ABSTRACT

“Isolation, Characterization and Biological Activity with Validation and Standardization of Active molecule from a medicinal plant – Cassia auriculata L”

The work described in the present thesis is concerned with the isolation, characterization and biological activity with validation and standardization of active molecule from a medicinal plant Cassia auriculata L. The thesis is divided in four chapters.

Chapter 1

Chemical investigation of Cassia auriculata L

Cassia auriculata Linn, commonly known as Tanner’s cassia belongs to family Caesalpiniaceae. It is an annual shrub found throughout India. Literature survey revealed that all parts of the plant are used for the treatment of various diseases as reported in Ayurveda. The leaves are bitter, astringent, constipating, expectorant. It is used in the treatment of jaundice, ulcer, leprosy and skin diseases. Seeds are bitter, astringent, acrid, cooling, ophthalmic and diuretic. Young twigs are used as chewing sticks where as bark powder is used for fixing teeth. Flowers are used in diabetes and throat troubles.

Section I

Isolation, characterization and thermal parameters study of bioactive anthraquinone – Compound 1

The plant, Cassia auriculata L was collected and authenticated at Botanical survey of India, Government of India, India. Its voucher specimen number is BSI/WC/Tech/2009/95.

Quath of fresh leaves used in Ayurveda was prepared as mentioned. It was fractioned using column chromatography and fractions were evaluated for their activity. The active sub-fraction was chromatographed and compound was isolated by preparative TLC. It showed sharp meting nature. The structure was assigned by modern spectral techniques as substituted anthraquinone, Compound 1 (C_{15}H_{10}O_{5}). This was isolated for the first time from quath. Thermal parameters like stability and energy of activation etc. of molecule was studied.
Catalytic hydrogenation of Compound 1 was carried out to produce Compound 2 (C_{15}H_{12}O_5) & Compound 3 (C_{15}H_{14}O_5) and activity was assayed.

Section II
Isolation, characterization of steam volatile bioactive component — Compound 4.

Fresh leaves were employed for steam distillation to acquire steam volatile matter. GC-MS study of this was illustrated presence of four major compounds. Isolated brown crystalline solid, Compound 4 (C_{13}H_{16}O_5), as a novel molecule exhibited pronounced antimicrobial activity than crude matter. This molecule was isolated for the first time from this plant material.

Compound 4

2-(hexyloxy)-3,6-dioxocyclohexa-1,4-dienecarboxylic acid
Section III

Isolation and characterization of an aromatic di-ester—Compound 9.
The bioguided fractionation of active acetone extract was furnished by column chromatography. Fractions were evaluated for their activity. The active sub-fraction was re-chromatographed to obtain Compound 9 (C₁₂H₁₄O₄) as an oily colourless liquid. The structure was confirmed by modern spectral techniques. The novel compound is isolated for the first time from this plant source.

**Compound 9**

![Diethyl phthalate](image)

Chapter 2

Validation and standardization of active molecules.

Section I

Standardization & quantification of Compound 1 by UV-VIS & IR.

A simple, accurate, sensitive, precise & reproducible UV spectrometric method has been developed for the standardization of Compound 1. Estimation of it was performed in methanol at maximum absorption wavelength over a range of 10-100 µg/ml. The method was validated for various parameters according to the ICH guidelines. The stability was examined. The presence of polyfunctional groups at specific positions in a molecule by their shifts in absorption were acknowledged using spectral reagents by UV and IR spectra. This type of the work has been carried out for the first time.

**Comparative UV-VIS & IR scans using spectral reagents for Compound1**
Section II

Standardization & quantification of Compounds 1 & 4 by HPTLC.

A simple, precise High Performance Thin Layer Chromatographic (HPTLC) method has been developed for the analysis. A suitable solvent system was acquired by attempting various mobile phases on pre-coated aluminium plates for quantification of analytes. The densitometric scanning at specified wavelength of standard with Camag TLC Scanner and WINCAT software was executed. The quantification of Compound 1 & 4 in plant parts was estimated by comparing peak areas. Various analytical parameters were noticed for the first time. The proposed HPTLC method was found to be simple, faster and reliable for quantification of analytes.

![Regression analysis graphs for Compound 1 and Compound 4](image_url)

Section III

Assessment of redox potential of Compound 1 by CV.

Compound 1 is a naturally occurring anthraquinone present in most of the species of cassia as an active ingredient, which was assigned as chemotherapeutic anticancer agent. In present study electrochemical characteristic of Compound 1, as biological molecule, have been studied at a platinum electrode using cyclic voltammetry (CV) method in the presence of dissolved oxygen. The redox response of Compound 1 was investigated by voltammetric data at various scan rates and in different concentrations. The heterogeneous charge transfer rate constant was calculated for the first time in protic as well as aprotic solvents. The proposed method was applied for quantification.
Chapter 3

Biological activity: An *In-vitro* study.

Section I

Comparative Anthelmintic Activity study of plant parts.

Helminth infections are among the most common infections in humans, affecting a large population of the World. Use of herbal products as antimicrobial agents may provide the best alternative. In present study various extracts of *C.auriculata* L leaves, stems and fruits along with Compound 1 & 4 were investigated for their anthelmintic activity against *Echinia foetida* using saline as control and albendazole, as a standard. The test samples had illustrated a dose dependant inhibition of paralysis and cause death of worms. This work was committed for the first time.

Section II

Clinical Trials -Dental Consequences.

Oral diseases are major health problems with dental caries and periodontal disease. In this sense, aqueous and acetone extract of leaves and young stems of *C.auriculata* L were studied against dental pathogens. The efficacy of the plant extracts has been assessed by testing on 10 salivary samples of patients suffering from dental caries using well diffusion method. Acetone extract of leaves was found to be more effective as anticariogenic medicine as compare to stem and fruit. Compound 1 was found to be more active against salivery samples as well as *S.Mutans*. This study has confirmed the antibacterial potentials of the plant, thus supporting its folklore application as a medical remedy for odontopathy, inflammation and bleeding of gums.
Section III
Antimicrobial Activity study.
Microbial diseases rank as number one cause for almost half of the deaths in underdeveloped and tropical countries. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Antimicrobial studies for various extracts were carried out against gram +ve and gram -ve bacterial strains. The present study indicated that the acetone and ethanol extracts exhibited indicative activity. Bioactivity of Compound 4 exhibited more activity than crude mass, which indicates that other components present in the crude mass must be suppressing the tested activity.

Chapter 4
Proximate analysis.

Section I
Phytochemical assessment of plant parts.
Plant analysis has been considered a very promising tool to assess nutritional requirements of plants for cost effective. Elemental analysis is probably the most fundamental test in the plant analysis, which supports the production of amino acids. This plant may become a good source of calcium, potassium and zinc. Amino acids are the building blocks of proteins. This encompasses the detection of amino acids. Total eleven amino acids were detected from two different mobile phases. This work has been carried out for the first time from this plant source.

Section II
Antioxidant potential of plant parts.
The present work is carried out in order to evaluate the efficacy of the plant in view of phenolic and flavonoid contents. The various plant parts were screened for their antioxidant potential. Quantitative determination of phenols and flavonoids were carried out using spectrophotometric method. Total flavonoid content was determined as quercetin and phenolic content as pyrocatechol equivalent using Folin-Ciocalteu reagent.
Free radical scavenging assays were determined by: DPPH and NO. The percent radical activity for both the assays was determined using ascorbic acid as a standard. This systematic study is performed for the first time.

Section III

GC-MS study & Computational analysis of compound 1.

a. GC-MS study
GC-MS is used for the qualitative and quantitative detection and estimation of saturated/unsaturated hydrocarbons, fatty acids, esters, alcohols, lactones etc. The hexane extract of fruits was analyzed by GC-MS using NIST and Willey libraries. Major seven compounds were identified.

b. Computational analysis of Compound 1
Compound 1, a bioactive molecule used as an anticancer drug was studied by using Gussian-09 computational software for the first time to spectral parameters. This shows good agreement to the experimental data and calculated data using Gussion-09 software.