EVALUATION OF NON-ENZYMATIC AND ENZYMATIC ANTIOXIDANT ACTIVITIES IN LEAVES AND FLOWERS OF Cassia auriculata. N.thangaraj¹ and C. jayalakshmi²
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Abstract
Nature has been a source of medicinal agents since times immemorial. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. The blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security.

I. INTRODUCTION

Among ancient civilisations, India has been known to be rich repository of medicinal plants. The indigenous system of medicine in India is known by many names such as Ayurveda, Unani, Siddha and Homeopathy. The common element in the different branches of the system is the use of medicinal plants (Tomoko N 2002). The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products.

Plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. Since ancient times, plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999). Increased scientific interest and consumer demand have promoted the development of herbal products, presently as dietary supplements. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. (Bishnu Joshi et al., 2011)

Medicinal plants have been used for centuries before the advent of orthodox medicine. The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties. They synthesise and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines. (Nonita P. Peteros et al., 2010).

The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body. These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. These phytochemicals are extensively found at different
levels in various medicinal plants and used in herbal medicine to treat diverse ailments such as cough, malaria, wounds, and toothache and rheumatism diseases. (Harborne, 1998). However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, and selection of the superior plant stock and over exploitation by pharmaceutical industry.

Antioxidant may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, arthritis, inflammation, neurodegeneration, parkinson’s diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties (Polterait et al., 1997). Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components (Makari et al., 2008).

*Cassia auriculata* L. is one such herb, profoundly used in Ayurvedic medicine, known locally as 'avaram' and belonging to the family Caesalpiniaeae. *Cassia auriculata* is a shrub with smooth brown bark and is a common plant in Asia, India and Sri Lanka. The leaves are anthelmintic, good for ulcers, leprosy and skin diseases. The flowers are used in urinary discharges, diabetes and also for throat infection. The fruit is useful in thirst and in vomiting. The seed is used in diabetes, dysentery and chronic conjunctivitis. The bark is considered as astringent. The main objective of the present study was designed to investigate the antimicrobial efficacy of *Cassia auriculata* leaves and flower extracts. Since antioxidant and membrane stabilizing activity have not been systematically studied in the plant parts, therefore in *vitro* studies were undertaken which could be the major mechanism involved in the protective effect.

**Scientific classification**

- **Kingdom**: Plantae
- **(unranked)**: Angiosperms
- **(unranked)**: Eudicots
- **(unranked)**: Rosids
- **Order**: Fabales
- **Family**: Fabaceae
- **Subfamily**: Caesalpinoideae
- **Tribe**: Cassieae
AIM

- To study the non-enzymatic and enzymatic antioxidant activities in flowers of *Cassia auriculata*

OBJECTIVE

- To screen qualitatively the methanolic extract of the leaves and flowers of *Cassia auriculata*.
- To analyse quantitatively the phytochemical constituents of the leaves and flowers of *Cassia auriculata* evaluated by non enzymatic and enzymatic methods.
- To determine the phytochemical content of the leaves and flowers of *Cassia auriculata* by standard method quantitatively.

II. MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The plant material namely leaves and flowers of *Cassia auriculata* were collected from the field areas of Namakkal, Namakkal district, Tamilnadu, India.

EXTRACTION

The leaves and flowers of *Cassia auriculata* were shad dried in below 60 degree celcius for 2hrs. They were finely powered and extracted with 80% acetone chloroform methanol using soxhlet apparatus at 55 degree celcius. The soluble part was concentrated over water bath maintained below 60 degree celcius and dried in vacuum oven to obtain free flowing reddish brown powder. The extract obtained was termed as methanolic extract of *Cassia auriculata*.

PHYTOCHEMICAL ANALYSIS

The phytochemical in each sample was determined qualitatively and quantitatively.

QUALITATIVE ANALYSIS

The phytochemical analysis of the plant was carried out by the standard method.

Test for Tannins:
0.1 ml extraction solution 0.5 ml distilled water 2 drops of Ferric chloride solution added observed for the blue or green black coloration.

Test for Saponins:
0.2 ml of extract and 0.2 ml of distilled water shaked well and small bubbles on surface of liquid.
Test for Flavonoids:
0.5 ml of methanol added to 0.5 ml of extract and 1 ml of NaOH added shaken well yellow coloration.

Test for Glycosides:
0.4 ml of extract solution added glacial acid 5 drops and few drops of FeCl3 and concentration sulphuric acid and observed reddish brown coloration 2 layers and bluish green colour in upper layer.

Test for Alkaloids:
0.4 ml of extract 1 drops of Mayers reagents was added by the sides of the test tubes Creamy yellow or white precipitate indicates test for positive.

Test for Anthraquines:
1.0 ml of extract and few drops CCl4 (Carbon tetrachloride) from layer of solution remove the layer solution. Added 5 drops (HCl+FeCl3) mixing solution cherry red colour.

Test for Phenolic compounds:
0.2 ml of extract was diluted to 0.5 ml with distilled water. To this few drops neutral 5% ferric chloride solution was added A dark color indicate phenolic compounds.

QUANTITATIVE ANALYSIS

Tannins
500 mg of plant sample was weighed and transferred to 50 ml flask. Then added 50 ml of distilled water and stirred for 1 h. Sample was filtered into a 50 ml volumetric flask and the volume was made up to the mark. 5 ml of the filtered sample was pipette into test tube and then mixed with 2 ml of 0.1 M ferric chloride. The absorbance was measured using spectrophotometer at 395 nm wavelength within 10 min (Tyler 1994; Harborne et al., 1973).

Saponins
20 g of each ground plant samples were put into a conical flask and 100 ml of 20% ethanol was added to the plant sample. The said sample is heated over a water bath for 4 h at about 55°C with continuous stirring. The extracted mixture is then filtered and the residue is then re-extracted again with 200 ml of 20% ethanol. The collective residues are reduced to 40 ml over a hot water bath. The concentrated is then transferred to a separating funnel and 20 ml of diethyl ether is added to the plant extract and shaken vigorously. The aqueous layer was recovered while the organic layer was discarded and the process of purification was repeated. 60 ml of n-Butanol was added and combined n-Butanol extract were washed twice with 10 ml of 5% sodium chloride. The remaining solution was then heated on water bath and after evaporation; the samples were dried in oven to a constant weight.

Flavonoids
Extracted 10 g of the plant sample with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered and the filtrate was then transferred into a water bath. The solution was evaporated to dryness and weighed to a constant weight (Mattila and Hellström, 2007, Williamson and Manach, 2005).

Alkaloids
5 g of the plant sample was prepared in a beaker and 200 ml of 10% CH3CO2H in C2H5OH is added to the plant sample. The mixture is covered and allowed to stand for 4 h. The mixture was then filtered and the extract is allowed to become concentrated in a water bath until it reaches ¼ of the original volume. Concentrated ammonium hydroxide was added until the precipitation is complete. The whole solution is allowed to settle and the precipitate is collected and washed with dilute ammonium hydroxide and then filtered. The residue is alkaloid, which is then dried and weighed.
Phenols

Plants sample was boiled for 15 min with 50 ml of (CH$_3$CH$_2$)$_2$O. 5 ml of the sample was pipetted into 50 ml flask, and 10 ml of distilled water was added. Then 2 ml of NH$_4$OH solution and 5 ml of concentrated CH$_3$ (CH$_2$)$_3$CH$_2$OH was added to the mixture. The sample was made up to the mark and left to react for 30 min for color development and measured for 505 nm wave length using a spectrophotometer (Tyler 1994; Harborne et al., 1973).

Estimation of Protein by Lowry’s method (Sadasivam and Manickam, 1996)

- Pipetted out 0.2 - 1 ml of the working standard solution into a series of test tubes.
- 1ml of the samples was taken in other test tubes and the volume of all the test tubes was made upto 1ml with distilled water.
- 5ml of alkaline copper reagent was added into all the tubes, mixed well and allowed to stand for 10 minutes.
- Then 0.5ml of Folin’s reagent was added and incubated at room temperature in dark for 30 minutes.
- The color developed and it was read at 660nm.
- A standard graph was drawn and the amount of protein in the samples was calculated.

Determination of total carbohydrates by anthrone method. (Sadasivam and Manickam, 1996)

- Weigh 100mg the sample into a boiling tube .
- Hydrolyse by keeping it in a boiling water bath for 3hrs with 5ml of 2.5N Hcl and cool to room Temperature. Neutralise it with solid sodium carbonate until the effervescence ceases.
- Make up the volume to 100ml and centrifuge.
- Collect the supernatant and take 0.5 and 1ml aliquotes for analysis.
- Prepare the standards by taking 0,0.2-1ml of the working standard ‘0’ serves as blank.
- Make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water.
- Then add 4ml of anthrone reagent .
- Heat for 8 minutes in a boiling water bath.
- Cool rapidly and read the green to dark green colour at 630nm.
- Draw a standard graph by plotting concentration of the standard on the X-axis verses absorbance on the Y-axis.
- From the graph calculate the amount of carbohydrates present in the sample tubes.

Extraction and estimation of total free amino acids

Extraction

500mg of the plant samples Halodule pinifolia, Cymodocea serrulata were weighed and ground in a marter and pestle with a small quantity of acid-washed sand. To this homogenate 5 to 10ml of 80% ethanol was added, filtered and centrifuged. The supernatant was saved. The extraction was repeated. The supernatants were pooled together and used for the estimation of total free amino acids.

Estimation

- 0.1ml of extract was taken in a test tube.
- To this was added 1ml of ninhydrin solution.
- The tube was heated in boiling water bath for 20 min.
- 5ml of the ethanol:water (1:1) was added and the contents were mixed well.
- After 15 minutes the absorbency was recorded with colorimeter at 570nm. By using the standard graph the concentration of the given samples were calculated and expressed as µg/100mg on dry weight basis.
III. ENZYMATIC METHOD

CATALASE

Total catalase (CAT, E.C. 1.11.1.6) activity was measured as described (Beers and Sizer, 1952). The reaction mixture (1.5 mL) consisted of 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 20 mM H2O2 and samples of CgLP or PrLP or EtLP. The reaction was started by adding the enzyme extract, and the decrease in H2O2 was monitored at 240 nm and quantified by its molar extinction coefficient (36 M-1cm-1). The results were expressed as μMol H2O2 min-1 g-1DM.

\[
2 \text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2 \text{H}_2\text{O} + \text{O}_2
\]

**Calculation**

\[
\text{Units/mg} = \frac{\Delta \text{A}_{240}/\text{min} \times 1000}{4.36 \times \text{mg enzyme} / \text{ml reaction mixture}}
\]

ACTIVITY OF PEROXIDASE

Total peroxidase activity (APX, E.C. 1.11.1.1) was performed by two methods. Colorimetric assays were performed according to (Nakano and Asada, 1981). The reaction mixture (1.5 mL) composed of 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 1.0 mM H2O2 and samples of CgLP or PrLP or EtLP. The reaction was started by adding H2O2 and ascorbate oxidation was measured at 290 nm for 1 min. Enzyme activity was measured using the molar extinction coefficient for ascorbate (2.8 mM-1 cm-1) and the results expressed in μMol H2O2 min-1 g-1DM, taking into consideration that 2 mol ascorbate are required for a reduction of 1 mol H2O2. Peroxidases were also detected by zymography. Samples of CgLP, PrLP and EtLP were separated by electrophoresis in 12.5% polyacrylamide gels in presence or absence of SDS. The gels were then immersed in 100 mL phosphate buffer pH 7.0 containing 0.03% H2O2, 0.2% guaiacol and 0.03% 3-amino-9-etil-carbazole. Peroxidase isoforms were detected as brown bands (Johri et al., 2005).

ACTIVITY OF SUPEROXIDE DISMUTASE (SOD)

The assay of SOD was performed according to Madamanchi et al.,(1994). 1g of plant material was homogenized in 10mL ice-cold 50 mM potassium phosphate buffer (pH 7.8) and centrifuged at 10,000 rpm for 10 min at 4º C. The supernatant was used as the enzyme source. To about 50μl of crude enzyme was mixed 3mL reaction cocktail containing 50 mM potassium phosphate buffer (pH 7.8), 13mM methionine, 2 μM riboflavin, 0.1mM EDTA and 75 μM NBT and was exposed to 400 W bulbs for 15 minute. The optical density was absorbed at 560nm and 50% inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity and expressed as unit/mg of protein.

ASSAY OF POLYPHENOL OXIDASE (PPO)

Poly phenol oxidase was measured according to the method of Esterbaner et al.,(1997). wherein, the enzyme extract was prepared by grinding 5g of sample in about 20mL reaction medium containing 50mM Tris HCl (pH 7.2), 0.4M sorbitol and 10 mM NaCl respectively. The homogenate mixture was centrifuged at 20,000rpm for 10 minutes and the supernatant was added to the assay mixture containing 2.5mL of 0.1M phosphate buffer and 0.3mL of catechol solution (0.01M) and read at 495 nm at an interval of 30 seconds up to 5 minutes. Hence for the activity of PPO was calculated using the formula,

\[
\text{Enzyme unit} = K \times (D/min)
\]

Where, K for Catechol oxidase is 0.272, and for Laccase is 0.242.

IV. NON ENZYMATIC METHOD
DETERMINATION OF DPPH (2, 2-DIPHENYL-1-PICRYLHYDRAZYL) RADICAL SCAVENGING ACTIVITY

Free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured Oboh et al.,(2005) in about 50μL of the extract, where 1.5 mL of 0.1 mM DPPH was added and vortexed for 15 to 30 s and allowed to stand without any disturbance for 30 min at room temperature. Indication in the activity of DPPH was observed with a change in the colour from purple to yellow and was measured by reading the absorbance at 517 nm. Ascorbic acid was used as the standard, while the inhibition ratio for DPPH scavenging activity was calculated from the equation:

\[
\text{AA (%)} = \left( \frac{A_c - A_s}{A_c} \right) \times 100
\]

Where,
AA - Ascorbic acid,
AC - Absorbance of control
As - Absorbance of test sample

DETERMINATION OF TOTAL PHENOLIC CONTENT

The total phenolic content (TPC) of the plant extracts was determined spectrophotometrically using Folin-Ciocalteau’s reagent Kahkonen et al.,( ) . 50 μL of the samples in triplicate was added into the test tubes followed by 1.5 mL of 2N Folin-Ciocalteau reagent (diluted 10 times) and 1.2 mL of 20% sodium carbonate. The contents of the tubes were mixed thoroughly and stored at dark for 30 min. Phenols react with phosphomolibdic acid of Folin-Ciocalteau’s reagent in alkaline medium and produce blue colored complex, that could be measured at 765 nm and expressed as mg Gallic acid per gm of plant material with Gallic acid as the standard.

MEASUREMENT OF TOCOPHEROL

Tocopherol Rosenberg et al.,(1992) was measured by pipetting 1.5mL of each plant extract, standard (α tocopherol) and water respectively in tubes separately. To the test and blank was added 1.5mL ethanol and to the standard was added 1.5mL water and vortexed, to which was added 1.5 mL of xylene and centrifuged at 1000rpm for 10min. About 1.0 mL of xylene layer was transferred into another stopper tube, taking care not to include any ethanol or protein and 0.1 mL of 2, 2`-dipyridyl reagent was added and read at 460 nm for both the extinction of test and standard against the blank. Since, tocopherol is estimated by Emmerie- Engel reaction, which indicates the presence of tocopherols by reducing the ferric ions to ferrous ions and also with the change in colour (from colourless to red), 0.33 mL of ferric chloride solution was added and read at 520 nm after 15 min. It was calculated using the formula

\[
\text{Reading of test (at 520nm) – (Reading of test at 460nm ) Total volume of homogenate}
\]

\[
= \frac{\text{Volume used } \times \text{ weight of the tissue}}{0.29 \times 15}
\]

DETERMINATION OF FERRIC REDUCING ANTIOXIDANT POWER (FRAP) 

The FRAP assay was conducted using method of Wong et al. (2006). Two hundred microlitre of extract were added with 3 ml of FRAP reagent that was prepared with mixture of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM FeCl.6H2O at the ratio of 10:1:1. The reaction mixture was incubated in a water bath at 37°C for 30 min. The increase in absorbance was measured using spectrophotometer at 593 nm. The antioxidant capacity based on the ability to reduce ferric ions of the extracts was calculated as percent of
antioxidant. The percent of antioxidant was calculated using the formula, percent of antioxidant (%) = \[\frac{(A_{593 \text{ of sample}} - A_{593 \text{ of control}})}{A_{593 \text{ of sample}}} \times 100\]

V. RESULTS AND DISCUSSION

Table - 1 Qualitative analysis of phytochemicals on acetone, chloroform, methanol extract of *Cassia auriculata* leaves.

| S.No | Phytochemicals    | Acetone | Chloroform | Methanol |
|------|-------------------|---------|------------|----------|
| 1.   | Tannins           | +++     | +          | +++      |
| 2.   | Saponins          | ++      | +++        | +++      |
| 3.   | Flavonoids        | ++      | +          | +++      |
| 4.   | Steroids          | +       | +++        | +        |
| 5.   | Glycosides        | ++      | +          | ++       |
| 6.   | Alkaloids         | +++     | +          | +        |
| 7.   | Anthraquinones    | ++      | +++        | +        |
| 8.   | Phenolic compounds| ++      | +++        | +++      |

Table - 2 Qualitative analysis of phytochemicals on acetone, chloroform, methanol extract of *Cassia auriculata* flowers.

| S.No | Phytochemicals    | Acetone | Chloroform | Methanol |
|------|-------------------|---------|------------|----------|
| 1.   | Tannins           | +++     | +++        | +++      |
| 2.   | Saponins          | +++     | +          | ++       |
| 3.   | Flavonoids        | +       | +++        | ++       |
| 4.   | Steroids          | ++      | +          | +++      |
| 5.   | Glycosides        | +++     | ++         | +        |
| 6.   | Alkaloids         | +++     | +++        | +        |
| 7.   | Anthraquinones    | +++     | +          | +        |
| 8.   | Phenolic compounds| +++     | +++        | +++      |

Table - 3 Quantitative analysis of phytochemicals on acetone, chloroform, methanol extract of *Cassia auriculata* leaves.

| S.No | Phytochemicals    | Acetone | Chloroform | Methanol |
|------|-------------------|---------|------------|----------|
### Table 4: Quantitative analysis of phytochemicals on acetone, chloroform, methanol extract of *Cassia auriculata* flowers.

| S.No | Phytochemicals | Acetone | Chloroform | Methanol |
|------|----------------|---------|------------|----------|
| 1.   | Tannins        | 0.092   | 0.041      | 0.092    |
| 2.   | Saponins       | 0.082   | 0.078      | 0.094    |
| 3.   | Flavonoids     | 0.082   | 0.074      | 0.086    |
| 4.   | Steroids       | 0.036   | 0.086      | 0.042    |
| 5.   | Alkaloids      | 0.093   | 0.082      | 0.032    |
| 6.   | Protein        | 0.966   | 0.966      | 0.968    |
| 7.   | Carbohydrates  | 1.232   | 1.074      | 1.254    |
| 8.   | Amino acid     | 0.190   | 0.212      | 0.140    |

Table - 4 Quantitative analysis of phytochemicals on acetone, chloroform, methanol extract of *Cassia auriculata* flowers.

### VI. ENZYMATIC METHODS

Table - 5 Catalase scavenging activity of leaves and flowers of *Cassia auriculata*.

| Solvents | Leaf Units /mg | Flower Units /mg |
|----------|----------------|------------------|
|          |                |                  |

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Table - 6 Peroxidase scavenging activity of leaves and flowers of *Cassia auriculata*.

| Solvents   | Leaf Units /lit | Flower Units /lit |
|------------|----------------|------------------|
| Acetone    | 35.20          | 18.99            |
| Chloroform | 33.68          | 15.95            |
| Methanol   | 36.98          | 22.54            |

Table - 7 Superoxide dismutase scavenging activity of leaves and flowers of *Cassia auriculata*.

| Solvents   | Leaf Units /mg | Flower Units /mg |
|------------|----------------|------------------|
| Acetone    | 1.65           | 1.58             |
| Chloroform | 1.17           | 1.16             |
| Methanol   | 1.76           | 1.63             |

Table - 8 Assay of polyphenol oxidase scavenging activity of leaves in *Cassia auriculata*.

| Solvents | Acetone Units /min | Chloroform Units /min | Methanol Units /min |
|----------|---------------------|-----------------------|---------------------|
| 1        | 0.015               | 0.013                 | 0.015               |
| 2        | 0.007               | 0.007                 | 0.008               |
| 3        | 0.005               | 0.007                 | 0.005               |
| 4        | 0.004               | 0.003                 | 0.004               |
| 5        | 0.003               | 0.003                 | 0.003               |

Table - 9 Assay of polyphenol oxidase scavenging activity of flowers in *Cassia auriculata*.

| Solvents | Acetone Units /min | Chloroform Units /min | Methanol Units /min |
|----------|---------------------|-----------------------|---------------------|
| 1        | 0.013               | 0.011                 | 0.014               |
| 2        | 0.007               | 0.005                 | 0.007               |
| 3        | 0.004               | 0.005                 | 0.005               |
| 4        | 0.003               | 0.003                 | 0.003               |
| 5        | 0.003               | 0.002                 | 0.003               |

NON ENZYMATIC METHODS

Table - 10 Free radical scavenging activity of leaves and flowers of *Cassia auriculata* by DPPH assay.

| Solvents   | Leaf mg/g | Flower mg/g |
|------------|-----------|-------------|
| Acetone    | 8.13      | 30.62       |
| Chloroform | 9.56      | 35.40       |
Table - 11 Total phenolic content scavenging activity of leaves and flowers of *Cassia auriculata*.

| Solvents  | Leaf μg /g | Flower μg /g |
|-----------|------------|--------------|
| Acetone   | 0.165      | 0.125        |
| Chloroform| 0.175      | 0.135        |
| Methanol  | 0.185      | 0.150        |

Table - 12 Tocopherol scavenging activity of leaves and flowers of *Cassia auriculata*.

| Solvents  | Leaf μg /g | Flower μg /g |
|-----------|------------|--------------|
| Acetone   | 9.43       | 6.63         |
| Chloroform| 11.36      | 7.23         |
| Methanol  | 8.96       | 5.95         |

Table - 13 Free radical scavenging activity of leaves and flowers of *Cassia auriculata* by FRAP method.

| Solvents  | Leaf Μg | Flower μg |
|-----------|---------|----------|
| Acetone   | 39      | 30       |
| Chloroform| 38      | 33       |
| Methanol  | 43      | 36       |

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