of the world and it has been used in many parts of the world to treat a wide variety of disorders[2]. In India the leaves of the plant are boiled for tea and the decoction is a remedy against cough and it is used as a lotion for wounds and Pounded leaves are applied to cuts, ulcers and swellings[3]. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species. They are phytochemicals, vitamins and other nutrients that protect our cells from damage caused by free radicals. The principal agents responsible for the protective effects could be the presence of antioxidant substances that exhibit their effects as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers and metal ion chelators[4] .The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins[5]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [6].

Some of the reactive oxygen species, including hydrogen peroxide, singlet oxygen, hydroxyl and superoxide radicals, have positive roles in energy production *in vivo* systems, phagocytosis, intercellular signal transfer, regulation of cell growth and the synthesis of important biological compounds[7]. Additionally, reactive oxygen species modify DNA and membranes by attacking the lipids, proteins, and carbohydrates in cell membranes and tissues[8]. In the organism, the rates of production and removal of free radicals are in balance, known as oxidative balance. An increase in the rate of production or a decrease in the rate of removal disrupts this balance and increases the levels of reactive oxygen species. This condition, which is called oxidative stress, indicates a serious imbalance between the production of free radicals and the antioxidant defense systems, resulting in tissue damage[9].Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species.

L. camara contains lantadenes, the pentacyclic triterpenes which is reported to possess number of useful biological activities. Several previous reports have described antifungal[10], antiproliferative[11], and antimicrobial activities of *L. camara*[2] including termicidal activities[12] .Ethanolic extract of dried flowers of *Lantana camara* were screened for its protective effect against the Cisplatin-induced kidney damage in rats[13]. The antioxidant activity of methanolic extract of *Lantana camara* has been already studied using DPPH radical scavenging activity and nitric oxide free radical scavenging method[14]. The aim of present study is to determine the percentage of free radical scavenging activities of antioxidants using H_2O_2 radical scavenging and FRAP assay of leaf, unripe and ripe fruits of the plant, *Lantana camara,* in four different extracts viz. ethanol, methanol, ethyl-acetate and water .

MATERIALS AND METHODS

Plant Material

The aerial parts of *Lantana camara* L. were collected in March 2013 in Lawspet, Puducherry and identified at the French Institute, Puducherry for its correct identity. Voucher Specimen was deposited in the Herbarium of the Department of Plant science and Biotechnology, Kanchi Mamunivar centre for Post Graduate Studies, Puducherry.

Preparation of plant extracts

The leaves of the plants were air dried at room temperature for 10 days then powdered using a mixer grinder. Plant extracts were prepared for all the parts of the plant (ethanol, methanol, ethyl-acetate and water) following a standard protocol.

HYDROGEN PEROXIDE SCAVENGING EFFECT

The scavenging activity of hydrogen peroxide by the plant extracts was determined by the method of Ruch et al. (1989)[15].

Principle

The UV light absorption of hydrogen peroxide can be easily measured at 230nm. On scavenging of hydrogen peroxide by the plant extracts, the absorption decreases at this wavelength, which property can be utilized to quantify their H_2O_2 scavenging ability.

Reagents

1. Phosphate buffer (40mM, pH 7.4),
2. H_2O_2 in phosphate buffer (40mM)

Procedure

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Plant extracts at the concentration of 10mg/10µl were added to 0.6ml of H_2O_2 solution. The total volume was made up to 3ml with phosphate buffer. The absorbance of the reaction mixture was recorded at 230nm. The blank solution contained phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging by the plant extracts was calculated as

$$\% \text{ scavenged hydrogen Peroxide} = \frac{A_o - A_1}{A_o} \times 100$$

Where,

A_o - Absorbance of control

A_1 - Absorbance in the presence of plant extract

REDUCING POWER ASSAY –FRAP

The reducing power of different fractions was determined by the method of Oyaizu (1986)[16].

Principle

The reducing power assay was used to assess the reduction potential of fractions as this assay involves the reduction of ferricyanide ion i.e. $[Fe(CN)_6]^{3-}$ to ferrocyanide ion i.e. $[Fe(CN)_6]^{4-}$ by electron donation from polyphenols . The polyphenols are oxidized in this reaction. The ferrocyanide ions combines with Fe (III) in acidic medium to give a Prussian blue complex i.e. ferriferrocyanide complex, $Fe_4[Fe(CN)_6]^3$, the intensity of which is measured spectrophotometrically at 700 nm (Graham, 1992). The intensity of coloured complex increases with the electron or H. donating ability of extract and its different fractions. The redox reaction may be summarized as follows:

$$PP + 2[Fe(CN)_6]^{3-} \longrightarrow PP + 2[Fe(CN)_6]^{4-}$$

Ferricyanide ion — oxidized — Ferrocyanide ion

$$3[Fe(CN)_6]^{4-} + 4Fe^{3+} \longrightarrow Fe_4[Fe(CN)_6]^3$$

Ferric ion — Ferric Ferrocyanide (λ_{max} =700nm) (Prussian Blue)

Procedure

1 ml of extract of different concentrations was mixed with 2.5 ml of phosphate buffer (200mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. A volume of 2.5 ml of 10% TCA was then added to the mixture and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) and the absorbance was measured spectrophotometrically at 700nm. Increase in absorbance of the reaction mixture was interpreted as increase in reducing activity of the extract and the results were compared with Gallic acid that was used as a positive control. The percentage reduction of the sample as compared to standard was calculated using the formula:

$$\text{Reduction (\%)} = 1 - \frac{\text{Absorbance of Gallic Acid} - \text{Absorbance of Sample}}{\text{Absorbance of Gallic Acid}} \times 100$$

Here, absorbance of Gallic acid refers to the absorbance value exhibited by Gallic acid at the highest tested concentration i.e. 200µg / ml.

RESULTS AND DISCUSSION

All the four extracts viz. ethanol, methanol, ethyl-acetate and water of leaf, unripe and ripe fruits of the plant, *Lantana camara* shows antioxidant activity. Percentage of antioxidant activity of different extracts of *Lantana camara* leaf, raw and ripe fruit are given in Table 1 and 2. Percentage of antioxidant activity of different extracts of *Lantana camara* leaf, raw and ripe fruits are given in Fig 1 and 2.

H_2O_2 scavenging activity

The antioxidant activities of H2O2 scavenging ability of ethanol, methanol, ethyl-acetate and water extracts are reported in Table-1 and Fig-1. The antioxidant activity of unripe fruit decreased in the order of water extract (14.53%)> methanol extract (10.83%)> ethyl acetate extract (4.28%)> ethanol extract (2.79%).In ripe fruit the antioxidant activity decreased in the order of ethanol extract (6.81%)> methanol extract (4.67%)> ethyl acetate extract (3.24%)> water extract (2.79%).In the case of leaf powder the order of decrease in the antioxidant activity is ethanol extract (4.54%)> methanol extract , ethyl acetate extract , water extract (3.05%).Water extract of unripe showed the highest activity of 14.53% and water extracts of ripe fruit and ethanol extract of unripe fruit showed the least activity of 2.79%.

Antioxidant activity by using FRAP method

The results of the FRAP assay are reported in Table 2 and Fig.2. All the four extracts of leaf, unripe and ripe fruits of *Lantana camara* showed considerable antioxidant activity . The antioxidant activity of unripe fruit decreased in the order of ethanol extract (56.78%)> ethyl acetate extract (20.16%)> methanol extract (14.94%)> water extract (0.05%).In ripe fruit the antioxidant activity decreased in the order of methanol extract (60.70%)> water extract (53.32%)>ethanol extract (25.92%)> ethyl acetate extract (23.29%).In the case of leaf powder the order of decrease in the antioxidant activity is water extract (94.81%)>ethanol extract (71.22%)> methanol extract (65.59%)> ethyl acetate extract(60.38%) Water extract of powdered leaf showed a maximum activity of 94.81% where as aqueous extract of unripe fruit showed the least of 0.05% in FRAP activity .

Percentage of antioxidant activity of different extracts of Lantana camara leaf, unripe and ripe fruits.

TABLE 1
H_2O_2 scavenging activity

SAMPLE	SOLVENTS			
	ETHANOL	METHANOL	ETHYL ACETATE	WATER
Unripe fruit	2.79	10.83	4.28	14.53
Ripe fruit	6.81	4.67	3.24	2.79
Leaf powder	4.54	3.05	3.05	3.05

TABLE 2
FRAP Method

SAMPLE	SOLVENTS			
	ETHANOL	METHANOL	ETHYL ACETATE	WATER
Unripe fruit	56.78	14.94	20.16	0.05
Ripe fruit	25.92	60.70	23.29	53.32
Leaf powder	71.22	65.59	60.38	94.81

Figure 1
H_2O_2 scavenging activity of various extracts of parts of Lantana camara

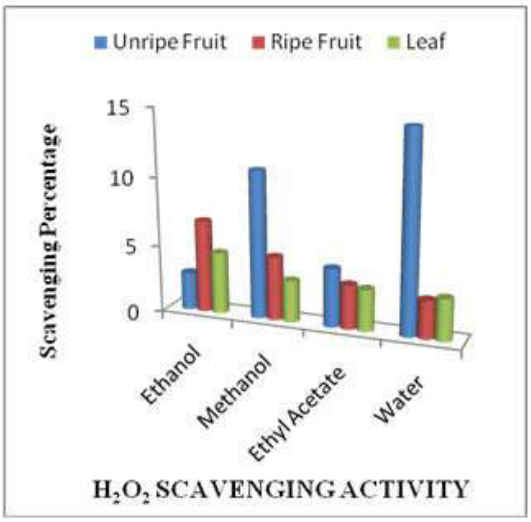
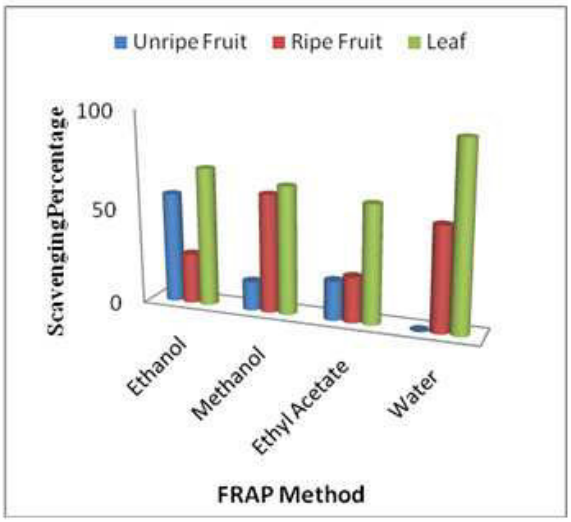


Figure 2
Antioxidant activity by using FRAP method of various parts of Lantana camara in various solvents



Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer[6]. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of food stuffs[17]. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Antioxidants thus play an important role of protecting the human body against damage by reactive oxygen species[18]. Based on the research finding leaf, unripe and ripe fruits of Lantana *camara* can be studied from pharmaceutical and nutraceutical aspects.

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