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Quantification of the Antioxidant Potential of Cassia Auriculata L.: As Phenolic Rich Compound

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ABSTRACT

The study was conducted to assess the phytochemical constituents in the aerial parts of *Cassia auriculata* Linn. extracts using standard methods. The quantitative analysis of bioactive compounds for five different extracts was performed in this study, revealing a wide range of phytochemical compounds. *Cassia auriculata* Linn. has been used therapeutically for a long period in the treatment of various chronic diseases. This work focuses on the phytochemical studies and extractive value of the aerial parts of *Cassia auriculata* L., highlighting their antioxidant properties of methanolic and aqueous extract of aerial part of *Cassia auriculata* L. by DPPH and FRAP standard methods. This plant is found in the dry regions of India and Sri Lanka. As traditional, mostly and most preferably it is used for the treatment of diabetes mellitus and as wound healing.

Keywords: Cassia Auriculata, Avar Taki, Phytochemicals Constituents, Extractive Value, Antioxidant Activity (DPPH, FRAP).

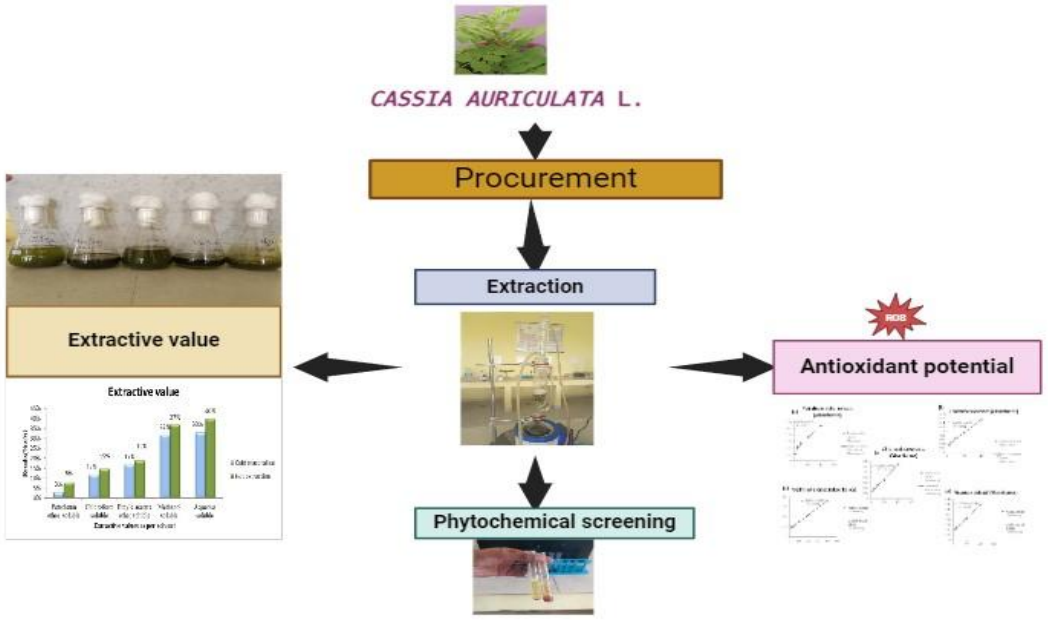
1. INTRODUCTION

Cassia auriculata, a plant traditionally used to treat various ailments, belongs to the Fabaceae family. Its synonyms include *Senna auriculata* Linn., and it's locally known as tarvad, Matura tea tree, and avaram. This kind of plant usually grows in the dry, arid areas of both Sri Lanka and India. The leaves of the plant are individual, tightly spaced, paripinnately complex like those of Senna, with a slightly pubescent rachis bearing rigid linear glands between leaflet pairs. The flower is big, bright yellow, bisexual, and uneven, with glabrous pedicels. The racemes are short and have few flowers, forming a large terminal inflorescence. Its fruit is a short legume carrying 12-20 seeds per fruit. This plant's various components are utilized in traditional medicine for their antioxidant activity (1,2,3).

Herbal medicines, used for decades, offer effective cures for common infectious diseases. These plants have proven therapeutic benefits against various illnesses, making them convenient and affordable, especially in rural areas (4,5). Plants produce an extensive range of bioactive compounds, which serve as the basis for many modern drugs. However, the overusing antibiotics, bacterial strains resistant to them have emerged, highlighting the need for new antimicrobial solutions from natural sources (6,7).

Medicinal plants, rich in compounds like tannins, phenolic compounds and flavonoids, show potential in fighting infections while reducing side effects. *Cassia auriculata* Linn., also known as tanner's cassia or "Avaram" in Tamil, is highly valued in traditional medicine and various industries. Its bark, leaves, flowers, and seeds are used to treat diseases like diabetes and skin diseases. According to Ayurveda various plant sections have distinct curative properties (8,9,10).

GRAPHICAL ABSTRACT



2. MATERIAL AND METHOD

2.1 Collection & identification of plant material

Cassia auriculata linn. aerial parts were gathered freshly from Jodhpur, India mainly in June month. The plant was recognized and verified at the raw material Herbarium & Museum CSIR- NIScPR Delhi.

2.2 Preparation of powder Material

Aerial part of the *Cassia auriculata* linn. collected & washed with water to remove dirt or debris. Later dried in the shade for 3 weeks, grind & passed through a 22-mesh sieve. The powdered crude plant was kept in an airtight container and used for further purpose (11).

2.3 Extraction of aerial part of plant:

2.3.1 Estimation of Extractive Values of aerial part Plant Material-

2.3.1.1 Hot Extraction- 4 g of powdered drug was poured in a conical flask which should be glass- stoppered. 100 ml of water was supplemented to the conical flask and weighed. Then, the mixture was shaked well and kept aside for about one hour. Then, the hot extraction assembly was subjected for setting and joined with the RBF & then the mixture was exposed for boiling for about 1 h which wasfurther processed for the cooling process and then again weighed. The total original weight was further accustomed by the specified liquid as per the testing method for concerned herb. Again, the mixture is agitated and strained through a dry filter paper rapidly. Now, the filtrate (25 ml) was poured in a previously tared porcelain dish and then the filtrate was subjected to evaporation till it attains semi consistent level over a water-bath till a residual consistency was attained. Then, evaporated extract was subjected to drying process at a temperature around 105°c for duration of 6 hrs, and further it is placed in a dessicator for 30 min, then the dish was subjected to weighing without delay. Then, the extractive value was determined in mg/g units.

2.3.1.2 Cold maceration- 4g of powdered drug was accurately weighed and transferred into a conical flask. 100 ml of the solvent was added and shaked occasionally or in every 10 minutes for about 6 hr duration. After shaking, conical flask was kept aside for 18 h. Filtration should be done carefully not to lose any solvent from the mixture. 25 ml of the filtrate was dispensed over a previously weighed porcelain dish and then the filtrate was evaporated over a water-bath. The extract was dehydrated at 105°c for about 6 h, and then cooled in a dessicator for around 30 mins. Then, the dish was weighed without delay because it will absorb moisture very soon. Then, the extractive value was determined in mg per g of the air-dried material used (11,12).

2.4 Phytochemical Analysis

The preliminary phytochemical Studies were completed to identify the presence of individual chemical group present in all the plant extracts which was extracted by using a mechanical grinder, the plant's dry aerial portion was pulverized. Then, the substance that was contained in powder form underwent an additional procedure of Soxhlet extraction by the use of different

solvents in escalating order of polarity viz. petroleum ether, chloroform, ethyl acetate, methanol and distilled water. prior to each process of soxhlet extraction, the medicinal material in powder form was dried in an oven with hot air that was set under 50 degrees Celsius.To end, the residue was further subjected to the maceration process with distilled water for four hours duration for the water-based extract preparation. Every extract was then subjected to concentrate in a water bath, then concentrated mixture were submitted to the process of freeze drying and the whole extract was stored at 4ºc till further use. All the prepared extracts were then weighed (11-13).

The plant extract was utilized to investigate qualitatively for the presence of Alkaloids, Carbohydrates, tannins, flavonoids, Saponins, Terpenoids, etc. by using the standard methods (14-19).

2.4.1 Alkaloids

i. Dragendorff's Test

5 ml of water that is distilled should be added to 2 milligrams of the extract. Added 1 milliliter of Dragendorff's reagent after that. Alkaloids are present when a precipitate that is orange or orange-red forms.

ii. Hager's Test

Added couple of drops of Hager's reagent to a test tube containing 2 milligrams of the extract. The presence of alkaloids is verified by the production of a yellow precipitate.

iii. Wagner's Test

2 milligrams of the extract are acidified with 1.5% v/v Sodium hydroxide and then Wagner's reagent is added in little amounts. When alkaloids are present, a precipitate that is yellow or brown forms.

iv. Mayer's Test

Added several drop of Mayer's reagent to 2 milligrams of the extract. The existence of alkaloids is indicated by the production of a yellow precipitate.

2.4.2 Carbohydrates

i. Molish's Test

Place two milliliters of the extract and two drops of a recently made 20% alcoholic naphthol solution into a test tube. To create a layer underneath the mixture, add 2 milliliters of sulfuric acid in its concentrated form. When too much alkali is added, a reddish-violet ring forms, signifying the existence of carbohydrates.

.**Benedict's Test**

In a test tube, add 5 ml of Benedict's solution to 0.5 milliliters of the extract. Boil in a bath of water for five minutes. Reducing sugars are present when a precipitate with a brick-red color form.

ii. Fehling's Test

Add 1 ml of a combination of equal parts Fehling's Solution A and B to 2 milliliters of the extract. For a few minutes, boil. The existence of reducing sugar is indicated by the production of a red or a brick red colored precipitate.

2.4.3 Glycosides

i. Kedde's Test

A single drop of 90 percent alcohol and a second drop of 2% 3,5-dinitrobenzoic acid in 90 percent alcohol rendered alkaline with 20% NaOH solution should be added to 2 mg of the extract. The development of a purple hue signifies the existence of glycosides.

ii. Keller-Killani Test

Add 0.4 milliliter of glaciated acetic acid with a hint of ferric chloride to two milligrams of the extract. Pour 0.5 ml of conc. H_2SO_4 slowly down the test tube's side. The existence of cardiac glycosides is indicated by the acetic acid layer taking on a blue hue.

2.4.4 Flavonoids

i. Shinoda Test

To the dried powdered extract, add 5 milliliters of 95 percent ethanol and just a few drop of conc. HCl. The appearance of a reddish-pink or brown tint suggests the existence of flavonoids.

ii. Zinc-HCl Reduction Test

To the test solution, added a mixture of strong Hydrochloric and zinc dust. After a few a few minutes, the appearance of red

shows the existence of flavonoids.

2.4.5 Proteins

i. Biuret Test

Added 6-8 droplets of 10 percent w/v the copper sulfate mixture as well as 2-3a couple of drops of 3 percent w/v copper sulfate sol. to 1 milliliter of the extract. The existence of protein is shown by the development of a violet-red hue.

ii. Millon's Test

Added 6-8 droplets of 10 percent w/v the copper sulfate mixture as well as 2-3a couple of drops of 3 percent w/v copper sulfate sol. to 1 milliliter of the extract. The existence of protein is shown by the development of a violet-red hue.

2.4.6 Resins

One milliliter of the extract should be dissolved in acetone before being added to water that was distilled. The turbidity that develops suggests the existence of resins.

2.4.7 Saponins

In a test tube, dilute 5 milliliters of the extracts in 5 ml of filtered water and give it a good shake. The existence of saponins is indicated by the production of steady, persistent foam.

2.4.8 Steroids

i. Liebermann-Burchard Test

Melt 2 milligrams of extracted substance in a solution of acetic anhydride, bring to a boil, then let cool. The test tube should then contain 1 milliliter of concentrated sulfuric acid. The development of a red hue signifies the existence of steroids.

ii. Salkowski Reaction

After shaking two milligrams of dried extract with chloroform, gradually add some sulfuric acid to the layer of chloroform that has formed along the test tube's walls. The development of a crimson hue signifies the existence of steroids.

2.4.9 Tannins

Dissolve 0.5 g of the extract in 5 ml of distilled water. Add a few drops of 1% ferric chloride solution. The formation of a brownish-green or blue-black precipitate confirms the presence of tannins.

2.4.10 Phenols

Dissolve 2 ml of the extract in 4 ml of distilled water and add a few drops of 10% ferric chloride solution. The formation of a blue or green color indicates the presence of phenols.

2.5 Total Phenolic Content Determination of *Cassia auriculata* Linn.

2.5.1. Standard Preparation

Following the dissolution of 10 milligrams of gallic acid was used in 100 ml of diluted methanol, additional dilute solutions (1, 2, 4, 8, and 16 mg/ml) were created. The test tubes were filled with 1 milliliter of each diluting, 10 milliliters of distilled water, and 5 milliliters of Folin-Ciocalteu reagent. After allowing the solution to stand at room temperature for five minutes, each test tube received four milliliters of a 20% w/w sodium carbonates solution. After shaking and diluting the mixture with 25 cc of distilled water, it was allowed to sit at room temperature. Using a spectrophotometer, the absorption of the standard sol. was determined at 756 nm in relation to a blank.

2.5.2. Test Preparation

The plant extraction (1 mg/ml) was dissolved in 10 ml of distilled water using a methanol solution. After adding 1.5 milliliters of Folin-Ciocalteu reagent, the mixture was allowed to sit at room temp for five minutes. Next, 4 milliliters of a 20 percent w/w sodium carbonate sol. were then added, diluting the mixture to yield 5 milliliters. After shaking, the mixture was allowed to sit at room temp for half an hour. Using distilled water as a blank, the sample's absorbance was measured at 756 nm using spectrophotometry (20,21).

2.6 IN VITRO ANTIOXIDANT STUDIES

All extract of *Cassia auriculata* linn. showed antioxidant activity using improving assay based on the decolorization of the radical monocation of ferric reducing antioxidant power (FRAP), and 1,1- diphenyl- 2- Picrylhydrazyl (DPPH) radical scavenging method.

2.6.1. DPPH (1, 1-diphenyl-2-picryl hydrazyl) method

2.6.1.1 Preparation of Extract Dilutions

50 mg from every plant extract of whole plant was weighed and a stock solution of 500 µg/ ml was made by adding a required quantity of extract in 100 ml of their plant extract prepared solvent separately. various dilutions at various dilutions of 10, 20, 40, 80, 160 µg /ml were made by preparing dilutions of stock solution with particular solvent.

2.6.1.2 Preparation of Standard Dilutions

Ascorbic acid was weighed (10 mg) separately and stock solution i.e., 100 µg/ml was made by dissolving ascorbic acid in 100 ml of particular solvent. Different concentrations of ascorbic acid (5, 10, 15, 20, 25 µg/ml) was prepared by making dilutions of stock solution with particular solvent.

2.6.1.3 Method

The stable method 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was utilized for determinations the Free Radicals Gathering Activities of the prepared extracts of the plant. Fresh preparation of the DPPH was made by dissolving the DPPH (0.1 mM) in particular solvent (i.e., 22.2 mg in 1000 ml). To this DPPH solution, equal volume of altered dilutions of different prepared plant extracts was added individually. After keeping the prepared solution at moderate temperature at which 517 nm, the absorbance was measured for around half an hour. Ascorbic acid was served as standard compound. The concentration of sample represented by IC_{50} values which were requisite for the 50% scavenging of D.P.P.H free radicals. Radical Scavenging power or % inhibition determined by the below mentioned formula

i.e.,

$$\% \text{ Inhibition} = \frac{\text{Absorbance [Control- (Sample – Blank)]}}{\text{Absorbance of Control}} \times 100$$

IC_{50}value was evaluated by plotting a graph between radical scavenging power v/s different concentrations of different prepared extracts of whole plant. Three readings were taken and their scavenging power was estimated depending upon the percentage of DPPH scavenged (22,23).

2.6.2. FRPA Method

2.6.2.1. Preparation of Extract Dilutions`

50 mg of all the plant extracts of whole plant material was weighed separately and 500 µg/ml solution in stock was created by dissolving the extract in 100 ml of particular solvent individually. Different dilutions of variable concentrations like 25, 50, 100, 200 and 400 microgram /milliliters were prepared by preparing dilutions of solvents used for preparing the extract.

2.6.2.2. Preparation of Standard Dilutions

50 mg of ascorbic acid was weighed and a stock solution of 500µg/ml was made by dissolving ascorbic acid in 100 ml of the prepared solvent. Various concentrations of 25, 50, 100, 200 and 400 microgram /milliliters were made by utilizing the specific solvent to dilute the stock solution.

2.6.2.3. Method

1 ml of various plant extracts of whole plant material which means 25–400 µg/ ml was subjected to mixing with addition of phosphate buffer (2.5 ml) which should be prepared and maintained at pH 6.4 and addition of 1 percent C6N6FeK3 (2.5 ml). Then, resulting solution subjected to the incubation process at about 50 ºc for around 30 minutes of duration and combined with 2.5 milliliters of ten percent trichloroacetic acid. Further the mixture subjected to the centrifugation process at around 3000 RPM for about 10 mins. Then, around 2.5 ml of the upper marc was diluted through the same quantity of distilled water & shaked with 0.5 ml of 0.1% of ferric chloride solution. Then, the absortion was finds at 700 nanometers. Among which L-Ascorbic acid was served as the standard compound. Three readings were recorded and graph was plotted as an average of three readings (22-24).

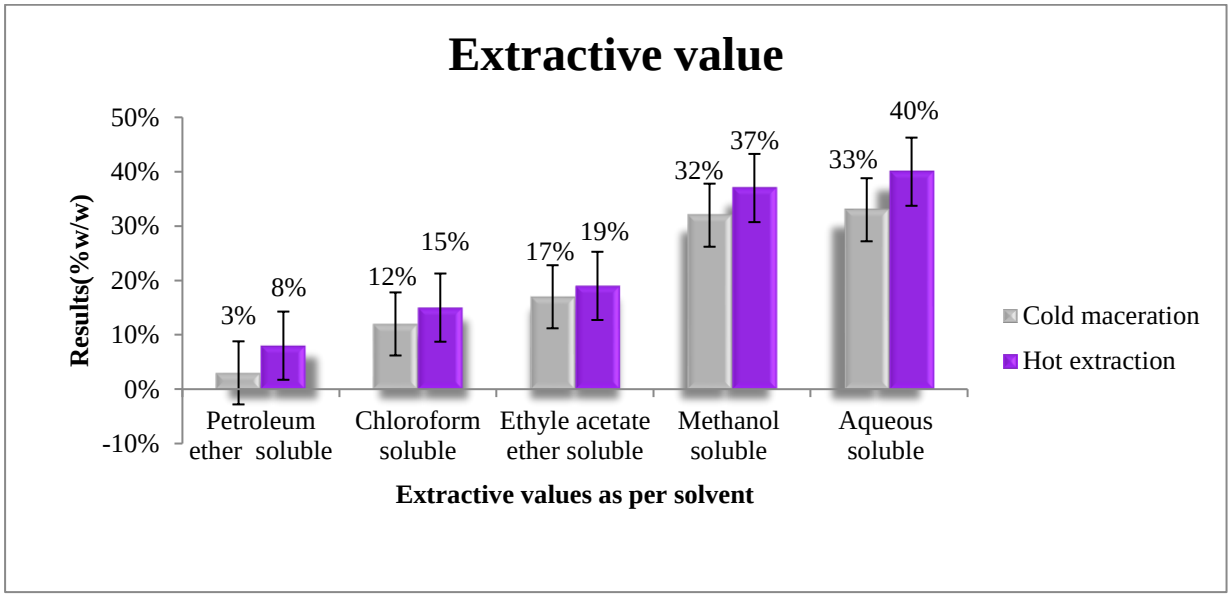
3. RESULT AND DISCUSSION

3.1 Extractive value

Table.1. Extractive value of Cassia auriculata L. in different solvent by hot extraction and cold maceration.

S.No.	Extractive values	Result (% w/w)	
		Hot extraction	Cold maceration
1.	Water soluble	40%	33%
2.	Methanol soluble	37%	32%

3.	Ethyle acetate soluble	19%	17%
4.	Chloroform soluble	15%	12%
5.	Petroleum ether soluble	8%	3%



Graph no. 1. Extractive value of *Cassia auriculata* L. in different solvent by hot extraction and cold maceration.

3.2 Phytochemical analysis:

The extract obtained was subjected to the qualitative chemical test to detect the major primary & secondary active constituents. Result showed that plant was rich in phenolic and flavonoid content.

Table.2 Result of Preliminary Phytochemical investigation of *Cassia auriculata* L. aerial part.

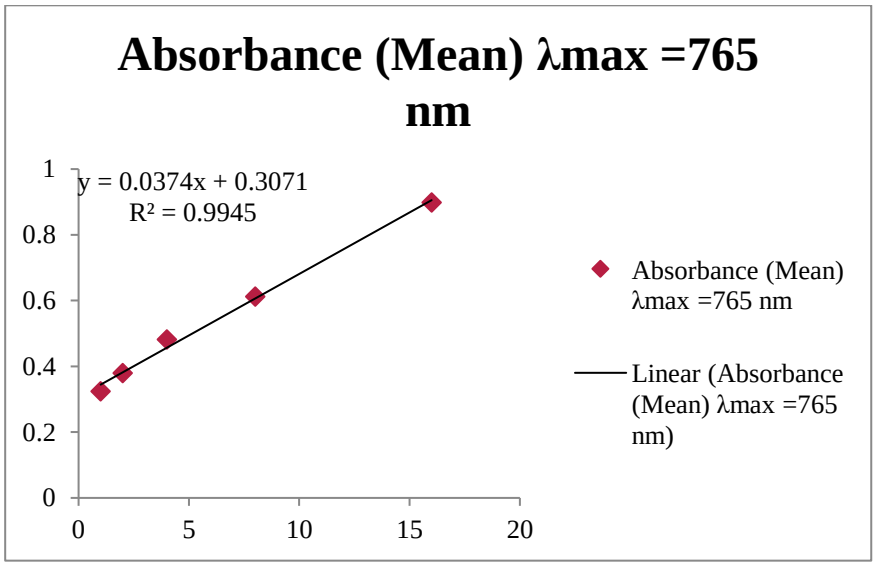
S. no	Chemical constituent	Petroleum ether	chloroform	Ethyl acetate	Methanol	Aqueous
1	Carbohydrates	-	-	-	+	+
2	Alkaloids	-	+++	+	++	-
3	Glycosides	-	+	+	+	-
4	Phenolic compound and tannin	-	-	+	+++	++
5	Saponins	-	-	+	+	-
6	Flavonoids	+	_	+	+++	++
7	Fixed oil and fat	++	+	-	-	-
8	Protein and amino acid	-	-	-	-	+
9	Steroids	-	-	-	+	-

3.3 Determination of total phenolic compound

The total phenolic content in *Cassia auriculata* linn. extract can depend on factors like the extraction method, plant age & environmental conditions. Typically, Spectrophotometric assays such as the Folin– Ciocalteu method are used to determine this content. In the present study total phenolic content of methanolic extract was found to be highest as significant as standard (Gallic acid).

Table No. 3: Absorbance of Standard Compound (Gallic Acid)

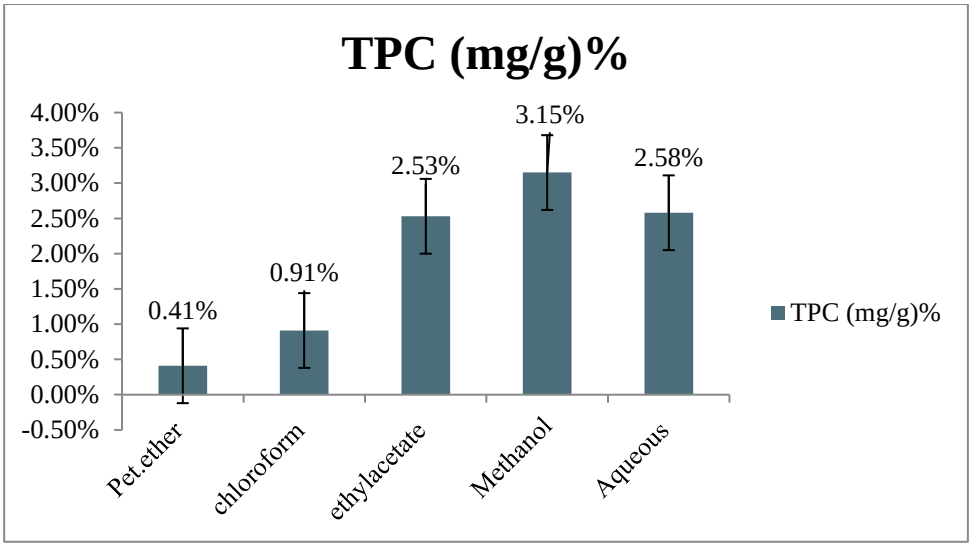
S. No.	Concentration (µg/ml)	Absorbance (Mean) λmax =765 nm
1.	1	0.324
2.	2	0.379
3.	4	0.482
4.	8	0.612
5.	16	0.898



Graph. No. 2: Standard Gallic Acid Calibration Curve for the evaluation of TPC of the plant *Cassia auriculata* L.

Table 4: TPC of *Cassia auriculata* L. Plant extracts

S.no.	Name of Plant extract	TPC (mg/g)%
1	Petroleum ether	0.41%
2	Chloroform	0.91%
3	Ethyle acetate	2.53%
4	Methanol	3.15%
5	Aqueous	2.58%



Graph. No. 3: TPC of the plant *Cassia auriculata* L. in different solvent

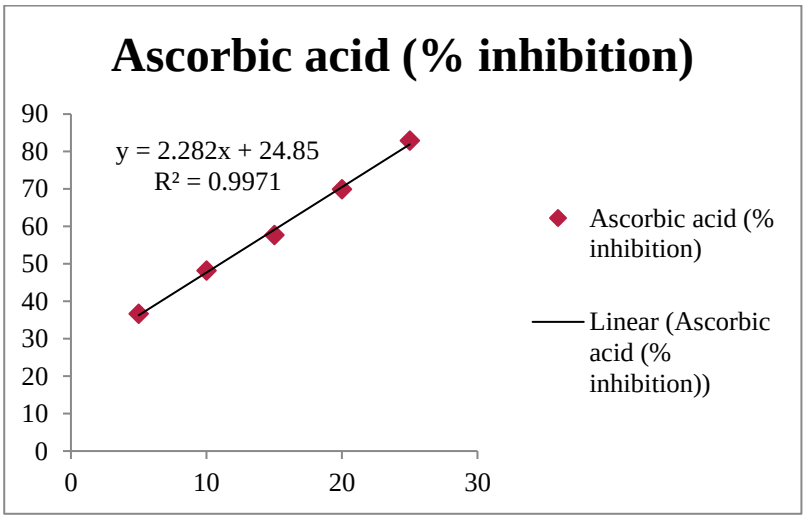
3.4 INVITRO ANTIOXIDANT ACTIVITY

3.4.1 DPPH (1,1 diphenyl -2- picrylhydrazyl) method

The antioxidant activity of the aerial part of *Cassia auriculata* L. measured by DPPH assay showed that the antioxidant activity varied in different solvents, the DPPH activity was found to be highest in methanolic extracts of *C. auriculata* (IC_{50} 37.34mg/ml) which was lies near to standard IC_{50} value (11.05 mg/ml).

Table 5. %Inhibition of Ascorbic acid in DPPH Activity

S.no.	Concentration (µg/ml)	Ascorbic acid (% inhibition)
1	5	36.7
2	10	48.2
3	15	57.7
4	20	69.9
5	25	82.9

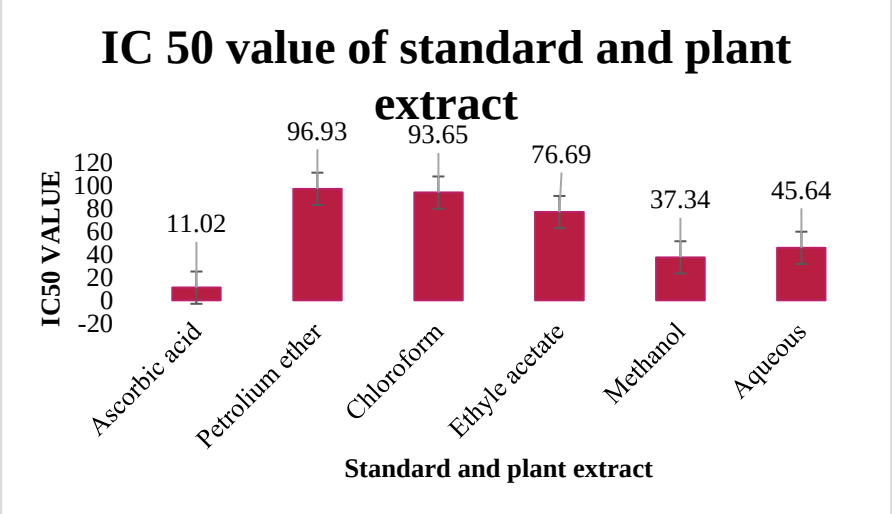


Graph no. 4: %Inhibition of Ascorbic acid in DPPH Activity

Table 6.- %inhibition of *Cassia auriculata* L. by DPPH method

Concentration (µg/ml)	Petroleum ether extract (% inhibition)	Chloroform extract (% inhibition)	Ethyl acetate extract (% inhibition)	Methanol extract (% inhibition)	Aqueous extract (% inhibition)
10	17.89	27.89	20.89	36.89	33.89
20	24.93	31.93	28.93	43.93	40.93
40	32.73	39.83	33.83	51.83	48.83
80	47.23	40.24	57.23	68.24	65.21
160	69.24	69.24	80.24	99.24	92.24
IC_{50}	96.93mg/ml	93.65 mg/ml	76.69 mg/ml	37.34 mg/ml	45.64 mg/ml

IC_{50} value of ascorbic acid was found to be 11.02 %



Graph no.5: IC_{50} value of different standard and different solvent of plant extract

3.4.2 Ferric reducing antioxidant power (FRAP) method

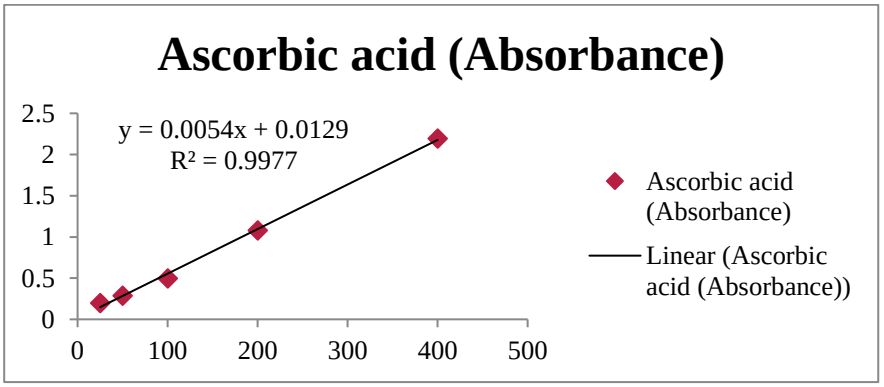
By measuring a substance's ability to convert ferric ions into ferrous ions through a redox-linked colorimetric process, the frap technique quantifies its antioxidant capability. This reduction if monitored spectrophotometrically, usually at 700nm. And the FRAP activity was found to be highest in methanolic extract of aerial part of the *Cassia auriculata* L. as shown in table no. 8.

Table No.7 Absorbance of standard ascorbic acid by FRAP method

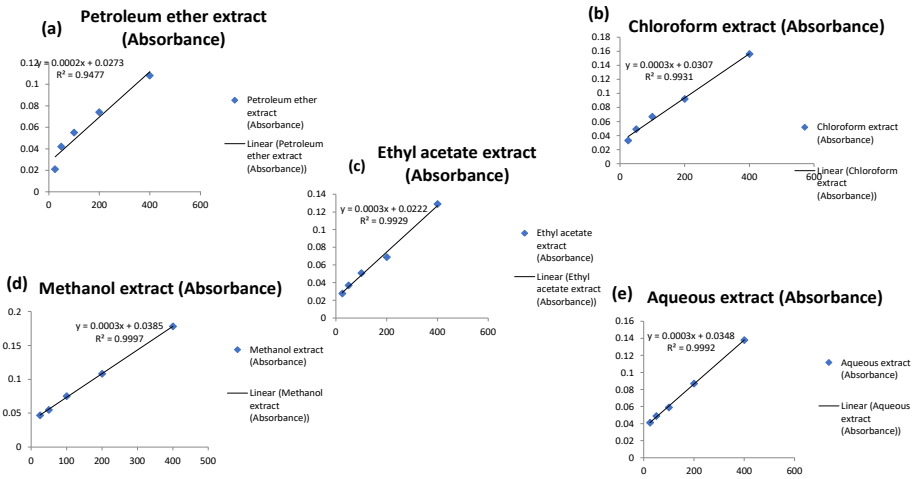
S.no.	Concentration (µg/ml)	Ascorbic acid (Absorbance)
1	**25**	0.198
2	**50**	0.287
3	**100**	0.499
4	**200**	1.078
5	**400**	2.196

Table 8. Antioxidant activity of *Cassia auriculata* by FRAP method

Concentration (µg/ml)	Petroleum ether extract (Absorbance)	Chloroform extract (Absorbance)	Ethyl acetate extract (Absorbance)	Methanol extract (Absorbance)	Aqueous extract (Absorbance)
25	0.021	0.033	0.028	0.047	0.041
50	0.042	0.049	0.037	0.055	0.049
100	0.055	0.067	0.051	0.075	0.059
200	0.074	0.192	0.069	0.108	0.087
400	0.108	0.156	0.129	0.178	0.138



Graph no. 6: Absorbance of Ascorbic acid in FRAP Activity.



Graph no.-7: FRAP of different extract of *Cassia auriculata* l. (a) pet ether extract, (b) chloroform extract, (c) ethyl acetate extract, (d) methanol extract and (e) aqueous extract.

4. CONCLUSION

The phytochemical analysis showed that methanolic extract of *Cassia auriculata* have phenolic and flavonoids in rich quantity. The methanolic extracts of C. auriculata L. aerial part showed antioxidant activity which was concluded by DPPH and FRAP method, due to the presence of high level of phenolic compounds as compared to other extracts. Therefore, the antioxidant property may be one of the mechanisms by which this drug is useful for pharmacological activity.

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