

DECODING NATURAL ANTIOXIDANTS: PHYTOCHEMICAL RICHNESS AND DPPH SCAVENGING ACTIVITY OF *Cassia auriculata* FLOWER ETHANOLIC EXTRACT

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ABSTRACT

The present study aimed to evaluate the phytochemical constituents and *in-vitro* antioxidant potential of the ethanolic flower extract of *Cassia auriculata*, a medicinal plant widely used in traditional systems of medicine. *Cassia auriculata* belongs to the family Fabaceae and is traditionally employed for the management of diabetes, skin disorders, inflammation and urinary diseases. The dried flower extract was prepared using ethanol and subjected to quantitative estimation of total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical scavenging assay to assess antioxidant activity. The results revealed that the *Cassia auriculata* flower ethanolic extract possessed significant phytochemical constituents, with total phenolic content of 134.61 ± 14.80 mg Gallic acid equivalents/g and total flavonoid content of 107.20 ± 11.79 mg Quercetin equivalents/g. The antioxidant activity evaluated by DPPH assay demonstrated concentration-dependent radical scavenging potential with IC_{50} value of $53.53 \mu\text{g/ml}$ which was comparable to the standard ascorbic acid ($IC_{50} = 51.54 \mu\text{g/ml}$). These findings indicate that the antioxidant activity of *Cassia auriculata* flower ethanolic extract may be attributed to its rich phenolic and flavonoid content. Therapeutic relevance of *Cassia auriculata* as a promising natural antioxidant source. The plant extract could be utilizing in the development of herbal formulations for preventing oxidative stress-related disorders.

Keywords: *Cassia auriculata* flower ethanolic extract, Total Phenolic content, Total Flavonoid content, Antioxidant activity, DPPH Radical Scavenging assay.

INTRODUCTION

Medicinal Plant

Medicinal plants also referred to as therapeutic herbs, have been widely used in traditional systems of medicine for centuries to prevent and treat various diseases. They are considered safer and more compatible with the human body compared to synthetic drugs because of their natural origin and minimal side effects. Medicinal plants have been used since ancient times as the primary source of health care. The World Health Organization (WHO) estimates that more than 80% of the population in developing countries relies on herbal medicine for treating various ailments due to their low cost, accessibility and safety compared to synthetic drugs. Traditional system of medicine like Ayurveda, Siddha, Unnai and Chinese medicine have documented thousands of plants for the management of disease ranging from infection to chronic conditions such as diabetes, cancer, and cardiovascular disorder. WHO has listed over 21000 plant species used around the world for medicinal purpose. Nearly 50% of pharmaceuticals in current use for derived from natural source, demonstrating the importance of phytochemicals leads to novel drug development.

Cassia auriculata

The *cassia auriculata* commonly known as a Avaram poo. It belong to the family Fabaceae (Leguminosae) and is a small, branched shrub native to dry region of India and Sri Lanka. The plant typically growth in dry zones, open forests and wastelands. It also reach about 1- 1.5 in height. It is characterized by bright yellow colour flowers, paripinnate green leaves and flat leguminous buds containing 7-10 seeds.

The plant is commonly known as Tanner's Cassia in English and Avaram senna in Tamil. Almost every part of the plant has medicinal value. Traditionally Avaram poo has been used for treating diabetes, urinary disorders, skin diseases and wounds. Women in Southern India have long used the flowers for maintaining ethnomedicinal relevance as a natural cosmetic agent. The plant have a phytochemical investigation that contains several bioactive constituents such as flavonoids, phenolic compounds, tannins, saponins, alkaloids and glycosides.



Figure 1: *cassia auriculata*

PLANT PROFILE

Botanical name : *Cassia auriculata*

Common name : Avaram poo

Family : Fabaceae

Genus : Cassia

Species : *C. auriculata*
Symbol : CAUR
Legal status : Commonly found wild shrub in dry regions

SYNONYMS & VERNACULAR NAMES

Tamil : Avaram poo
English : Tanner's Cassia
Malayalam : Avaram poo
Hindi : Tarwar
Sanskrit : Pitapuspa
Kannada : Avarike, Tangedi
Telugu : Tangedupuvvu

TAXONOMICAL STATUS

Kingdom : Plantea
Phylum : Tracheophyta
Class : Magnoliopsida
Family : Fabales
Genus : *Cassia*
Species : *Cassia auriculata* Linn.

BIOLOGICAL SOURCE

Biological source of Avaram Poo consists of the dried flower of *Cassia auriculata* Linn.

It belongs to the family Fabaceae.

ETHNOBOTANICAL USES

Table 1: Ethnobotanical uses

Plant parts	Ethnobotanical uses
Leaves	Used in treatment of skin diseases, eczema and wounds. It act as antimicrobial and cooling agent when applied a paste.
Flowers	Used for body cooling and treating skin disorder. Exhibits antioxidant and anti-inflammatory. It commonly used in herbal teas and cosmetics.
Seeds	It also used in diabetes management due to hypoglycaemic activity. When applied externally for treating ulcers, skin infection.
Pods	Traditionally given to women after childbirth for uterine cleaning and to enhance recovery.
Steam bark	It used for wound healing. It helps in controlling skin irritation and inflammation.
Root	Decoction used in fever, diabetes and urinary tract infection. Possesses antimicrobial and diuretic effect.

MATERIALS AND METHODS

Preparation of different concentrations of *Cassia auriculata* flower ethanolic extract

The *Cassia auriculata* flower ethanolic extract was dried in water bath at 37°C for 6 hrs. The *Cassia auriculata* flower ethanolic extract was then concentrated until the solvents was completely removed. A semi solid extract was obtained after complete elimination of solvent was stored in refrigerator. 10 gm sample dissolve in 100ml solvent prepare in different concentrations of sample (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml) and

similarly standard ascorbic acid. The different concentration of *Cassia auriculata* flower ethanolic extract was prepared and used for analysis of *in vitro* antioxidant assay.

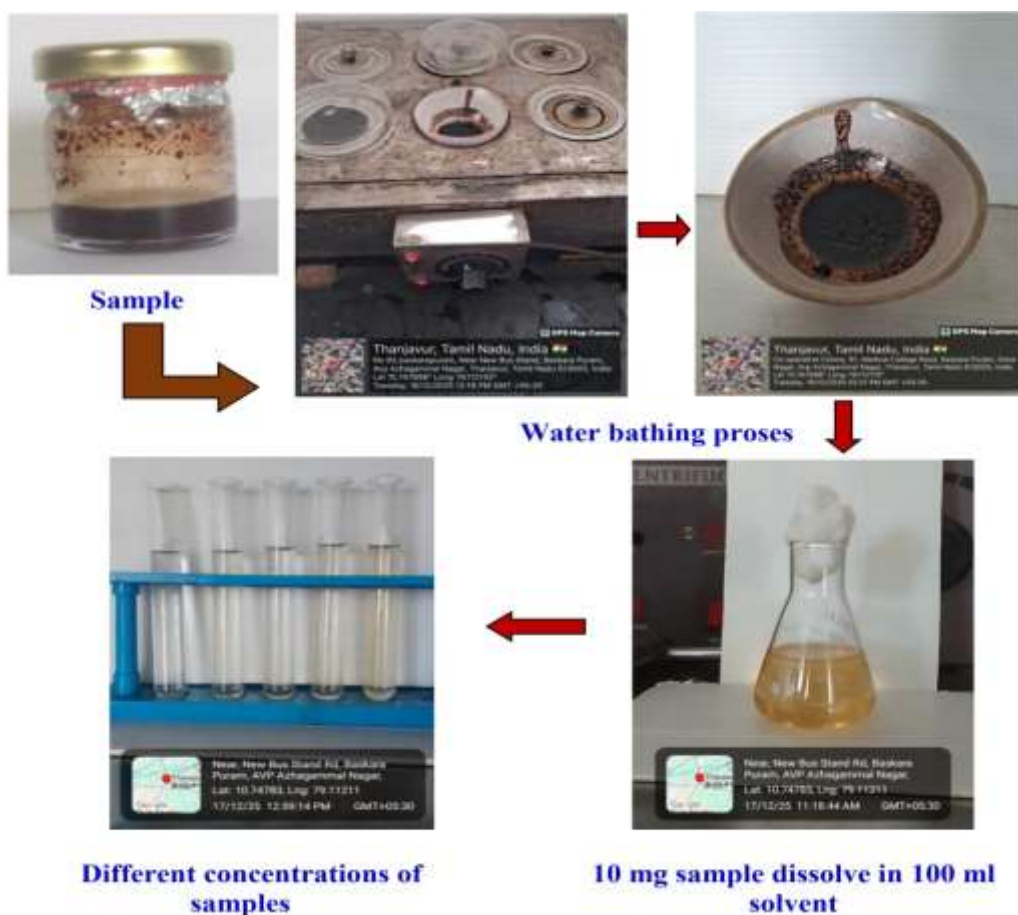


Figure 2: preparation of different concentrations of *Cassia auriculata* flower ethanolic extract

Total Phenols Determination

The amounts of total phenolic contents of different sample of *Cassia auriculata* flower ethanolic extract were determined.

Reagents

1. Folin-Ciocalteu's phenol reagent
2. 7% sodium carbonate
3. Galic acid (standard)
4. Preparation of plant extract

0.250g of plant powder (made or dried powder) with 2.5 ml of ethanol for 15 mins and filtered.

Procedure

A diluted filtrate different sample *Cassia auriculata* flower ethanolic extract (1ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin - Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 mins, 10 ml of 7% Na₂CO₃ solution mixed in to the test sample solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 mins at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by prepare in Gallic acid solution (10 to 100 µg/ml). The estimation of the phenolic compounds was carried out in triplicate. The total Phenol content was expressed as milligrams of Gallic acid equivalents per gram dried sample.

Total Flavonoids Determination

The total Flavonoids content was determined by using Aluminium chloride colorimetric method.

Reagents

1. 10% Aluminium chloride
2. 1M Potassium acetate
3. Quercetin (Standard)
4. Preparation of bark extract

0.250g of different leaves powder (dried powder) with 2.5 ml of water for 15 mins and filtered.

Procedure

Different sample of *Cassia auriculata* flower ethanolic extract (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV – Visible spectrophotometer. The calibration curve was prepared by preparing Quercetin solutions at concentration 10 to 100 µg/ml in methanol. The Total flavonoids Content was expressed as milligrams of Quercetin equivalents per gram of dried sample.

IN VITRO ANTIOXIDANT ACTIVITY

DPPH Radical – Scavenging Activity

DPPH Radical - Scavenging Activity was determined.

Reagents

1. DPPH : 25 µg/ml in methanol
2. Methanol

Procedure

Briefly, a 2 ml aliquot of DPPH methanol solution (25 µg/ml) was added to 0.5 ml different (C11 to C15) *Cassia auriculata* flower ethanolic extract solution at different concentrations (20 to 100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 mins. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free – radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = \frac{Ac - As}{Ac} \times 100$$

Where; Ac = Control is the absorbance and As = Sample is the absorbance of reaction mixture.

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of sample needed to inhibit free radicals concentration by 50%, IC₅₀, was graphically determined by a linear regression method using Ms – Windows based graph pad InStat (version 3) software. Results were expressed as graphically / mean ± standard deviation.

RESULTS

TFC, TPC Quantitative analysis

The significant amount of Flavonoids and polyphenol in *Cassia auriculata* flower ethanolic extract (Table 1 and Figure 1).

Table 2: Quantitative analysis of TFC, TPC

Name of Sample	Total phenol (Milligrams of Gallic acid (GAE) equivalents per gram)	Flavonoid (Milligrams of Quercetin equivalents per gram)
<i>Cassia auriculata</i> flower Ethanolic Extract	134.61 ± 14.80	107.20 ± 11.79



Figure 3: Quantitative analysis of TFC, TPC

DPPH

DPPH of *Cassia auriculata* flower Ethanolic extract and compared with ascorbic acid. The *Cassia auriculata* flower ethanolic extract and ascorbic acid showed the minimum DPPH in 20 µg/ml concentration range (20.53% and 22.40%) while maximum inhibitory concentration (IC₅₀) of *Cassia auriculata* flower ethanolic extract and ascorbic were 53.53 µg/ml and 51.54 µg/ml respectively. The lower the IC₅₀ value of a compound, the higher its DPPH. *Cassia auriculata* flower ethanolic extract has potential DPPH and near to standard.

Table 3: DPPH of *Cassia auriculata* flower ethanolic extract

Concentrations (µg/ml)	% of inhibitions	
	Sample extract	Std. (Ascorbic acid)
20	20.53±0.14	22.40±0.15
40	36.26±0.25	40.80±0.28
60	50.13±0.35	57.06±0.39
80	67.20±0.47	74.40±0.52
100	85.60±0.59	91.73±0.64
IC ₅₀ (µg/ml)	53.53	51.54

Values were expressed as mean ± Standard deviation for triplicates

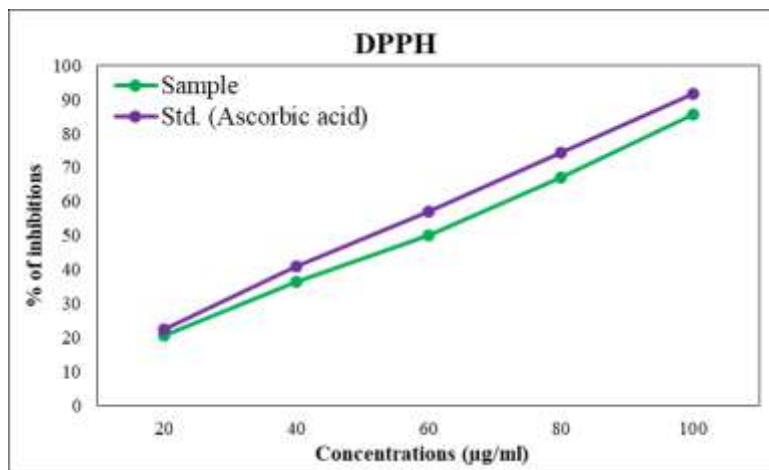


Figure 4: DPPH of *Cassia auriculata* flower ethanolic extract

CONCLUSION

The present investigation confirmed that the ethanolic flower extract of *cassia auriculata* possesses significant phenolic and flavonoid content along with notable antioxidant activity. The DPPH radical scavenging assay demonstrated that the *cassia auriculata* flower ethanolic extract exhibited dose dependent antioxidant potential comparable to ascorbic acid, indicating its ability to neutralize free radicals. These findings scientifically support the traditional use of *cassia auriculata* in treating various oxidative stress – related disorder and highlight its importance as a natural therapeutic agent.

Further studies should focus on isolation and characterization of active phytoconstituents responsible for antioxidant activity. *In – vitro* pharmacological evaluation, toxicity studies and formulation development are required to establish safety and efficacy. Additionally *Cassia auriculata* flower ethanolic extract can be explored for incorporation into herbal cosmetics, nutraceuticals and functional foods aimed at managing oxidative stress and chronic diseases.

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