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# Phytochemical and Biochemical Screening of Lantana Camara L. A Major Invasive Species

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**Abstract**

Plants and plant extracts have an important role in modern medicine as their chemical and medicinal constituents are found in natural form. In the present study, the phytochemical screening of the powder of the leaf of *Lantana Camara* L. methanolic extract was performed. The results of qualitative phytochemical screening of methanolic extract of *Lantana camara* root, stem and leaves revealed that the presence of various bioactive secondary metabolites constituents in alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, amino acids, steroids, terpenoids and protein were respectively. The quantitative phytochemical analysis of methanolic extract of *Lantana camara* root, stem and leaves showed the maximum concentration of carbohydrates, followed by phenols, starch, proteins, total chlorophyll and amino acids. Hence, *Lantana camara* extracts are used for discovering and analysis of secondary metabolites which are precisely helpful for the manufacturing of new drugs for the treatment of various diseases.

**Keywords:** *Lantana camara* L., phytochemical and biochemical screening, Root, stem and leaves.

## Introduction

Medicinal plants have been used as sources of medicine since the time immemorial by indigenous communities in different parts of the world (Pandita *et al.*, 2021). The use of traditional herbal medicine and phytotherapy remains popular, despite the increasing growth and development of the pharmaceutical industry. The World Health Organisation (WHO) reported that approximately 80% of the populations of developing countries rely upon traditional medicine for primary health care (Khan *et al*., 2019). India is endowed with a rich wealth of medicinal plants, being perhaps the largest producer and rightly acclaimed as the Botanical garden of the world (Ingole, 2019). It has a strong base of many systems of medicines including Ayurveda, Yunani, Siddha and other health practices (Raveendran and Bazzul, 2021). The Indian subcontinent is being inhabited by over 54 million tribal people dwelling in about 5000 forest dominated villages spreading across the country comprising 15% of the total geographical area (Singh and Kumar, 2019). Due to constant association with forest, ethnic groups have immense plantlore which they inherit and pass on from generation to generation just through oral conservation (Gebeyehu, 2020).

Over the last many decades, a number of invasive species have been introduced in India from their native areas either accidentally or deliberately as fodder crops or ornamentals (Johnson *et al*., 2020). Alien plants have various effects on the environment and economy of non-native areas, many of the exotic plants are of economic benefit and some have severe negative impacts (Rai *et al*., 2020). Some alien species, often cultivated, may provide food, medicine, fuel, or fodder to local communities (Makhlouf, 2019). Several studies also show the importance of

exotic plants in traditional medicine system (Astutik *et al*., 2016). A comprehensive study by yossoufou *et al* (2021), which focused on exotic species in the medicinal floras revealed a remarkable discovery. They found that of the 2401 North American taxa that are used medicinally by native Americans, 620 are exotics. Furthermore, of 1178 species declared as weed or exotics Rıos and Garcıa (1998) in the Highland Maya of Chiapas, 35 are used by traditional healers as medicine. In India, research has been done to identify the invasive species and their impact on ecosystem and biodiversity (Mungi *et al*., 2019), Therefore, the present study aimed to document the ethnobotanical uses of the invasive alien plants. This paper further attempted to explore presence of phytochemical and biochemical compounds and to study the importance of the invasive plant species in the traditional medicinal system.

# Material and Methods

### Collection and preparation of extract:

*Lantana Camara* L. root, stem and leaves were collected in its natural habitat from M. L. B. college, Bhopal (M.P.). 100 g of *Lantana camara* root, stem and leaf powder were extracted with 100 ml of methanol using Soxhlet apparatus. Extraction was considered to be completed when the root, stem and leaf material became exhausted of their constituents that were confirmed. The filtrates obtained are dried at temperature of 40 ±2°C to have gummy concentrate of the crude extract. The extracts were kept in suitable containers with proper labeling and then stored in cold and dry place for further use.

### Preliminary phytochemical investigations

Preliminary phytochemicals was determined by following the standard protocols of Harborne (1984) and Ajaiyeoba (2000).

### Test for carbohydrate:

**Molisch test:** Few drops of α-naphthol solution in alcohol were added to 2-3 ml aqueous extract. It was shaken and conc. H2SO4 was added from the side of test tube. Violet ring at junction of two liquids appeared, confirms the presence of carbohydrates.

**Fehling s solution:** 1 ml of extract was mixed with 1 ml of Fehling-A and 1 ml of Fehling-B. The mixture was heated in water bath for 5 minutes. Appearance of green yellow or red colour indicates the presence of carbohydrates in test solution.

### Test for protein:

**Biuret test:** 3 ml. test solution + 4% NaOH and few drops of 1 % CaSO4 solution. Solution appeared violet in colour indicates presence of proteins

**Millon's test:** 2 ml. solution and Millon's Reagent were mixed properly. It gave brick red colour.

### Test for tannins and phenolic compounds:

**Lead acetate Test:** 2-3 ml. of aqueous extract was added to few drops of Lead acetate White ppt. appeared and it determined the presence of phenolic compounds.

### Test for amino acid:

**Ninhydrin test:** Three drops of 5% ninhydrin were added to 3 ml test solution and heated for 10 minutes in boiling water bath. Purple or bluish colour indicated the presence of amino acids.

### Test for steroid:

**Salkowski reaction:** 2 ml. of extract solution was added to 2 ml of chloroform and 2 ml. of conc. H2SO4 and shaken well. Chloroform layer appeared red in colour and acid layer appeared greenish yellow fluorescence indicated the presence of steroids.

### Test for flavonoids:

**Lead acetate test:** 2 ml of test solution was added to small quantity of reduced lead acetate solution (10%). Solution formed yellow colored ppt. Addition of increasing amount of sodium hydroxide to the residue. Presence of flavonoids is indicated by Yellow colour of solution decolorization of yellow-colored solution after addition of acids.

### Test for alkaloids:

**Mayer's Test**: Few drops Mayer's reagent was added to 2- 3 ml. filtrate extract solution. Formation of ppt. indicated the presence of alkaloids.

**Wagner's test:** Take 2-3 ml. filtrate extract solution with few drops Wagner's reagent. Solution formed reddish brown ppt. indicated the presence of alkaloids.

### Test for organic acid:

Few drops of 5% lead acetate were added to 2 ml. test solution. Formation of white ppt indicated presence of organic acids.

**Test for sulphate:** With lead acetate reagent gives white ppt. that is soluble in NaOH solution, confirms the presence of sulphates in solution.

### Test for chloride:

5-7 ml filtrate plant extract solution was added to 3-5 ml. lead acetate solution. White ppt. heat soluble in hot water was observed indicating the presence of chloride.

### Test for nitrate:

Plant extract solution with solution of ferrous sulphate yield no brown colour but when sulfuric acid was added brown colour was produced at the junction of two liquids, indicated the presence of nitrates.

### Test for starch:

**Iodine test:** 3 ml test solution was mixed with few drops of iodine solution; blue black colour indicated the presence of starch.

### Test for triterpenoids:

2 ml test solution was added to few drops of H2SO4.Then shaken well and allowed to stand. Golden yellow layer appeared at bottom indicated the presence of triterpenoids.

## Biochemical estimation of Root, stem and leaf extracts of *Lantana camara* L.

### Carbohydrate estimation

Total carbohydrate content in root, stem and leaf extracts of *Lantana camara* was estimated by Dubois *et* al., method, (1956).

**Chemicals required**: D-glucose as standard, Phenol, conc.H2SO4, Distilled water.
Sulphuric acid used: 2.5 ml (concentrated), Phenol was taken: 0.5%.

Standard Glucose: Stock 1 mg/ml –. Working standard – 0.3 g in 30 ml of distilled water.
Sample: 1 g was dissolved in 10 ml distilled water. Blank was prepared with distilled water.
Absorbance: 490 nm.

**Procedure**

100 mg of weighed sample was taken in a boiling tube, hydrolyzed and kept in water bath for 3 hours with 5 ml of 2.5 N-HCl and cooled at room temperature. It was neutralized with solid sodium carbonate until the effervescence ceases. The volume was made up to 100 ml and centrifuged. After centrifugation, supernatant was collected. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard.'0' served as blank. The final volume 10 ml was made by addition of distilled water in all the tubes including the sample tubes.0.5 ml of phenol solution and 2.5 ml of conc.H2SO4 was added to each test tube and the reaction mixture was allowed to stand for incubation period of 30 minutes in dark. The absorbance of different dilutions was determined by spectrophotometer to a wave length of 490 nm. A graph was plotted by taking concentration on X-axis and absorbance on Y- axis.

**Total phenolic content**

Total phenolic content in root, stem and leaf extracts of *Lantana camara* was determined by the method described by Singleton and Rossi (1965).

**Requirements:** Tannic acid as standard, Sodium bicarbonate, phenol-Folin reagent.
35% Sodium bicarbonate solution used: 1.75 g in 1 ml distilled water. Phenol-Folin reagent: 1 ml in 9 ml distilled water. Working standard: 1 ml of tannic acid in 10 ml of distilled water Absorbance: 760 nm.

**Procedure**

1.0 ml of each sample was mixed with 1.0 ml of Folin and Ciocalteu's Phenol reagent. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard.'0' served as blank. The final volume of 10 ml was made by addition of distilled water in all the test tubes including the sample tubes. After 3 min, 5.0 ml of saturated Na2CO3 (5 %) was added to all the test tubes. Tannic acid was used as a standard. The test tubes were kept in the dark for 90 minutes and observed under UV-Vis spectrophotometer at 760 nm absorbance. The results were expressed as mg of Tannic acid equivalents/g of extract and same procedure was done with extracts of both plants.

**Total protein content**

Protein estimation in the root, stem and leaf extracts of *Lantana camara* was done by Lowry *et al.,* method, (1951).

**Preparation**

Solution A was prepared by dissolving 2% Sodium bicarbonate in 50 ml Sodium hydroxide.
Solution B was prepared with 0.5% CuSO4 in 1% Potassium sodium tartarate.
Solution C was prepared by dissolving 49 ml solution A in 1 ml solution B.

Solution D was Phenol-Folin Ciocalteu's reagent in a ratio (v/v), 5 ml of Phenol: 5 ml distilled water.
Standard: Bovine Serum Albumin 1mg/ml. Absorbance: 640 nm - 660 nm.
These solutions were prepared freshly.

**Procedure**

BSA (0.2 ml) as working standard was added to all test tubes of extract samples of both plants and volume was made up to 1 ml using distilled water. The test tube with 1 ml distilled water served as blank. Solution C (0.5 ml) was added to all the test tubes and incubated for 5 minutes in dark. After incubation, 0.5 ml of solution D was added and incubated for 15 minutes. Absorbance was measured at 640 nm- 660 nm and standard graph was plotted. The amount of proteins present in the given samples from the standard graph was estimated with Bovine serum albumin as standard.

## Estimation of amino-acids:

Total free amino acids are known as ninhydrin positive substances in tissues has been estimated in *Lantana camara* (root, stem and leaf extracts) by the method of Moore and Stein, (1954).
Requirements: Ninhydrin solution, ethanol, distilled water.
Standard: Bovine serum albumin.
Absorbance: 570 nm.

## Procedure:

2 g of samples were added to 10% Bovine Serum Albumin solution and were centrifuged at 1000 rpm for 15 minutes. From that residue, 0.5 ml of supernatant was taken and 1 ml of ninhydrin was added to all test tubes. These test tubes were kept in water bath for 5 minutes. After incubation, the test tubes were cooled down immediately. The final volume of 10 ml was made by addition of distilled water in all the test tubes including the sample tubes. Blank was made by 0.5 ml of BSA and 1 ml of ninhydrin reagent. the intensity of purple colour against the reagent blank in a colorimeter at 570 nm was noted. A standard curve using absorbance *vs* concentration was obtained from which the total free amino acid concentration in all samples of both plants were estimated.

## Total starch content

The total starch content in the root, stem and leaf extracts of *Lantana camara* was done by the method of Jensen, (1962).

**Chemicals required**: iodine, potassium iodide and distilled water.
Standard: Starch; 1mg/ml.

## Procedure

2 g potassium iodide was added to 20 ml. of distilled water.2 g of iodine was also added to 20 ml distilled water. It was heated at 50°c gently with constant mixing until iodine gets dissolved completely. Starch was taken as a standard. It was diluted to 100 ml. From that residue 1ml. solution was added to all the test tubes including all root, stem and leaf samples of both plants. Absorbance was taken at 660 nm.

## Estimation of chlorophyll and carotenoid content

The total chlorophyll and carotenoid content in the leaves of *Lantana camara* was estimated by the Arnon method (1949). 200 mg leaf sample was ground in pre-chilled mortar and pestle and to this 1 mole HCl and 80% pre-chilled acetone was added. It was centrifuged at 5000 rpm for 5minutes. The supernatant was transferred to a 20 ml volumetric flask. The volume of sample was 20ml with 80% acetone and the absorbance of solution was recorded at 645nm for chlorophyll a, 663 nm for chlorophyll b and 470 nm for carotenoid against the solvent (80% acetone) blank, using UV visible spectrophotometer. The amount of chlorophyll present in the extract mg chlorophyll per gm was calculated by the following formula as µg/ml of extract.
Chlorophyll a (mg/g) = (12.7* O.D. 663) – (2.69*O.D. 645) *V/1000* W.
Chlorophyll b (mg/g) = (22.9* O.D. 645) – (4.68* O.D. 663) * V/1000*W.
Total chlorophyll = a + b
Total Carotenoid (mg/gm) = (1000*O.D. 470) – 2.27 (Chl. a) – 81.4 (Chl. b) / 227

Where; V= Total volume of extract.
W = Weight of leaves in grams.

# Results and Discussion

## Qualitative phytochemical analysis of methanolic extracts of Root, stem and leaf extracts of *Lantana camara*

Plants are made up of secondary metabolites which are formed as products of primary metabolism and produced for defence against predators (Divekar *et al*., 2022). Examples of such metabolites are tannins, flavonoids and alkaloids; they are known to be the brain behind the healing potentials of plants (Khuntia *et al*., 2022). Secondary metabolites are generally not important for the growth and reproduction of organisms, but they play an important role in pharmaceutical field (Bachetti *et al*.,2022). Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development (Maitra *et al*., 2022).
In the present study, qualitative phytochemical tests were performed for detection of common secondary metabolites in methanolic root, stem and leaf extracts of *Lantana camara* The primary phytochemical screening of the plant confirmed presence of carbohydrates, proteins, phenols, starch, amino acids, alkaloids, flavonoids, triterpenoids, steroids, essential oils, chloride, nitrate, inorganic and organic acids in the methanol extracts. Results of the phytochemical analysis are presented in **Table 1:**

**Table 1: Preliminary phytochemical analysis of methanolic root, stem and leaf extracts of *Lantana Camara* L**

| S.No. | Name of the phytochemical test | Root extract | Stem extract | Leaf extract |
|---|---|---|---|---|
| 1. | Carbohydrate | Positive | Positive | Positive |
| 2. | Proteins | Positive | Positive | Positive |
| 3. | Phenols | Positive | Positive | Positive |
| 4. | Starch | Positive | Positive | Positive |
| 5. | Amino acids | Positive | Positive | Positive |
| 6. | Alkaloids | Positive | Positive | Negative |
| 7. | Flavonoids | Negative | Positive | Positive |
| 8. | Triterpenoids | Negative | Negative | Positive |
| 9. | Steroids | Negative | Positive | Negative |
| 10. | E. oils | Negative | Negative | Positive |
| 11. | Chloride | Positive | Positive | Negative |
| 12. | Nitrate | Negative | Negative | Negative |
| 13. | Inorganic acids | Positive | Negative | Negative |
| 14. | Organic acids | Negative | Positive | Negative |

The above mentioned (**Table 1)** confirms the presence and absence of various secondary metabolites dissolved in methanol extracts of root, stem and leaf extracts of *Lantana camara* it was revealed that carbohydrates, proteins, phenols, starch and amino acids were present in all extract samples. Alkaloids and chloride were present in root and stem extracts. Flavonoids were present in stem and leaf extract. Triterpenoids and essential oils were present in leaf extract. Steroids and organic acids were present in stem extract. Inorganic acids were present only in root extract sample.

## Quantitative biochemical analysis of methanolic root, stem, and leaf extracts of *Lantana camara* L.

The quantitative analysis of root, stem and leaf extracts of *Lantana camara* are described below as under:

### Total carbohydrate content

Total carbohydrate content was found in root, stem and leaf extracts calculated with standard curve of D-glucose. Concentration of D-glucose was observed at absorbance of 490 nm at variable range of concentration from 20-100 µg/ml to establish standard value as depicted in Table 2; Fig.1. Compared to standard value concentration of total carbohydrates was established in stem, root and leaf, and it was found to be highest in root extract 91.66 mg/g followed by leaf extract 91.036 mg/g and stem extract 90.537mg/g samples of *Lantana camara* L. (Fig.2).

**Table 2: Absorbance of D-glucose at concentration ranges from 20 -100 µg/ml**

| Concentration µg/ml | Absorbance at 490 nm |
|---|---|
| 20 | 0.375 |
| 40 | 0.728 |
| 60 | 1.197 |
| 80 | 1.434 |
| 100 | 1.554 |

**Figure 1: Standard curve of D-glucose at concentration ranges from 20 -100 µg/ml**

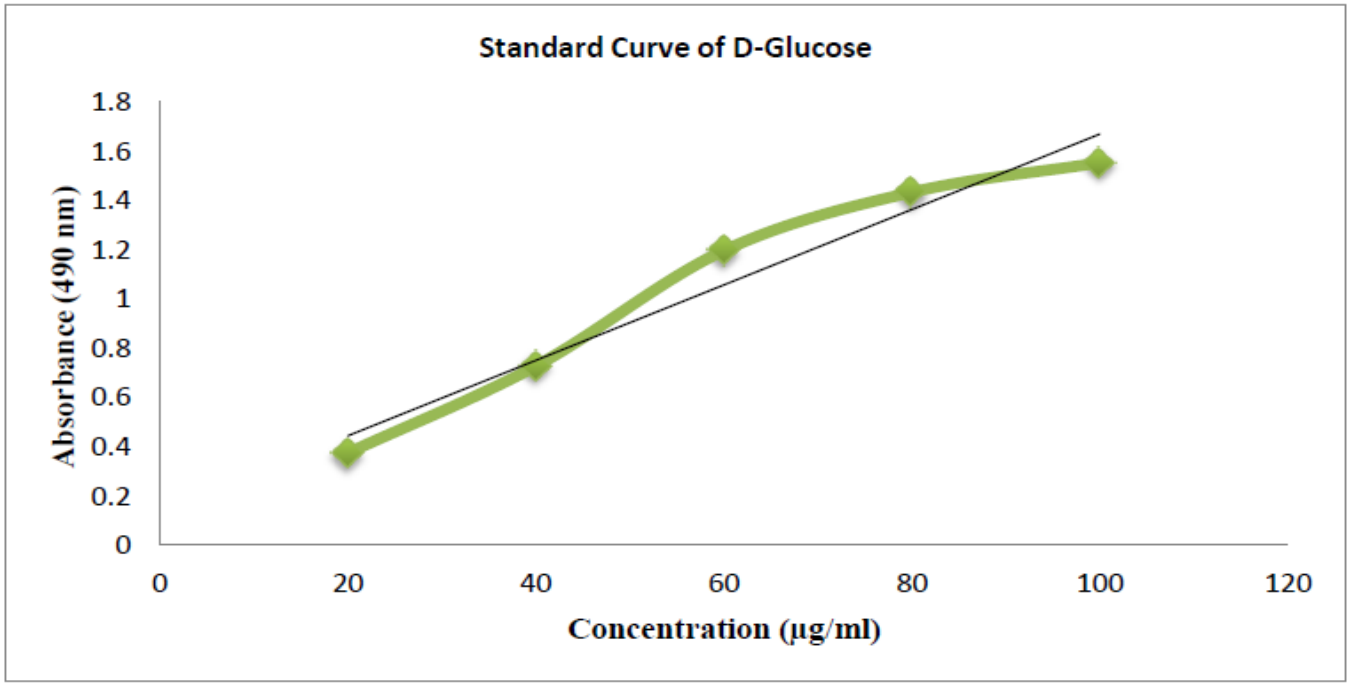
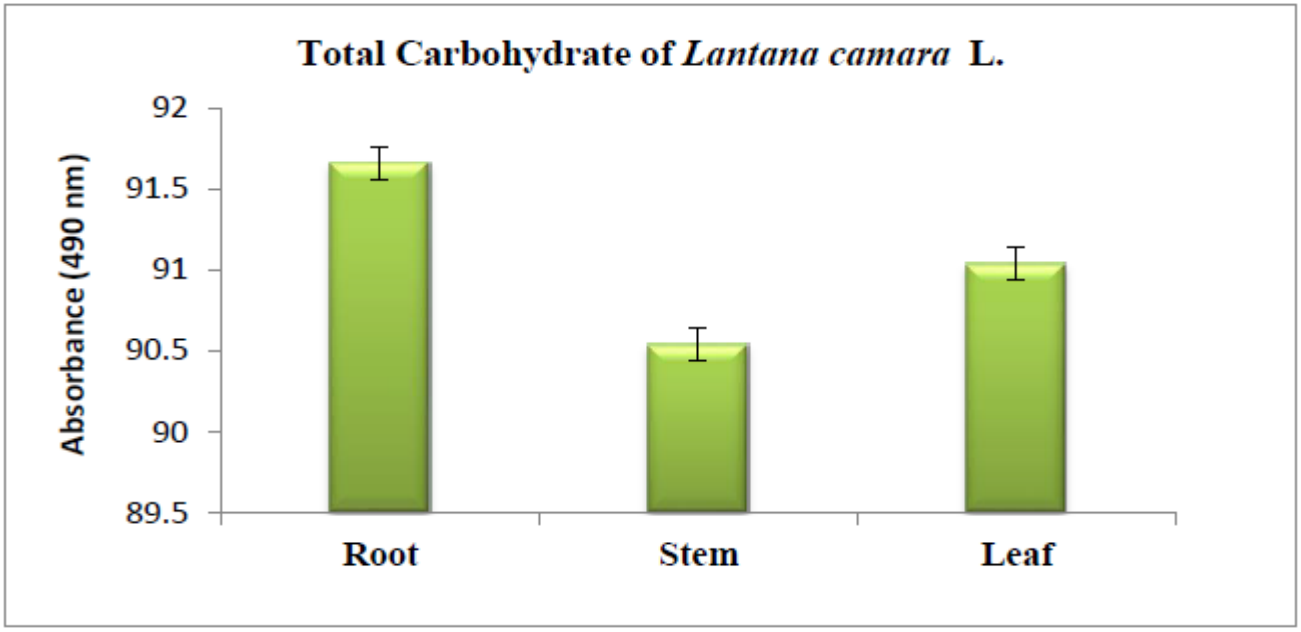



**Figure 2: Determination of Total Carbohydrate Content in root, stem and leaf extracts of *Lantana camara***

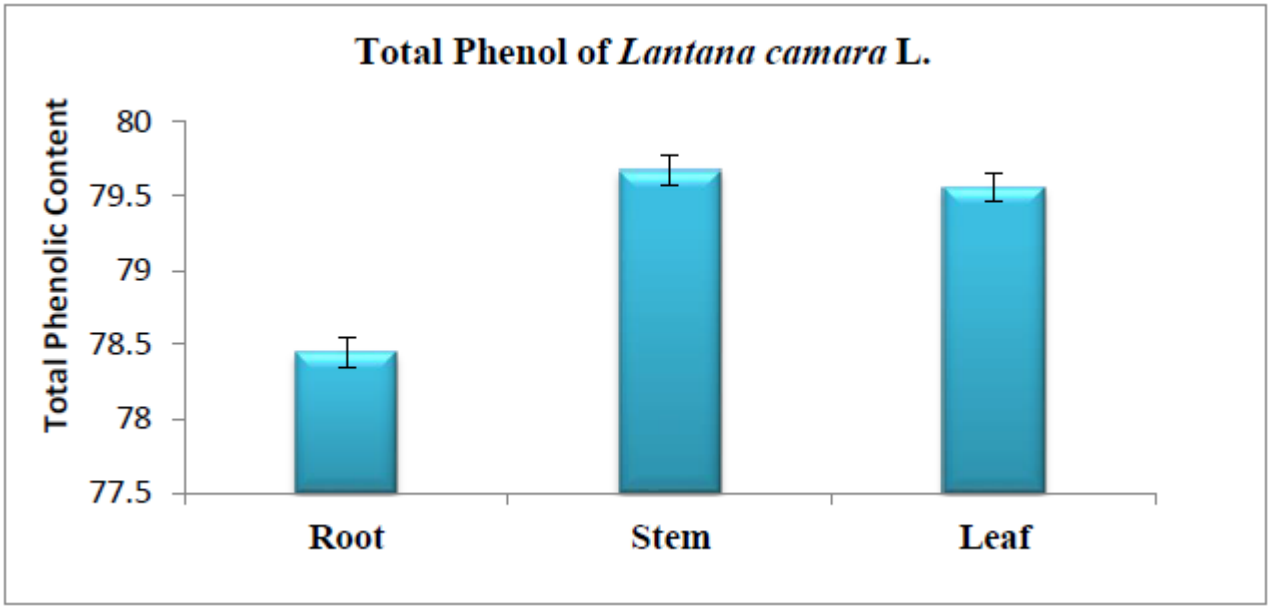



## Total Phenolic Content

Phenolic content was found in root, stem and leaf extract samples of *Lantana camara* calculated with standard curve of Tannic acid. Concentration of Tannic acid was observed at absorbance of 760 nm at variable range of

concentration from 20-100 µg/ml to establish standard value. Compared to standard value total phenolic content was established in stem, leaf and root. The value was found to be highest in stem extract 79.673 mg/g followed by leaf extract 79.558 mg/g. whereas root extract sample was found to be 78.448 mg/g respectively (Fig.3).

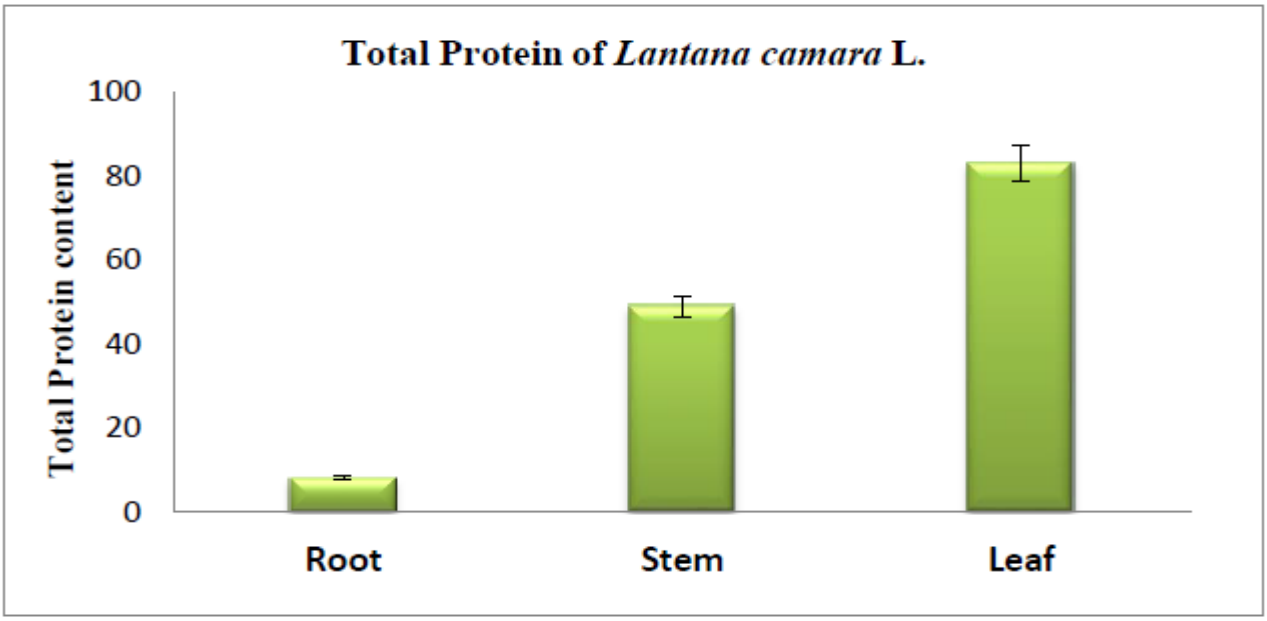
**Figure 3: Determination of Total Phenolic Content in root, stem and leaf extracts of Lantana camara**




## Total Protein content

Fig.4 represents the total protein content found in root, stem and leaf extract samples of *Lantana camara* calculated with standard curve of Bovine Serum Albumin. Concentration of BSA was observed at absorbance of 640-660 nm at variable range of concentration from 20-100 µg/ml to establish standard value. Compared to standard value total protein content was established in root, stem and leaf. The value was found to be highest in extract leaf 83.043 mg/g followed by stem extract 49 mg/g. whereas root extract sample was found to be 7.99 mg/g respectively.

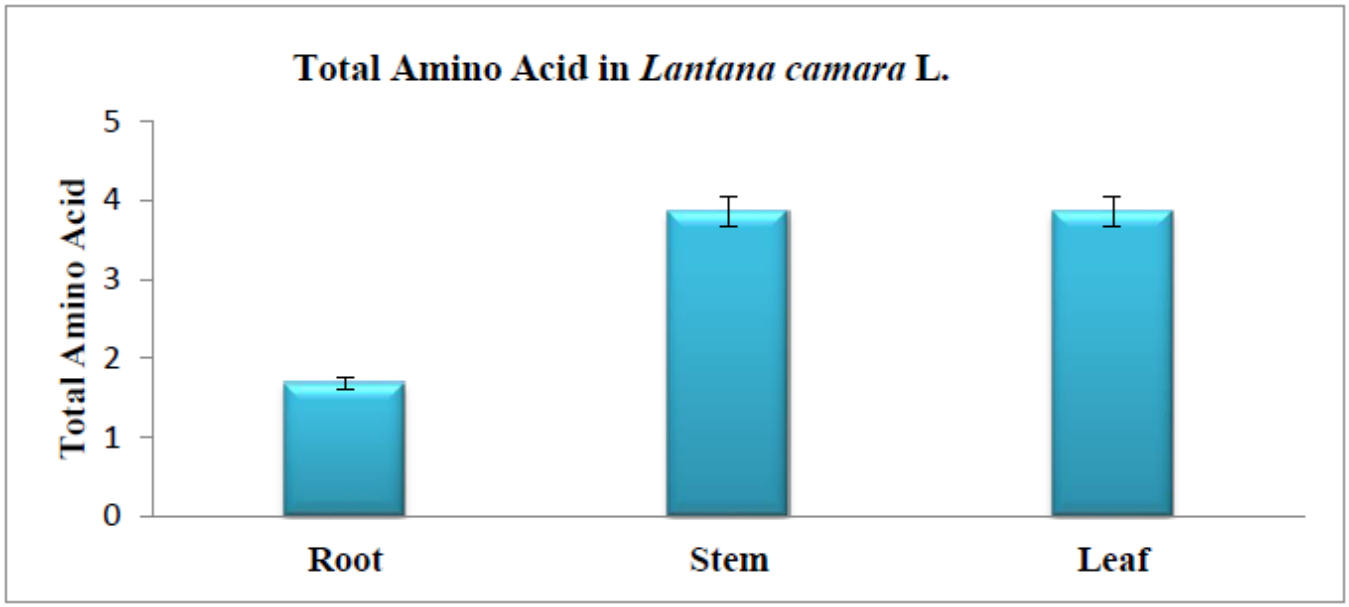
**Figure 4: Determination of Total Protein Content in root, stem and leaf extracts of** Lantana camara




## Total amino acid content

Total amino acid content was found in root, stem and leaf extract samples of *Lantana camara* calculated with standard curve of Bovine serum albumin. Concentration of BSA was observed at absorbance of 640-660 nm at variable range of concentration from 20-100µg/ml to establish standard value. Compared to standard value total amino acid composition in root, stem and leaf was established. The value was found to be highest and same in stem extract 3.866 mg/g and leaf extract 3.866 mg/g samples. Whereas root extract sample was found to be 1.685 mg/g respectively (Fig.5).

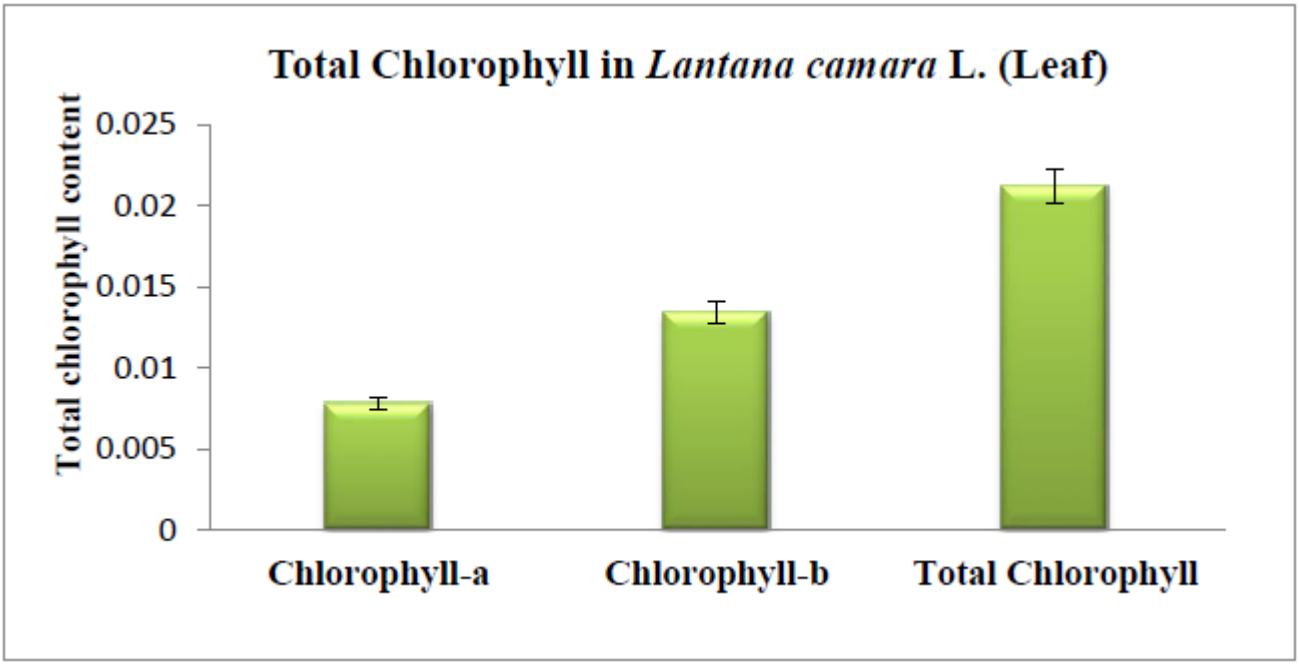
**Figure 5: Determination of Amino acid Content in root, stem and leaf extracts of Lantana camara**




## Chlorophyll and Carotenoid Content

Chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) and carotenoid content was estimated in fresh leaves of *Lantana camara* as shown in fig.6. The value of chlorophyll-a was found to be 0.212 mg/g, chlorophyll-b was found to be 0.134 mg/g, total chlorophyll was found to be 0.212 mg/g. whereas total carotenoid content was found to be 5.034 mg/g respectively.

**Figure 6: Determination of Chlorophyll Content in root, stem and leaf extracts of** Lantana camara




# Conclusion

It can be concluded that the qualitative phytochemical analysis of methanolic extract of *Lantana camara* root, stem and leaves confirms the best source of secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, amino acids, steroids, terpenoids and proteins. Furthermore, quantitative analysis of *Lantana camara* leaves showed that higher amounts of flavonoids when compared to other secondary metabolites such as alkaloids, phenols and tannins were respectively. Therefore, the medicinal value of these plants lies in some chemical substances that produce a definite physiological action and thus can be a source of novel therapeutics which may help in protection against countless diseases.

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