# COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS IN LEAF AND FRUIT EXTRACTS OF *Lantana camara L.*

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**ABSTRACT**

***Lantana camara L.*is a flowering ornamental plant widely distributed in Kerala. It is a well-known plant in traditional medicinal system. The various phytochemicals like the total Phenols, Flavonoids and other antioxidants vary in their concentration and composition in different plant part. So, the present study aims in the comparative study of Antioxidant activity, total phenolic and flavonoid contents in leaf and fruit extract of *Lantana camara* L. Ethanolic extract of leaves and fruits of *Lantana camara* L. was prepared by solvent extraction method. The preliminary quantitative phytochemical analysis of leaf and fruit extract was done. For quantitative phytochemical screening, Total phenolic content, Total flavonoid content and antioxidant activity of *Lantana camara* L. was estimated. The ethanolic extract of fruits of Lantana camara L. depicted highest total phenolic and total flavonoid content and thus greater antioxidant activity when compared to leaf extract and has a promising application as nutraceuticals for herbal drug formulations.**

**Key words: *Lantana camera*, Total phenol, Total flavonoid, Antioxidant activity**

## INTRODUCTION

Medicinal plants represent an important source of medically important compounds. Since ancient time , medicinal plants are used to cure several types of health problems. Systemic analysis of these plants provides a variety of bioactive molecules for the development of newer pharmaceutical products . Recently, there is a growing interest in the pharmacological evaluation of various plants used in different traditional system of medicine. In last few decades, many of traditionally known plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer activity, anti-inflammatory activity, anti-diabetic activity, Anti-helmintic, antibacterial activity, anti-fungal activity, hepatoprotective activity , antioxidant activity, larvicidal activity etc. *Lantana camara L.* Is a flowering ornamental plant belonging to family Verbenaceae . *L. Camara* is also known as *Lantana*, Wild Sage, Surinam Tea Plant, Spanish flag and West Indian lantana . *L. camara* is a well known medicinal plant in traditional medicinal system and recent scientific studies have emphasized the possible use of the. *L.camara* in modern medicine. [1,2] *L.camera* is a low erect or subscandent vigorous shrub with tetrangular stem, stout re curved pickles and a strong odour of black currents. Plant grows up to 1 to 3 meters and it can spread to 2.5 meter in width. Leaves are ovate or ovate oblong, acute or sub acute, crenate serrate, rugose above, scabrid on both sides. The leaves are 3-8 cm long by 3-6 cm wide and green in colour. Leaves and stem are covered with rough hairs. Small flower held in clusters (called umbels). Colour usually orange, sometime varying from white to red in various shades and the flower usually change colours as they ages. Root system is very strong and it gives out new fresh shoots even after repeated Cuttings.Ghana, infusion of the whole plant is used to cure bronchitis and the powdered root in milk was given to children for stomach-ache and as a vermifuge. *Lantana* oil is used in the treatment of skin, itches, as an antiseptic for wounds. In leprosy and scabies decoctions were applied externally. Phytochemical composition of the *L. camara* has been extensively studied in last few decades. Different parts of *L. camara* are reported to possess essential oils, phenolic compounds, flavonoid, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, quinine, saponins, steroids, triterpens, sesquiterpenoides and tannin as major phytochemical groups. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage . These antioxidants delay or inhibits cellular damage mainly through the free radical scavenging property [3]

## MATERIALS AND METHODS

### Collection of specimen

The plant specimens (leaves and fruits) for the proposed study were collected from plants located at pampady, Thrissur district. Leaves and fruits were collected from plant and were carefully examined and authenticated by Dr.RANJUSHA A.P, Botany Department,NSS COLLEGE OTTAPALAM.

### Preparation of powder.

The leaves and fruits of *lantana camara* was collected and dried under shade. These dried materials were mechanically powdered, sieved using 80 meshes and stored in an air tight container. These powdered materials were used for further studies

**Preparation of extracts**

The shade dried coarse powder of the leaves and fruits of *Lantana camera* (50g) was taken in conical flask and was macerated with 70% ethanol(1000 ml) for 7 days with occasional shaking and filtered. Then the extract was distilled in vacuum in order to remove the solvent completely, dried in a desiccator and calculated the percentage yield. [4]

**Qualitative Phytochemical analysis**

Phytochemical examinations were carried out for all the extracts as per the standard methods. **Detection of alkaloids:**
About 50mg of solvent-free extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrate was tested with various reagents for the detection of alkaloids as follows:
**a) Mayer's Test:** A few ml of the filtrates was treated with one or two drops of Mayer's reagent (Potassium Mercuric Iodide).
**b) Wagner's Test:** A few ml of the filtrates was treated with one or two drops of Wagner's reagent (Iodine in Potassium Iodide).
**c) Dragendroff's Test:** A few ml of the filtrates was treated with one or two drops of Dragendroff's reagent (solution of Potassium Bismuth Iodide).
**d) Hager's Test**: A few ml of the filtrates was treated with one or two drops of Hager's reagent (saturated picric acid solution).
**e) Tannic acid test:** A few ml of the filtrates was treated with one or two drops of freshly prepared tannic acid solution
**Detection of carbohydrates:**
About 100mg of the extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
**a) Molisch's Test:** 2 ml of the filtrates were treated with 2 drops of alcoholic α- naphthol solution in a test tube. The mixture was shaken well, and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand.
**b) Benedict's test**: 0.5ml of the filtrates were treated with 0.5ml of Benedict's reagent and heated on a boiling water bath for 2 minutes.
**c) Seliwanoff's test :** A few ml of the sample solution is treated with seliwanoff's reagent in conc.Hcl and boiled for two minutes.
**d) Fehling's Test:** 1ml of the filtrates were treated with 1ml of each of Fehling's solutions A and B and boiled on a water bath.

**Detection of Proteins and Amino acids**
**(a) Biuret test:** To 2ml the extracts, a few drops of Biuret reagent were added.
**(b) Ninhydrin test:** To 2ml of the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes.
**(c) Millon's test:** To 2 ml of the extracts, a few drops of Millon's reagent were added.
**d) Xanthoproteic Test:** 3 ml of the extracts were treated with 1ml of conc. Nitric acid and Sulphuric acid. Cooled the solution and made alkaline with 10 % sodium hydroxide.
**Detection of Terpenoids**
**(a) Salkowski test:** To the 5 ml of extract 2 ml of chloroform and drops of conc.sulphuric acid is added.
**Detection of Tannins** :
**(a) Ferric Chloride Test:** 50 mg of the extracts were treated with 3-4 drops of 5%ferric chloride solution.
**(b) Gelatin Test**: To 50mg of the extract, 2ml of 1% gelatin solution containing 10% sodium chloride was added.
**Detection of Phenolic compounds** :
**(a) Ferric Chloride Test:** 50 mg of the extracts were treated with 3-4 drops of 5%ferric chloride solution.
**(b) Lead acetate test**: To 50mg the extracts 10% Lead acetate was added.
**Detection of Coumarins**:
**(a) Sodium sulphate test:** Extracts were treated with 3-4 drops of Sodium sulphate solution.
**1.3.2.8. Detection of flavonoid**
**(a) Shinoda Test:** 50mg of the extract was dissolved in alcohol, a few Magnesium turnings were introduced, and a few drops of Hydrochloric acid was added
**(b) Sulphuric acid Test:** Concentrated Sulphuric acid was added to the sample.
**(c) Alkali test:** Added Aqueous solution of Sodium Hydroxide to the Sample.
**Detection of glycosides:**
Extracts were hydrolyzed with dil. Hcl, and then subjected to test for glycosides.
**(a) Saponin-Foam Test:** 50mg of extract was diluted to 20 ml with distilled water and shaken in a graduated cylinder for 15 minutes.
**(b) Anthraquinone Glycosides Modified Borntrager's Test:** 2 ml of the extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution.
**(C) Cardiac Glycosides Keller Killani Test:** 2 ml of the extract were dissolved in mixture of 1 % ferric sulphate solution in 5 % glacial acetic acid. Add one or two drops of Conc.$H_2SO_4$[2]
**Quantitative Estimations**
**Estimation of Total Phenolic Content**
Phenolics include simple phenols, phenolic acids (benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins. These compounds are among the most widely occurring secondary metabolites in the plant kingdom, acting mainly as phytoalexins, attractants for pollinators, contributors to plant pigmentation, antioxidants, and protective agents against UV light, among others .Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry.The reaction of Folin-

Ciocalteu reagent forms a blue chromophore constituted by a phosphotungstic phosphomolybdenum complex where the maximum absorption of the chromophore depends on the alkaline solution and the concentration of phenolic compounds.

**Principle**

The Folin-Ciocalteu reagentor Folin- phenol reagent or Folin- Denis reagent is also called the Gallic Acid Equivalence Method (GAE). Mixture of phosphomolybdate and phosphotungstate is useful for the colorimetric assay of phenolic and poly phenolic antioxidants. It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent. This reagent does not only measure total phenols and will also react with any reducing substance. The reagent therefore measures the total reducing capacity of a sample, not just the level of phenolic compounds.

**Reagents**

Folin- Ciocalteu reagent,7% Sodium carbonate solution

**Procedure**

The total phenolic content (TPC) of dry methanolic extract was performed with **Folin-Ciocalteu assay**. 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu phenol reagent. After 5 minutes, 10 ml of 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23 $^0$C, after which the absorbance was read at 760nm. The total phenolic content was determined from extrapolation-of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out five times. The

TPC was expressed as milligrams of Gallic acid equivalents (GAE)/ g of dried sample. [5, 6, 7]

**Estimation of Total Flavonoid Content**

Flavonoids comprise a large group of secondary plant metabolites. Their function in plants involves screening of UV light, in situ radical scavenging, anti-feeding effects (astringency), etc. Proanthocyanidins mostly occur in green tea (Camelliasinensis), grape seeds and skin (Vitis vinifera), or cacao (Theobromacacao). The distinct occurrence of flavonoids makes them good candidates for taxonomic studies. The antioxidant effect of flavonoids can reside both in their radical-scavenging activity or in their metal chelating properties, of which the former may dominate.

**Principle**

The principle of aluminium chloride colorimetric method is that aluminium chloride forms stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonoid. In addition, aluminium chloride forms acid liable complexes with the orthodihydroxil groups in the A or B-ring of flavonoid.

**Reagents**

Quercetin, methanol, aluminium chloride solution (10%), NaNO2 solution (10%), Na OH solution (1%)

**Procedure**

**Preparation of standard solution**

10mg Quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pipetted out and made up to 10ml with Methanol to get 100mcg/ml of Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 25, 50, 75, 100, 125 and 150 mcg/ml were prepared. To each of these 4ml water was added followed by 0.3ml of 5% sodium nitrite. After 5minutes, 0.3ml of 10 % Aluminium chloride solution and at the 6$^{th}$ minute, 2ml of 1M Sodium hydroxide was added. The total volume was made up to 10ml with distilled water. A blank was prepared without addition of aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 510nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

**Preparation of sample solution**

The total flavonoids content of each plant extract was estimated by method described by Zhishen et al. Based on this method, each sample (1.0 ml) was mixed with 4 ml of distilled water and subsequently with 0.30ml of NaNO2 solution (10%). After 5 minutes, 0.30ml AlCl3 solution (10%) was added followed by 2.0ml of NaOH solution (1%) to the mixture. Immediately the mixture was thoroughly mixed and absorbance was then determined at 510 nm versus blank. Standard curve of quercetin was prepared (0- 12 mg/ml) and the results were expressed as quercetin equivalents (mg quercetin/ g dried extract).The estimation of flavonoid content was carried out five times and the final results were calculated. **[**5,6,7]

***In vitro* Pharmacological Activities**

**Antioxidant Activity**

**DPPH assay (2, 2-diphenyl -1-picrylhydrazyl)**

The radical scavenging activity of ethanolic extract of leaves of *lantana camara*. was determined by using DPPH assay.The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

**Principle**

1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

DPPH + [H-A] →DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as a consequence, the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

**Reagent Preparation**
0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of methanol.

**Procedure**
0.1 mM solution of DPPH in methanol was prepared and it was protected from light by maintaining the dark condition and also by covering the test tubes with aluminium foil. 3ml of this solution was added to 1 ml various concentrations (100- 500 µg/ ml) of extracts and standard solution of ascorbic acid (25-500µg/ ml). Absorbance was taken after 30 minutes at 510 nm. The percentage inhibition activity was calculated. The procedure was repeated five times and the final results were calculated.
**Calculation**
**Percentage inhibition = (OD of control-OD of test/OD of control) X 100**
Where OD – optical density
**Statistical analysis**
Statistical analysis was done by using one way ANOVA followed by Dunnets test. P values lesser than 0.05 were considered as significant. [7,8,9]

**RESULT**
**Extraction by Maceration**
Extraction of the powdered leaves&fruits of *Lantana camara*. were done by Maceration method by using 70 % ethanol and the percentage yield of the extracts obtained were tabulated in Table 1.
**Table 1: Colour, nature and percentage yield of leaf and fruit extracts of *Lantana camara***

**Table 2:**

| Sl.no | Extracts | Colour | | Nature | | Percentage yield(%w/w) | |
|---|---|---|---|---|---|---|---|
| | | Leaf | Fruit | leaf | fruit | Leaf | Fruit |
| 1 | 70 % Ethanol | Brownish green | Brownish black | sticky | crystalline | 5.2 | 2.3 |

**Phytochemical Screening of *Lantana camara* by Maceration**

| Sl No | Qualitative test | 70%ethanol extract of leaf. | 70%ethanol extract of fruit. |
|---|---|---|---|
| **1.TEST FOR ALKALOIDS** | | | |
| **A.** | Mayer's test | - | - |
| **B.** | Wagner's test | - | - |
| **C.** | Dragendroff's test | - | - |
| **D.** | Hager's test | - | - |
| **E.** | Tannic acid test | - | - |
| **2.TEST FOR CARBOHYDRATES** | | | |
| **A.** | Molisch's test | - | + |
| **B.** | Benedict's test | - | + |
| **C.** | Fehling's test | - | + |
| **3.TEST FOR PROTEINS AND AMINOACIDS** | | | |
| **A.** | Biuret test | - | - |
| **B.** | Ninhydrin test | - | - |
| **C.** | Millon's test | - | - |
| **D.** | Xanthoproteic test | - | - |
| **4.TEST FOR PHYTOSTEROLS** | | | |
| **A.** | Salkowski test | - | + |
| **5.TEST FOR TANNINS** | | | |

| | | | |
|---|---|---|---|
| **A.** | Braymer's test | + | + |
| **B.** | Gelatin test | + | + |
| **6.TEST FOR PHENOLS** | | | |
| **A.** | Ferric chloride test | + | + |
| **7.TEST FOR FLAVONOIDS** | | | |
| **A.** | Alkali test | + | + |
| **B.** | Shinoda test | + | + |
| **8.TEST FOR SAPONINS** | | | |
| **A.** | Foam test | + | - |
| **B.** | Froth test | + | - |
| **9.TEST FOR GLYCOSIDES** | | | |
| **A.** | Liebermann's test | + | + |
| **B.** | Salkowski's test | + | + |

**Quantitative Estimations**

**Estimation of Total Phenolic Content**

Quantitative estimation of 70 % ethanolic extract of leaves & fruits of *Lantana camara* were carried out to determine the amount of total phenolic content. Total phenolic content was determined by Folin-Ciocalteau method. Gallic acid was used as standard and the total phenolic content was expressed as Gallic acid equivalent. Absorbance was measured at 760nm. The results obtained were as follows.

**Table 3 : Absorbance of standard Gallic acid and sample at 760nm**

| Drug | Concentration (µg/ml) | Absorbance |
|---|---|---|
| Gallic acid [standard] | 100 | 0.7214± 0.0005 |
| | 200 | 1.4435 ± 0.0002 |
| | 300 | 1.9913± 0.0005 |
| | 400 | 2.392 ± 0.0015 |
| | 500 | 2.7873± 0.0002 |
| Ethanolic extract of leaf | 100 | 0.5981± 0.0004 |
| Ethanolic extract of fruit | 100 | 0.6125± 0.0002 |

Fig 1 : Standard curve of Gallic acid

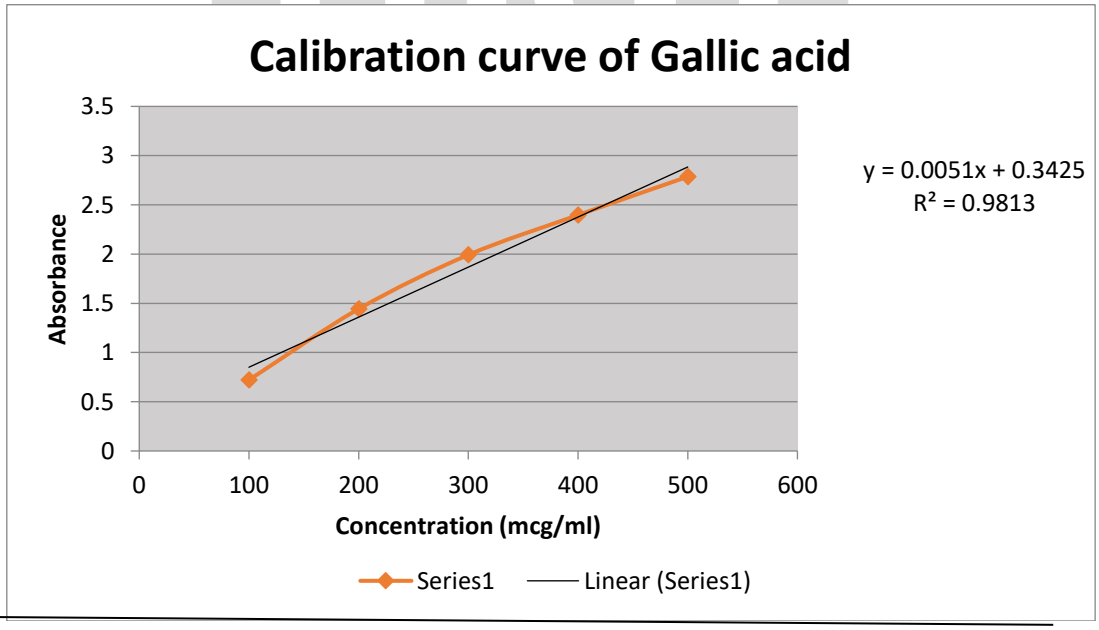



**Table 4 : Total Phenolic Content of ethanolic extract of *Lantana camara*.**

| Sample *Lantana camara* | Total phenolic content GAE mcg/ml |
|---|---|
| Ethanolic extract of leaf | 50.11± 0.015 |
| Ethanolic extract of fruit | 52.94± 0.030 |

**Estimation of Total Flavonoid Content**

Estimation of Total Flavonoids was carried out by aluminium chloride colorimetric method. The absorbance values obtained for different concentration of the standard quercetin are tabulated in table and standard graph is given below.

**Table 5: Absorbance of quercetin and ethanolic extract**

| Sl No | Concentration of quercetin(µg/ml) | Mean absorbance |
|---|---|---|
| 1 | 25 | 0.294 ± 0.0012 |
| 2 | 50 | 0.435 ± 0.0012 |
| 3 | 75 | 0.596 ± 0.0012 |
| 4 | 100 | 0.778 ± 0.0012 |
| 5 | 125 | 0.909 ± 0.0021 |
| 6 | 150 | 1.166 ± 0.0012 |
| 7 | *Lantana camara* Ethanolic extract of leaf(100µg/ml) | 0.1456.±0.0025 |
| 8 | *Lantana camara* Ethanolic extract of fruit(100µg/ml) | 0.2459±0.0025 |

**Fig 2 : Standard curve of quercetin**

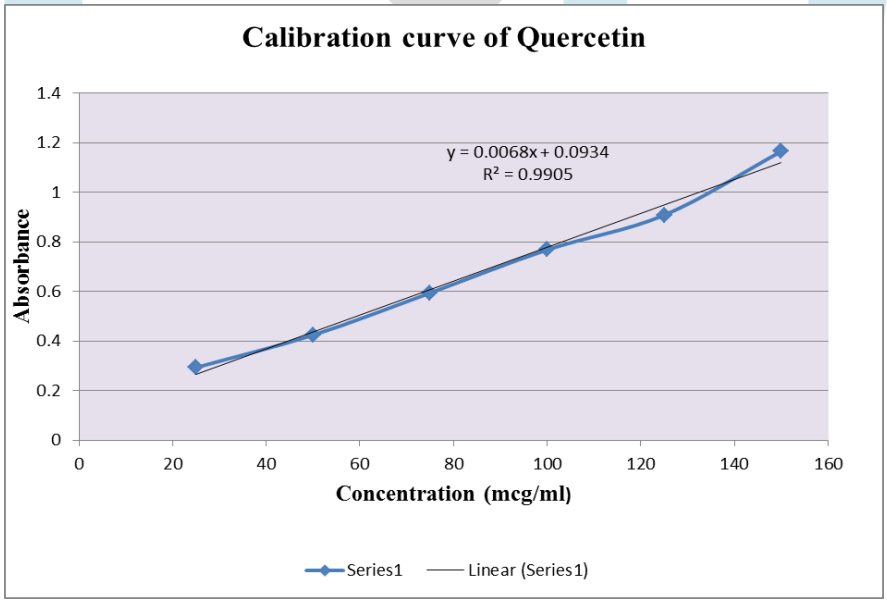



**Table 6 : Total Flavonoid Content of ethanolic extract of *Lantana camara*.**

| Extracts100 µg/ml | Flavonoid content quercetin equivalent mcg/ml |
|---|---|
| *Lantana camara* Ethanolic extract Of leaf | 7.67±0.0088 |
| *Lantana camara* Ethanolic extract of fruit | 22.42±0.0052 |

**Antioxidant Activity**

**DPPH assay (2, 2-diphenyl -1-picrylhydrazyl)**

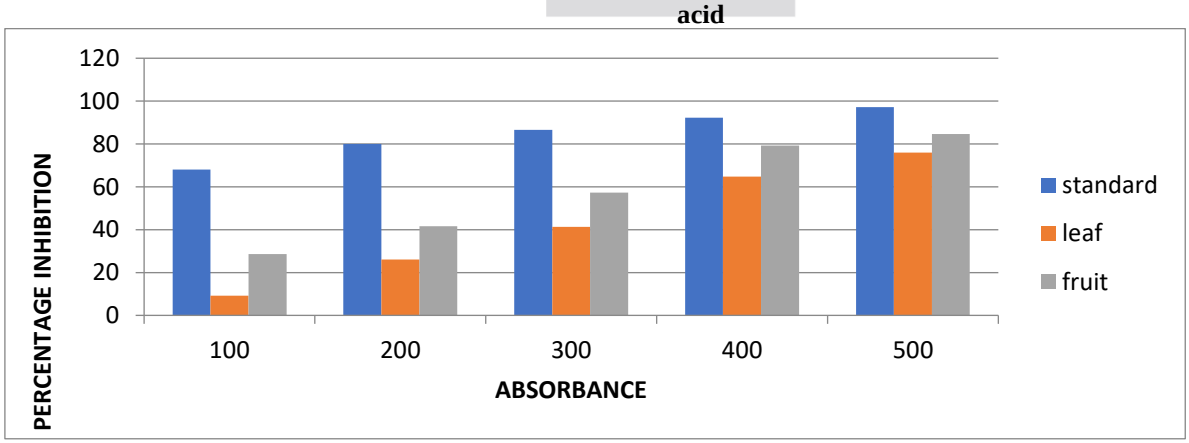
The percentage inhibition obtained in the different concentrations of ethanolic extract was compared to the percentage inhibition obtained with the standard and it is depicted below.

**Table 7 : Percentage inhibition of Ascorbic acid and ethanolic extracts of leaves and fruits of *Lantana camara* L.**

| Sl.No | Sample | Concentration µg/ml | Mean Absorbance | Percentage Inhibition |
|---|---|---|---|---|
| 1 | Control | - | 0.6185±0.0002** | - |
| 2 | Standard Ascorbic Acid | 100<br>200<br>300<br>400<br>500 | 0.1980±0.0001**<br>0.1238±0.0001**<br>0.0833±0.0001**<br>0.0478±0.0002**<br>0.0176±0.0001** | 67.98<br>79.97<br>86.53<br>92.26<br>97.14 |
| 3 | Ethanolic extract of leaf | 100<br>200<br>300<br>400<br>500 | 0.5472±0.0002**<br>0.4574±0.0001**<br>0.3631±0.0002**<br>0.2178±0.0002**<br>0.1491±0.0003** | 9.16<br>26.04<br>41.29<br>64.78<br>75.89 |
| 4. | Ethanolic extract of fruit | 100<br>200<br>300<br>400<br>500 | 0.4413±0.0002**<br>0.3612±0.0001**<br>0.2645±0.0004**<br>0.1287±0.0002**<br>0.0947±0.0003** | 28.64<br>41.6<br>57.23<br>79.19<br>84.68 |

**Fig 3**

**:Comparison of percentage inhibition of ethanolic extract of leaves and fruits of *Lantana camara* with standard ascorbic acid**



**CONCLUSION**

n the present investigation it was observed that different plant parts of Helicteres isora L. varied in their phytochemical constituents. The high phenolic content was positively correlated with free radical scavenging activity of the extracts. These results were supported by quantitative assays as well as phenolic profiling of extracts using RP-HPLC. Although aqueous extract of dry root showed maximum phenolic content and antioxidant potential as observed through TPC and FRAP assay, DPPH radical scavenging activity was exceptionally high in methan the present investigation it was observed that different plant parts of Helicteres isora L. varied in their phytochemical constituents. The high phenolic content was positively correlated with free radical scavenging activity of the extracts. These results were supported by quantitative assays as well as phenolic profiling of extracts using RP-HPLC. Although aqueous extract of dry root showed maximum phenolic content and antioxidant potential as observed through TPC and FRAP assay, DPPH radical scavenging activity was exceptionally high in meth an the present investigation it was observed that different plant parts of Helicteres isora L. varied in their phytochemical constituents. The high phenolic content was positively correlated with free radical scavenging activity of the extracts. These results were supported by quantitative assays as well as phenolic profiling of extracts using RP-HPLC. Although aqueous extract of dry root showed maximum phenolic content and antioxidant potential as observed through TPC and FRAP assay, DPPH radical scavenging activity was exceptionally high in metha In the present investigation it was observed that the 70 % methanolic leaf and fruit extract of *Lantana camera* vary in their phytochemical constituents. Fruit extract seems to response for more phytoconstituents when compared to leaf extract. High phenolic and flavonoid content was observed in fruit extract than leaf extract and a potential antioxidant activity was observed with the fruit extract compared to leaf extract. This study demonstrated the potency of 70% methonlic fruit extract of *Lantana camara* as significant source of phytoconstituent and antioxidant supplements, indicating their strong potential to be used as nutraceuticals in herbal formulations.

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