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AIR POLLUTION TOLERANCE INDEX AND ANTIOXIDANT ACTIVITY OF PLANT LEAVES COLLECTED NEAR RAILWAY JUNCTION, KARRUPUR, SALEM, TAMIL NADU, INDIA.

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ABSTRACT

Trees and plants keep the place green and it is essential to protect and preserve plants for various reasons. Hence, the present study was carried out to know about the tolerance, sensitivity of the plants located in the experimental site. **Method:** Aqueous leaf extract was used for the whole study. Biochemical components, secondary metabolites, antioxidant activities were assessed by following standard procedures. Fresh leaves were collected from the study area daily until the completion of the study. The collected leaves were weighed with dust and without dust i.e after cleaning. The difference gives an idea about the pollution load in the particular place. **Results:** The ascorbic acid content was high with *Tecoma stans*, all the other plants showed moderate level of ascorbic acid. The total chlorophyll content was higher with *Psidium guajava* 45.47±0.00mg/g, *Tecoma stans* 43.30±0.02mg/g, *Tamarindus indica* 41.14±0.41mg/g. pH observed in the study area was found to be acidic. The relative water content was high with *Citrus limon* 95.60±2.90%, *Lawsonia inermis* 93.18±1.72%. The aminoacid content was higher with *Manilkara zapota* 2.90±0.12 and moderate with rest of the plants studied. The carbohydrate content was high with *Azadirachta indica* 8.60±4.50. Air pollution tolerance index of the plants studied were found to be sensitive in the selected experimental site. The phenolic and flavonoid content of the selected plants were significantly high except *Tamarindus indica.* But, plants such as *Citrus limon, Tamarindus indica, Manilkara zapota* showed less flavonoid content compared to other plants. The

antioxidant activity was high with *Citrus limon* showing 19.1±1.09mg/g, The nitric oxide scavenging activity was high with *Manilkara zapota* 22.60±3.35mg/g, The reducing power activity was high with *Azadirachta indica* 17.8±0.35mg/g, While, the metal chelating activity was high with all the plants studied. Higher carotenoid content with *Psidium guajava* 16.27±0.02mg/g, likewise, protein content of *Lawsonia inermis* was 21.7±8.19mg/g. **Conclusion:** All the plants were of sensitive in nature but contain phenolics and flavonoids, antioxidant activities in higher amount which finds application in therapeutics.

KEYWORDS: APTI, Antioxidant activities, Biochemical parameters, Carotenoids, Secondary metabolites.

INTRODUCTION

Plants are essential in determining, preserving ecological stability via cycling of gases and nutrients. Air pollution is a major problem arising mainly by the use of more vehicles, industrialization etc. Pollutants in the air directly affect plant leaves causing physiological changes in leaf followed by damage that has been viewed through an open eye. The effect of air pollution could be assessed by means of biochemical parameters such as ascorbic acid, chlorophyll, pH, aminoacid, carbohydrate, relative water content. The effect of pollution on plant leaves in the particular location depend upon the resident time of pollutants residing in the atmosphere. Identification and plantation of tolerant species may have a marked effect on varied aspects of the quality of the urban environment and the cleanliness of life in a city.[1] Air pollution tolerance index of plants is an innate value of plants in encountering pollution a most important stress factor in the environment. Since, the response of plants to pollution varies from location to location, climatic condition, soil nutrients, type of pollution etc. It was decided to study the air pollution tolerance index of plant leaves collected from the experimental site i.e railway junction, Karrupur, Salem, Tamil Nadu, India.

OBJECTIVES

To determine the air pollution tolerance index of plant leaves through its various components like pH, ascorbic acid, chlorophyll, aminoacid, carbohydrate, relative water content and also to analyse the secondary metabolites, antioxidant activities, protein content.

MATERIALS AND METHODS

1. Materials

1.1. Leaf sample collection

For the present study, fresh leaves from each plants were collected from the experimental site i.e, near railway junction, Karrupur, Salem, Tamil Nadu, India during the month of December 2015 and January- 2016. Common plants identified were selected from the study areas. All the selected plants were identified by comparing with book named Dictionary of Medicinal Plants written by Dr. A. Balasubramanian, Executive Director, ABS Botanical garden, Salem, Tamil Nadu, India.

1.2. Extract preparation

Fresh leaves were used according to the standard prescribed methods adopted. Aqueous extract was used for the whole study.

2. Methods

2.1. Biochemical parameters

2.1.1. pH

100 mg of the fresh leaves was homogenized in 10ml deionized water. This was filtered and the pH of the leaf extract was determined after calibrating pH meter with buffer solution pH 4 and pH 9.

2.1.2. Relative water content

Fresh weight was obtained by weighing the leaves. The leaf samples were then immersed in water over night blotted dry and then weighed to get the turgid weight. The leaves were then dried overnight in a hot air oven at 70^0c and reweighed to obtain the dry weight. RWC was determined and calculated by the method as described by Singh 1977.[2]

RWC=[(FW-DW)/(TW-DW)] x 100.

Where: FW-Fresh weight, DW-Dry and TW-Turgid weight

2.1.3. Ascorbic acid content

Ascorbic acid content was measured by Titrimetric method of Sadasivam 1987[3] using 2,6, Dichlorophenol indo phenol dye. 500mg of leaf sample was extracted with 4% oxalic acid and then titrated against the dye until pink colour develops. Similarly a blank is also developed.

2.1.4. Total chlorophyll content

The total chlorophyll content was determined according to the method of Arnon (1949).[4] For TCh analysis, 0.5 g fresh leaves material was grounded and diluted to 10 ml in distilled water. A subsample of 2.5 ml was mixed with 10 ml acetone and filtered. Optical density was read at 645 nm (D645) and 663 nm (D663). Optical density of TCh (CT) is the sum of chlorophyll a (D645) density and chlorophyll b (D663) density as follows: CT = 20.2 (D645) + 8.02 (D663). TCh (mg/g DW) was calculated as follows: TCh = 0.1CT x (leaf DW/leaf fresh weight).

2.1.5. Calculation of APTI

The air pollution tolerance indices of the selected plants were determined by following the method of Singh and Rao (1983)[5] and modified by the addition of amino acid and carbohydrate.

The formula of APTI is given as: APTI= [A (T+P) + R+ Aminoacid + TC] / 10.

Where: A=Ascorbic acid content (mg/gm), T=Total chlorophyll (mg/gm), P=pH of the leaf extract, R=Relative water content of leaf (%), TC = Total carbohydrate.

2.2. Analysis of phytonutrients

Total carbohydrates, proteins, aminoacids were performed according to the standard prescribed methods.

2.2.1. Estimation of carbohydrate

The total carbohydrate was estimated by Anthrone method Hedge (1962).[6] To 0.1 ml of extract added 4ml of anthrone reagent and the contents were heated in a boiling water bath for 8 minutes. The tubes were cooled and read at 630nm using spectrophotometer Schimadzu Model - UV 1800. The standards were developed with glucose. Standard graph plotted was used to find out concentration of glucose present in the unknown/ sample.

2.2.2. Estimation of aminoacids

The amino acid was estimated by Ninhydrin method Yemm et.al (1955).[7] To 0.1 ml of sample added 1 ml of ninhydrin solution dissolved in Butanol: Acetone. Cover the test tube with a piece of paraffin film to avoid the loss of solvent due to evaporation. With gentle stirring, the reaction mixture was heated at 80-100ºC for 4-7 minutes. Cool the test tubes and the color developed was read at 570nm. Tyrosine was used for developing standards.

2.2.3. Estimation of protein

The total protein was estimated by Lowry's method Lowry et.al (1951).[8] To 0.1ml of extract added 2ml of alkaline copper reagent, mixed well and incubated for 10minutes. After the incubation period 0.2ml of Folin ciocalteau reagent (diluted in the ratio of 1: 2) was added and allowed for 30minutes incubation, then read at 660nm using spectrophotometer Schimadzu - Model UV 1800. The standards were developed with Bovine serum albumin. Standard graph plotted was used to find out concentration of protein present in unknown/ sample.

2.3. Secondary metabolites

The phenol and flavonoid content of aqueous leaf extract was analysed.

2.3. 1. Determination of Total phenol content

Total phenolic content were determined by Folil-ciocalteau method. Nabavi et.al (2008).[9] The extract samples 0.1ml were mixed with folinciocalteau reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous $NaCo_3$ (4ml, 1M) were added. The mixture was allowed to stand for 15min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared. Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

2.3. 2. Estimation of flavonoids

The aluminium chloride method Mervat et.al (2009)[10] was used for the determination of the total flavonoid content. Extract solution were taken and then 0.1ml of $AlCl_3$ (10%) were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30min of incubation. A standard caliberation plot was generated at 415nm using known concentration of quercetin. The concentration of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

2.4. Antioxidant assays

Nitric oxide scavenging assay, Reducing power, Total antioxidant assay, Metal chelating activities were performed.

2.4. 1. Nitric oxide scavenging activity

This was estimated by the method of Ebrahimzadeh et.al (2009d).[11] The procedure is based on the principle that, sodium nitroprusside in aqueous solution, at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline was mixed with extract and incubated at room temperature for 150min. After the incubation period 0.5ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. Quercetin was used as positive control.

2.4. 2. Reducing power assay

Reducing power assay was performed according to the method of Yen et.al (1995).[12] Aqueous extract was mixed with phosphate buffer (2.5ml, 0.2M, P^H 6.6) and potassium ferriccyanide (2.5ml %). The mixture was incubated at 50^0c for 20min. 1.0 ml of trichloro acetic acid (10%) was added to stop the reaction, which was then centrifuged at 3000rpm for 10min. The upper layer of solution (1.5ml) was mixed with distilled water (1.5ml) and $FeCl_3$ (0.1ml, 0.1%) after mixing, the contents were incubated for 10 min and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

2.4. 3. Total antioxidant capacity

Total antioxidant capacity by phospho-molybdenum method assay is based on the reduction of Mo (V1) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as number of equivalents of ascorbic acid. Assay was carried out according to the method of Prieto P et.al (1999).[13]

2.4.4. Metal chelating activity

The chelating ability of ferrous ion was estimated by the method of Ebrahimzadeh MA (2008a).[14] Add extract to a solution of 2mM $FeCl_2$ (0.05ml). The reaction was initiated by the addition of 5mM Ferrozine (160µl), the mixture was shaken vigorously and left standing at room temperature for 10min. Absorbance of the solution was then measured spectrophotometrically at 562nm. Standard curve was plotted using ascorbic acid. Distilled water (1.6ml) instead of sample solution was used as a control. Distilled water (160µl)

instead of ferrozine was used as a blank, which is used for error correction because of unequal color of sample solution.

For all estimations readings were taken using UV spectrophotometer Schimadzu Model 1800. Standard graph was plotted for all experiments using their respective standards and the samples were plotted against the standard by taking concentration in X axis and OD in Y axis.

2.5. STASTICAL TOOL

The Mean and Standard deviation (S) was calculated by using the following formula:

Mean = Sum of x values / n (Number of values)

$$s = \frac{\sqrt{\sum(X-M)^2}}{n-1}$$

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2.6. RESULTS AND DISCUSSION

2.6.1. Dust in the collected leaf sample

Table1. Difference in leaf collected with dust and without dust

Botanical Name	Result(mg/g)
Citrus limon	0.008±0.001
Lawsonia inermis	0.011±0.002
Azadirachta indica	0.010±0.002
Tecoma stans	0.022±0.014
Moringa oleifera	0.003±0.001
Tamarindus indica	0.006±0.006
Carica papaya	0.187±0.10
Manilkara zapota	0.018±0.001
Psidium guajava	0.018±0.011

Values are Mean ± SD for three experiments

Table 1 indicates the difference in the leaf sample with dust and without dust. From this study, there is not as much variation between leaf samples when it has dust and not dust. More variation was found in *Carica papaya* 0.187±0.10. Other plants show minute variation.

2.6.2. Biochemical components in APTI

Table. 2. Air pollution tolerance index (APTI)

Botanical Name	Ascorbic acid (mg/g)	Total Chlorophyll (mg/g)	P^H	RWC (%)	Amino acid (mg/g)	Total Carbohydrate (mg/g)	APTI
Citrus limon	0.16± 0.00	27.88±4.06	6.0±0	95.60± 2.90	2.20± 0.00	2.50± 0.38	10.57

Lawsonia inermis	0.20± 0.00	23.89±0.08	6.0±0	93.18± 1.72	2.20± 0.00	2.10± 0.12	10.35
Azadirachta indica	0.28± 0.00	19.65±0.02	6.0±0	88.74± 8.43	2.20± 0.00	8.60± 4.50	10.67
Tecoma stans	0.36± 0.00	43.30±0.02	6.0±0	47.45± 9.38	2.50± 0.12	3.07± 0.23	7.08
Moringa oleifera	0.20± 0.00	27.59±2.51	6.0±0	66.69± 9.51	2.50± 0.12	2.67± 0.92	7.86
Tamarindus indica	0.24± 0.00	41.14±0.41	5.0±0	77.91± 0.75	2.10± 0.23	2.90± 0.46	9.40
Carica papaya	0.16± 0.00	18.16±0.04	6.0±0	61.22± 13.33	2.30± 0.46	4.40± 1.73	7.18
Manilkara zapota	0.20± 0.00	32.31±0.29	6.0±0	71.07± 6.75	2.90± 0.12	3.73± 1.85	8.54
Psidium guajava.	0.36± 0.00	45.47±0.00	7.0±0	51.69± 7.84	2.30± 1.16	4.93± 0.92	7.78

Values are Mean ± SD for three experiments

Table. 2. Shows the results of components involved in the analysis of air pollution tolerance index. The ascorbic acid content of the plant leaves studied are as follows: *Citrus limon* 0.16±0.00, *Lawsonia inermis* 0.20±0.00, *Azadirachta indica* 0.28±0.00, *Tecoma stans* 0.36±0.00, *Moringa oleifera* 0.20±0.00, *Tamarindus indica* 0.24±0.00, *Carica papaya* 0.16±0.00, *Manilkara zapota* 0.20±0.00, *Psidium guajava* 0.36±0.00. Similar result was reported by Krishnaveni et.al for *Azadirachta indica,*[15] *Moringa oleifera.*[16]

Total chlorophyll content of the selected plant leaves calculated are shown below: *Citrus limon* 27.88±4.06, *Lawsonia inermis* 23.89±0.08, *Azadirachta indica* 19.65±0.02, *Tecoma stans* 43.30±0.02, *Moringa oleifera* 27.59±2.51, *Tamarindus indica* 41.14±0.41, *Carica papaya* 18.16±0.04, *Manilkara zapota* 32.31±0.29, *Psidium guajava* 45.47±0.00. Similar result was reported by Krishnaveni et.al for *Azadirachta indica.*[17,18]

The pH of almost all the plants studied were found to have 6.0. Only *Tamarindus indica* showed pH of 5.0 and *Psidium guajava* 7.0. Similar result was reported by Krishnaveni et.al for *Psidium guajava,,*[19,20] *Azadirachta indica.*[21,22,23]

Observed relative water content was depicted in the Table.2: *Citrus limon* 95.60±2.90, *Lawsonia inermis* 93.18±1.72, *Azadirachta indica* 88.74±8.43, *Tecoma stans* 47.45±9.38, *Moringa oleifera* 66.69±9.51, *Tamarindus indica* 77.91±0.75, *Carica papaya* 61.22±13.33, *Manilkara zapota* 71.07±6.75, *Psidium guajava* 51.69±7.84. Similar result was reported by

Krishnaveni et.al for, *Moringa oleifera,*[18] *Manilkara zapota,*[24] *Azadirachta indica,*[20] *Psidium guajava.*[24, 25]

The aminoacid content of the plant leaves are given as follows: *Citrus limon* 2.20±0.00, *Lawsonia inermis* 2.20±0.00, *Azadirachta indica* 2.20±0.00, *Tecoma stans* 2.50±0.12, *Moringa oleifera* 2.50±0.12, *Tamarindus indica* 2.10±0.23, *Carica papaya* 2.30±0.46, *Manilkara zapota* 2.90±0.12, *Psidium guajava* 2.30±1.16.

The carbohydrate content of the plant leaves studied are shown below: *Citrus limon* 2.50±0.38, *Lawsonia inermis* 2.10±0.12, *Azadirachta indica* 8.60±4.50, *Tecoma stans*3.07±0.23, *Moringa oleifera* 2.67±0.92, *Tamarindus indica* 2.90±0.46, *Carica papaya* 4.40±1.73, *Manilkara zapota* 3.73±1.85, *Psidium guajava*4.93±0.92.

Results of APTI were shown in Table 2. Results showed that *Azadirachta indica* has highest APTI value of 10.67 and that of lemon tree and henna tree has 10.57 and 10.35 respectively. The remaining plants has moderate APTI value which include *Tamarindus indica* (9.40), *Manilkara zapota* (8.54), *Moringa oleifera* (7.86), *Psidium guajava* (7.78), *Carica papaya* (7.18) and *Tecoma stans* (7.08). The APTI obtained was compared with Lakshmi et.al. According to Lakshmi et.al air pollution tolerance index in the range of 30-100 was considered tolerant to pollution, 17-29 are intermediate to pollution and below 16 and up to 1 are sensitive to pollution. Our results showed, that all the plants studied in the experimental site was found to be sensitive to air pollution. Similar result was reported by Krishnaveni et.al for *Tamarindus indica,*[18] *Psidium guajava,*[26] *Azadirachta indica.*[20, 27-29]

2.6.3. Analysis of secondary metabolite

Table 3. Secondary metabolites of leaves collected at railway junction

Botanical Name	Total phenolics (mg/g)	Total flavonoid (mg/g)
Citrus limon	14.2±7.27	9.30±0.12
Lawsonia inermis	25.5±2.19	14.5±1.62
Azadirachta indica	18.0±0.69	18.7±2.14
Tecoma stans	23.5±1.16	26.7±0.58
Moringa oleifera	13.4±2.80	10.7±0.12
Tamarindus indica	6.50±0.12	8.50±1.27
Carica papaya	11.8±1.62	17.5±0.00
Manilkara zapota	27.9±0.12	9.50±1.27
Psidium guajava	27.1±0.58	16.5±0.58

Values are Mean ± SD for three experiments

Total phenolic content was higher in *Manilkara zapota* 27.9±0.12, *Psidium guajava* 27.1±0.58, *Lawsonia inermis* 25.5±2.19 and *Tecoma stans* 23.5±1.16. The phenolic content was moderate with *Azadirachta indica* 18.0±0.69, *Citrus limon* 14.2±7.27, *Moringa oleifera* 13.4±2.80 and *Carica papaya* 11.8±1.62. The phenolic content was lower in *Tamarindus indica* 6.50±0.12.

The flavonoid content was higher in *Tecoma stans* 26.7±0.58. Moderate amount was observed with *Azadirachta indica* 18.7±2.14, *Carica papaya* 17.5±0.00, *Psidium guajava* 16.5±0.58, *Lawsonia inermis* 14.5±1.62. Flavonoid content was low in *Moringa oleifera* 10.7±0.12, *Manilkara zapota* 9.50±1.27, *Citrus limon* 9.30±0.12, *Tamarindus indica* 8.50±1.27.

2.6.4. Antioxidant assays

Table. 4 Antioxidant activities of plant leaves collected at railway junction.

Botanical Name	Phosphomolybdenum assay (mg/g)	Nitric oxide scavenging activity (mg/g)	Reducing power assay (mg/g)	Metal chelating activity (mg/g)
Citrus limon	19.10±1.09	19.30±7.85	9.60±1.04	4.80±0.35
Lawsonia inermis	05.10±0.41	09.50±4.97	7.40±0.35	7.30±0.46
Azadirachta indica	14.10±5.08	05.70±2.89	17.8±0.35	5.90±0.46
Tecoma stans	07.50±3.69	26.20±1.44	14.5±3.58	5.80±0.69
Moringa oleifera	08.90±0.58	10.90±5.77	6.70±0.23	7.70±0.58
Tamarindus indica	16.70±0.52	15.30±9.59	13.4±3.81	8.10±0.58
Carica papaya	11.20±1.04	02.30±0.17	10.3±1.85	6.70±0.23
Manilkara zapota	7.30±2.31	22.60±3.35	10.0±1.28	8.70±1.62
Psidium guajava	12.90±5.08	14.50±10.74	9.70±6.69	9.50±0.92

Values are Mean ± SD for three experiments

Total antioxidant activity was higher for *Citrus limon* showing 19.1±1.09, whereas *Tamarindus indica***,** *Azadirachta indica***,** *Psidium guajava***,** *Carica papaya* showed moderate amount of total antioxidant activity which ranges from 11.2 to 16.7 mg/g. Other plants such as *Moringa oleifera* 8.90±0.58, *Tecoma* stans 7.50±3.69, Manilkara *zapota* 7.30±2.31, *Lawsonia inermis* 5.10±0.41shows low antioxidant activity. Similar result was reported by Krishnaveni et.al for *Psidium guajava*,[30] *Azadirachta indica.*[31]

Nitric oxide scavenging activity was higher in *Tecoma stans* 26.2±1.44, *Manilkara zapota* 22.6 ±3.35. Moderate amount was observed with *Citrus limon* 19.3±7.85, *Tamarindus indica* 15.3±9.59, *Psidium guajava* 14.5±10.74, *Moringa oleifera* 10.9±5.77 and *Lawsonia inermis* 9.50±4.97. Nitric oxide scavenging activity was low with *Azadirachta indica* 5.70±2.89 and

Carica papaya 2.30±0.17. Similar result was reported by Krishnaveni et.al for *Tamarindus indica,*[31`,32] *Azadirachta indica.*[32,33]

The reducing power assay was high with *Azadirachta indica* 17.8±0.35. Moderate amount was observed with *Tecoma stans* 14.5±3.58, *Tamarindus indica* 13.4±3.81, *Carica papaya* 10.3±1.85, *Manilkara zapota* 10.0±1.28, *Psidium guajava* 9.70±6.69, *Citrus limon* 9.60±1.04, *Lawsonia inermis* 7.40±0.35, *Moringa oleifera* 6.70±0.23. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. Similar result was reported by Krishnaveni et.al for *Psidium guajava.*[29]

Metal chelating activity was higher in *Psidium guajava* 9.50±0.92. Moderate activity was observed with *Manilkara zapota* 8.70±1.62, *Tamarindus indica* 8.10±0.58, *Moringa oleifera* 7.70±0.58, *Lawsonia inermis* 7.30±0.46, *Carica papaya* 6.70±0.23, *Azadirachta indica* 5.90±0.46, *Tecoma stans* 5.80±0.69. The Metal chelating activity was low with *Citrus limon* 4.80±0.35. Similar result was reported by Krishnaveni et.al for *Tamarindus indica,*[29] *Manilkara zapota,.*[34] *Citrus sp.*[35] *Azadirachta indica,*[32,36,37]

2.6.5. Carotenoids

Table.5. Analysis of Carotenoids

Botanical Name	Carotenoids (mg/g)
Citrus limon	06.99±0.04
Lawsonia inermis	12.33±0.09
Azadirachta indica	04.85±0.41
Tecoma stans	10.32±0.01
Moringa oleifera	06.94±0.64
Tamarindus indica	08.95±0.40
Carica papaya	06.99±0.04
Manilkara zapota	07.08±0.41
Psidium guajava	16.27±0.02

Values are Mean ± SD for three experiments

Table. 5. indicates the pigment analysis of plant leaves. Higher carotenoid content was observed in *Psidium guajava* as 16.27±0.02. Moderate amount was observed with *Lawsonia inermis* 12.33±0.09, *Tecoma stans* 10.32±0.01, *Tamarindus indica* 8.95±0.40, *Manilkara zapota* 7.08±0.41, *Carica papaya* 6.99±0.04**,** *Citrus limon* 6.99±0.04, *Moringa oleifera* 6.94±0.64. Carotenoid content was low with *Azadirachta indica* 4.85±0.41.

2.6.6. Protein assay

Table. 6. Analysis of Proteins

Botanical Name	Protein (mg/g)
Citrus limon	16.3±1.62
Lawsonia inermis	21.7±8.19
Azadirachta indica	11.1±1.96
Tecoma stans	05.0±0.00
Moringa oleifera	11.3±1.62
Tamarindus indica	06.60±0.00
Carica papaya	08.70±0.23
Manilkara zapota	07.10±1.50
Psidium guajava	03.10±0.23

Table. 6 shows the protein content of the plant leaves collected. Highest amount of protein was found in *Lawsonia inermis* 21.7±8.19. Moderate amount of protein content was present in *Citrus limon* 16.3±1.62, *Moringa oleifera* 11.3±1.62, *Azadirachta indica* 11.1±1.96, *Carica papaya* 8.70±0.23, *Manilkara zapota* 7.10±1.50, *Tamarindus indica* 6.60±0. The protein content was low in *Tecoma stans* 5.0±0 and *Psidium guajava* 3.10±0.23.

CONCLUSION

Plant based traditional knowledge has become a recognized tool in search for new sources of drugs and nutraceuticals. The medicinal value of plants are enhanced by its most important biologically active components like secondary metabolites, which induces antioxidant activities. Richer antioxidant property makes a plant medicinally important and finds its use in pharmacology. The sensitiveness of plants signifies that it has to be protected.

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