RESEARCH PAPER

In-vitro Antioxidant Study on the Aqueous Extract of Cassia auriculata Flowers

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ABSTRACT

Medicinal plants contain phytoconstituents, including sterols, phenolics, tannins, terpenoids, alkaloids, essential oils, polysaccharides, and anthocyanins. Antioxidants from nature protect harmful impacts caused by ROS, reactive oxygen species. In this study, antioxidant function of the crude extract of C. auriculata flowers using aqueous solvent has been studied using DPPH method. The aqueous extract exhibited significant potential with IC50 value of 92.43±1.14 in DPPH assay. Plant extracts exhibit free radical scavenging potential due to their rich content of phytochemicals, including flavonoids, polyphenols, and vitamins. These phytochemicals function as antioxidants by donating electrons to neutralize free radicals, thereby protecting cells from damage.

Keywords: Cassia auriculata flowers, DPPH, phytochemicals, aqueous extract, radical, antioxidant

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1. Introduction

Reactive oxygen species (ROS) are primarily generated from food through various metabolic and chemical processes. The oxidation of unsaturated fats during food processing, storage, or digestion can lead to ROS formation like superoxide anions and hydroperoxides. When ROS levels exceed the body's antioxidant defenses, oxidative damage occurs, affecting lipids, proteins, and DNA. This damage disrupts cellular function, promotes cell death, and weakens tissue integrity. Chronic oxidative stress is a significant contributor to cellular aging and age-related functional decline. Excessive ROS are also implicated in the development of numerous conditions, including cardiovascular diseases like atherosclerosis, hypertension, and cardiac failure; neurodegenerative illnesses; cancer, through DNA mutations and tumor progression; and diabetes, by worsening insulin resistance and its complications. Antioxidants, which inhibit oxidation, play a vital role in minimizing the damage caused by free radicals and ROS. They are crucial for maintaining cellular health and protecting the body from oxidative stress.

Cassia auriculata flowers belongs to Fabaceae family. This moderate-sized shrub is particularly striking when in bloom. Its leaves are pinnately compound, with glands located opposite the leaflets. The stipules are leaf-like and persistent. The golden-yellow flowers, measuring 3–3.5 cm across, are arranged in corymbose racemes. The fruit is a long, dehiscent pod. This plant thrives in highly disturbed environments and dry regions. It is widely found in the hot deciduous forests of India and holds a highly esteemed place in the Ayurveda and Siddha medicinal systems. The plant also exhibits a wide range of therapeutic properties, such as analgesic, antipyretic, antiulcer, antihypertensive, antihysteric, antibiotic, antiviral, antipsychotic, antigonorrheal, antikidney stone, and hypoglycemic activities.

2. Results and Discussion

Phytochemical Investigation

The phytoconstituents, including carbohydrates, steroids, alkaloids, flavonoids, and phenols are identified in the extract. Various concentration of the aqueous extract of *C. auriculata* flowers was scrutinized for their antioxidant activity.

Antioxidant activity

The mean optical density value at 517 nm for the control was 1.113, while for the extract of *Cassia auriculata* flowers, it was 0.330 at a concentration of 10 micro gram per milli litre. This value decreased to 0.215 at 500 µg/mL. The mean optical density was 0.103 for ascorbic acid.

The aqueous extract of *Cassia auriculata* flowers exhibited substantial DPPH scavenging action, with an IC_{50} value of 92.43±1.14, which was comparable to the reference standards. The mean percentage inhibition of the aqueous extract with various concentrations is presented in the table. It was observed that the scavenging actions of the extract depend on the

concentration. The mean percentage inhibition was calculated from triplicate analyses.

Table: Mean Percentage Inhibition of the Aqueous Extract with various Concentrations

S. No.	Concentration (µg/mL)	Mean value (%)
1.	Ascorbic acid	90.90
2.	500	81.00
3.	250	79.15
4.	100	76.83
5.	50	74.10
6.	10	71.72

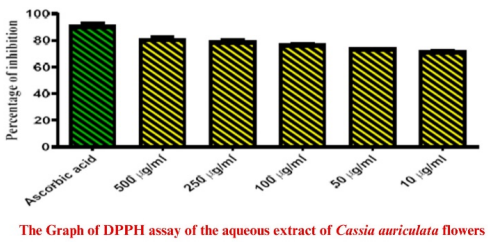

Figure: The Graph of DPPH assay of the aqueous extract of *Cassia auriculata* flowers

Oxidative stress by the free radical was linked to the development of an extensive range of clinical complaints, often due to insufficient natural antioxidant defenses. The antioxidant activity is assessed using the DPPH method, where DPPH is a constant free radical as compared with other compounds. This assay measures the scavenging potential of antioxidants against the stable DPPH radical. When it interacts with suitable reductant, the electrons pair off, causing the solution to lose its colour in a stoichiometric manner, on account of electrons taken up. Based on the investigation results, it recommended that *Cassia auriculata* flowers reduces the radical to the corresponding hydrazine by reacting with the H-donors in the antioxidant compounds.

3. Materials and Methods

Extraction

The dried flowers of *Cassia auriculata* was extracted successively with an aqueous solvent (1:10 w/v) using Soxhlet extraction method and then concentrated by boiling to yield a crude residue. This crude residue was used to assess its antioxidant potential.

Phytochemical Investigation

Cassia auriculata flower extract using aqueous solvent examined for phytoconstituents using the standard screening methods anticipated by J. B. Harborne.

Antioxidant activity

Preparation of Standard Solution

An exact amount of 3.9 mg of DPPH with molar mass, M = 394.32 was dissolved in methyl alcohol and then diluted in a 100.0 mL measuring flask with methyl alcohol to prepare a 0.1 milli Molar solution of DPPH. This solution was stored at -20 ˚C until needed.

Standard Ascorbic acid

As a reference material, 1 mg/1 mL of ascorbic acid was used.

DPPH method

The DPPH assay is widely functioned in the research of natural antioxidant due to its approachability and sensitivity. This method is based on the concept that antioxidants act as H-donors. It measures the capability of compounds to forage free radicals. The reaction pathway proton accepted by DPPH from a compound used against oxidizing agent is illustrated below. DPPH is a reliable and easily accessible organic N-radicals.

The activity of the extract is directly correlated to reduction of compound DPPH in the analysing sample. UV spectrometer was used to monitor DPPH that preferred method due to its effortlessness and precision. At 517 nm, DPPH shows a strong absorption peak in purple, which turned yellow as it accepts hydrogen which result the formation of reduced DPPH. Based on the number of absorbed H-atoms, this reaction is stoichiometric, allowing its effect to be simply assessed through tracing the diminution in Ultra-Violet absorption at 517 nm.

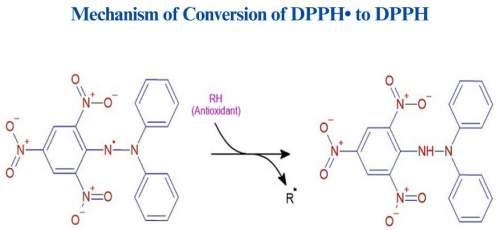

Figure: Mechanism of Conversion of DPPH˙ to DPPH

To 100 µL of prepared 0.1 milli Molar DPPH solution in methanol, add 300 µL of aqueous extract of *Cassia auriculata* flowers with dissimilar concentration. This solution mixture was shaken strongly and permitted to an undisturbed state for 30 min under standard temperature. The value of absorbance was identified

under 517 nm range using Ultra-Violet-Visible spectrophotometer. Minimum values indicate advanced scavenging potential. The software Graph Pad Prism 6.0 (USA) was used to calculate the percentage inhibition and IC_{50} value.

4. Conclusion

From the findings of this investigation suggest that aqueous extract of *Cassia auriculata* flowers exhibits significant DPPH scavenging action, likely ascribed to the presence of phytoconstituents like phenolic compounds.

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