Comparative Studies on Antioxidant Capacity , Total Phenolic and Total Flavonoid Contents of *Senna auriculate* and *Cissus quadrangularis*herbs extract

S. Divya* & Dr. S. Grace Annapoorani**

*Research Scholar, **Professor, Department of Textiles and Apparel Design, Bharathiar University, Coimbatore, Tamil Nadu India.
mail id:divya16091988@gmail.com

ABSTRACT

It is found that numerous plant species play an significant role in daily routine. The precious source of organic compounds are plant kingdoms, many of which have been used for medicinal purposes. There are many phytochemicals in a number of ways help the human body. Phytochemicals are compounds of herbs which are not rich in nutrients and have protective or preventive characteristics for disease. The present investigation was designed to evaluate the antioxidant properties of ethanolic extract of *Senna auriculate*and *Cassia auriculata.* The research was to explore the dye yield extract proportion, phytochemical analysis and antioxidant value quantification of complete phenolic and flavonoid content. The research results show the phytochemicals present and the polyphenols and flavonoids were quantified.

Keywords: phytochemicals, ethanol, antioxidant, total phenolics and flavonoids content.

1.Introduction

Antioxidant is a molecule capable of destroying chain reactions that harm cells by removing free radical intermediates and inhibiting other oxidation responses by decreasing stress caused by many degenerative disorders.*Cissus quadrangularis L*.(CQ) is one of the important medicinal plants, belonging to the family Vitaceae and common name is 'Hadjod'. *Senna auriculata (L.)Roxb* (SA) (Family: Fabaceae) is widely used in Indian traditional medicines and flowers are usedfor diabetes leaves and flowers to treat skin diseases leaf juice is used to reduce body heat. In Ayurvedic Medicine, these two herbs that have historically been used to treat a wide range of diseases.Medicinal plants are the wealthiest bio-resource of traditional medicine systems drugs, contemporary medications, nutraceuticals, food supplements, folk medicines, intermediate pharmaceuticals, and synthetic drug chemical entities. The Dye yield % was good in SA than CQ.The best results were obtained with SA extract concentration of 100 showing the highest IC50 value of 44.903 µg/ml.

2.MATERIALS AND METHODS

2.1 Chemicals

All routine chemicals such as Ascorbic acid, Gallic acid, Folin-Ciocalteu, were obtained from Presision scientific, Coimbatore, Tamil Nadu, India.

2.2 Collection and authentication of plant

Senna auriculate (SA) and *Cissus quadrangularis* (CQ) was collected from the local Therampalayam, Coimbatore district,Tamil Nadu. The herbarium of the plant was prepared, authenticated by Forest Genetic and Tree Breeding, Coimbatore, Tamil Nadu.

2.3 Preparation of Plant Extract

The collected plant was shadow dried and grinded in powder form. The powder was mesh sieve and exhaustively extracted with 90% (v/v) (200ml) ethanol by cold maceration process at room temperature for 90 hrs . The extract was filtered and the filtrate was evaporated until all the solvent had been removed and further removal of water was carried out by freeze drying to give *an* extract sample with the yield *Senna auriculate* of 15.5% (w/w) and *Cissus quadrangularis* extract yield was 9 % (w/w) The extracts were stored in refrigerator and used for the future investigation

Calculation of yields

The yields were calculated using the following formula:

Yield= (residue weight/ original dry weight) × 100

The residue represents the extract or the fraction following fractionation, while the dry weight becomes the residue after evaporation.

2.4 Preliminary Phytochemical Analysis of Plant Extract

2.4.1 Phytochemical screening

Preliminary phytochemical screening was studied for all the herb crude ethanol extracts were concentrated and several chemical tests were performed to test the presence of various phytochemicals like Carbohydrates, Proteins, Phenolic, Flavonoids and tannins.

2.4.2 Carbohydrates (Molish`s test)

About 10 mg of the samples were dissolved in 5 ml of distilled water and filtered using waterman No.1 fiilter paper.2drops of α-napthol (dissolved in ethanol were added to 2ml of the filtered and 1ml of conc.sulphuric acid (H_2SO_4) was added slowly and left at room temperature for few minutes. A development of violet ring indicate the presence of carbohydrates.

2.4.3 Proteins (Biuret test)

The sample 10mg were dissolved in 10ml of distilled water and filtered through Waterman No.1 filter paper. Then, 2 mL of the filters were treated with one drop of 2% copper sulphate solution. To this, 1mL of 95% ethanol was added, followed by excess of potassium hydroxide (KoH) pellets. The formation of pink color in the ethanolic layers indicated presence of proteins.

2.4.4 Phenolic Compounds (Ferric chloride test)

Few drops of neutral 5% ferric chloride ($FeCl_3$) solution were added to the sample filtrates (5 mg of samples was dissolved in 5mL of dissolved water). The presence of phenolic compounds was indicated by the presence of dark green color.

2.4.5 Flavonoids (Alkaline reagent test)

An aqueous solution (2 mg/mL) of the samples was suspended in 10% ammonium hydroxide solution. A bulky white precipitated indicate the presence of flavonoids.

2.4.6 Tannin (Potassium hydroxide test)

The samples (100mg) were added into 5mL of freshly prepared 10% of KoH and gently shaken. A dirty precipitate indicate the presence of tannin.

2.5 Phytochemical estimation

The most effective components responsible for antioxidant potential seem to be flavonoids and phenolic compounds of many plants raw materials. Their metal chelating capabilities and radical scavenging properties have enabled phenolic compounds to be thought of as effective free radical scavengers and inhibitors of lipid peroxidation.

2.5.1 Quantification of Total phenolic compound

The total phenol content was determined in the total extract 0.5mL of Folin – Ciocalteu reagent (1:1 with water) and 2.5mL of sodium carbonate solution (20%) were added sequentially in each test tube containing different extracts. Soon after vortexing the reaction mixture, the test tube were placed in dark for 40 minutes and the absorbance was recorded at 725 nm in a UV-spectrophotometer against blank. The total phenolic content were expressed as Gallic acid equivalents (GAEs). All experiments were performed in triplicates.

Total phenolic compound extract was determined by applying the following equation:

$C = C1 \times V/m$

Where; C= Total content of phenolic compound in mg/g, in GAE (Gallic acid equation),

C1 = Concentration of Gallic acid established from the calibration curve in mg/mL,

V =Volume of extract in mL,

m = Weight of plant extract in gram.

2.5.2 Quantification of total flavonoids

The flavonoid contents of all the extracts were quantified About 500 µL of different extracts were taken in different test tubes and 2ml of distilled water were added to each of the test tube .A test tube containing 2.5 mL of distilled water served as blank. Then, 150 µL of 5% $NaNO_2$ was added to all the testtubes followed by incubation at room temperature for 6 minutes. After incubation, 150 µL of 10% $AlCl_3$ was added to all the test tubes including the blank. All the test tubes were incubated for 6 minutes at room temperature. Then 2mL of 4% NaOH was added to all the test tubes which were made up to 5mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 min at room temperature .The mixture was shaken vigorously for thorough mixing the pink color developed due to the presence of flavonoids was read at 510 nm in a UV-spectrophotometer. Quercetin was used as the standard, and the total flavonoid content was expressed in terms of quercetin equivalent (QE).

2.6 Total antioxidant Capacity (TAC) Phosphomolybdate assay

Total antioxidant activity of SA and CQ was determined using ascorbic acid as the standard. Phosphomolybdate reagent was prepared by mixing 100 ml of 28 mM sodium phosphate, 100 ml of 0.6 M sulphuric acid and 100 ml of 4 mM ammonium molybdate solutions. To 3 ml of phosphomolybdate reagent, 300 µl of different concentrations (100 µg/ml) of extract solution were added and incubated in the

dark for 90 min at 95°. The absorbance was measured spectrophotometrically at 765 nm. TAC %scavenging = (A0–A1/A0)×100, where, A0 is the absorbance of the control after 10 min and A1 is the absorbance of the sample at 10 min. The concentration of extract at which 50% inhibition is observed (IC50) is calculated in µg/ml.

3. RESULTS & DISCUSSION

3.1 Extract Yeild %

Yield of extracts SA plant ethanol extract, CQ plant ethanol extract were shown below table 1 and figure 1.

S.No	Plant Name	Pre Weight (W1)	Post Weight (W2)	Extract Yeild %
1	SA	96.4	99.5	15.5
2	CQ	108.4	110.2	9

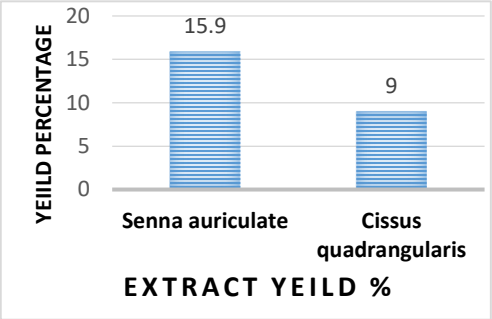


Table: 1 Extracts yield percentage of SA and CQ **Fig. 1:** Extracts yield percentage of SA and CQ.

The above result shows that SA contains maximum dye yield than CQ.

3.2 Phytochemical screening

Preliminary phytochemical screening color observation and the presents of components in SA and CQ plant showed the presence of proteins, carbohydrates, phenolic flavonoids and tannins. Was studied and the result were given in below table 2

Carbohydrats	Violet color ring (alpha napthol, ethanol, Conc. Sulphuric acid)
Proteins	Pink color layer (Copper Sulphate, ethanol, potassium hydroxide)
Phenolic	Dark Green (Ferric Chloride)
Flavonoids	Bulky white present (ammonium hydroxide)
Tannins	Dirty Presence (Potassium hydroxide)

S.No	Plant Name	Proteins	Carbohydrates	Phenolic	Flavonoids	Tannins
1	SA	+	++	+	+	+
2	CQ	-	+	+	++	+

(+) shows the Present (-) shows the absent

Table: 2 Phytochemical screening

The results of quantitative estimation of total phenolic content of 100 concentration given below as fig 2a and 2b.

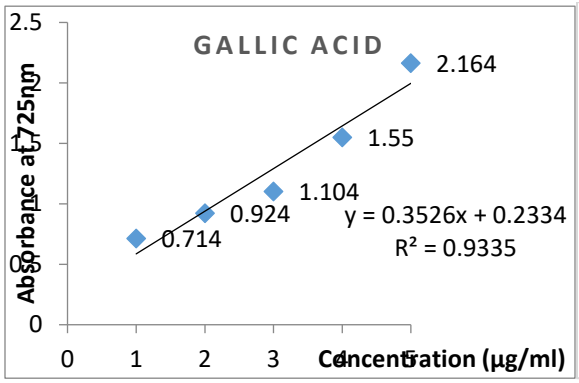


Fig. 2a: Standard curve of gallic acid

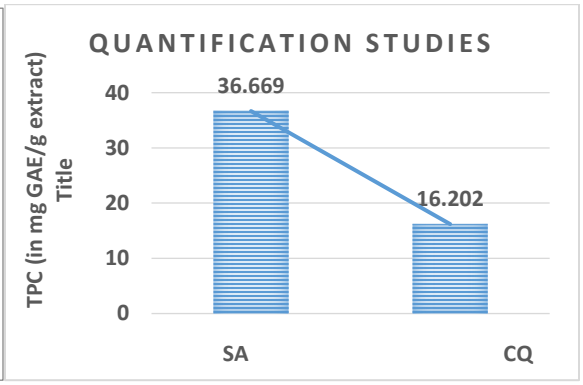
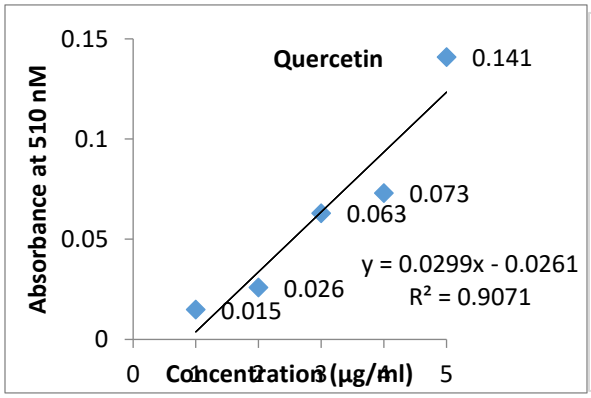


Fig 2b: Quantification of estimation of total phenolic content of SA and CQ

It is clearly evident from the results that TPC using Gallic acid extract. SA total phenolic content extract was higher than CQ.

3.4 Quantification of total flavonoids

The results of quantitative estimation of total flavonoids content of 100 concentration given below as figure 3a and 3b.



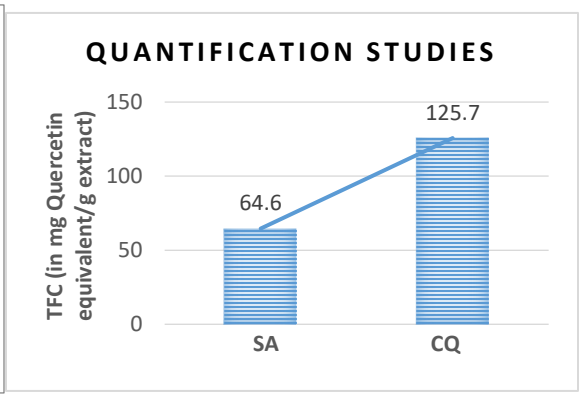
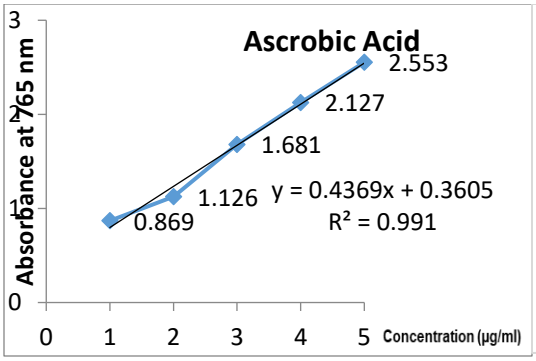


Figure. 3a: Standard curve of Quercetin Figure 3b: Quantification of estimation of total flavonoids content of SA and CQ

It is clearly evident from the results that TPC using quercetin extract. CQ total flavonoids content extract was higher than SA.

3.5 TAC using Phosphomolybdate assay

Total antioxidant capacity of SA and CQ was determined using ascorbic acid as the standard and the results are shown in figure 4a and 4b.



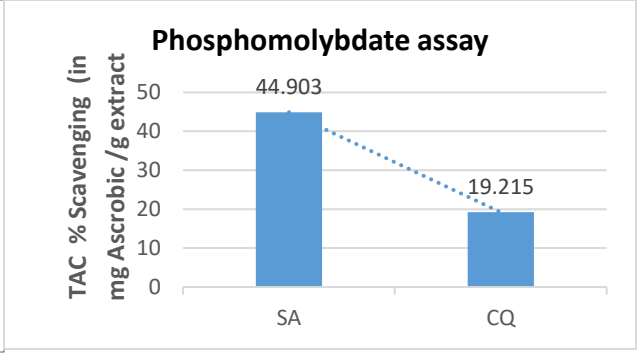


Fig. 4a: Standard curve of Ascrobic acid Fig 4b: Total Antioxidant Capacity% % scavenging of SA and CQ

Phosphomolybdate assay measures the capacity of an extract to destroy a free radical by transferring an electron to the later. Antioxidants present in the extract can be measured spectrophotometrically at 765 nm. Result of phosphomolybdate assay was illustrated in figure. 4a and 4b.

CONCLUSION

The findings of this inquiry revealed the existence of many phytochemicals, among which flavonoids were the predominant that could be necessary for the antioxidant property of the same in the ethanol extract of *Senna auriculate* (SA), *Cissus quadrangularis* (CQ) could be the necessary solvent for recovering elevated antioxidant-capacity polyphenolic compounds.Since this medicinal plant's ethanolic extract has excellent antioxidant characteristics, the oxidative stress that should be explored using appropriate animal models in the future may be prevented.It will be useful for human society to explore the medicinal value of such indigenous herbs to fight chronic diseases.The best results were obtained with SA extract concentration of 100 showing the highest IC50 value of 44.903 µg/ml. The IC50 value can be further reduced by using purified extracts. The total reducing capacity increased in dose dependent manner of the extracts.

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Corresponding Author

S.Divya

Research Scholar,

Department of Textiles and Apparel Design,

Bharathiar University,

Coimbatore,

Tanil Nadu -641046

Mobile no:09626214958

E.Mail id: divya16091988@gmail.com