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Metabolic Profile of Cassia Auriculata L. Extracts by High Performance Liquid Chromatography

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ABSTRACT

Present study involvescharacterizationof medicinallysecondary metabolitessuch as phenolic compounds namely Ellagic acid, Catchol, Gallic acid, Quercetin, anthrquinone were detected byqualitative High Performance Liquid Chromatography (HPLC) analysis. *Cassia* family is well known source of anthrquinone glycosides and its derivatives in the various parts of plants. The simple high performance liquid chromatography method was developed and validated for the determination of anthrquinone in the extract from *Cassia auriculata.* The extracts were analyzed on C-18 column isocratic mobile phase in HPLC equipped with UV detector at 270 nm. The limits of detection obtained for the analyte were in the range of 2.5 to 1.5µg/ml.

KEYWORDS: HPLC, *Cassia auriculata*, phenolic compounds.

I. INTRODUCTION

Plants up regulate and down regulate their biochemical paths in response to the local mix of herbivores, pollinators and microorganisms (Lin coln. 2006). The secondary metabolites and pigments can have therapeutic actions in humans which can be refined to produce drugs. Plants synthesize wildering variety of phytochemical but most are derivatives of a few Bio chemicals such as alkaloids, phenols and their derivatives terpenoids, glycosides and others. The secondary metabolites possess some therapeutic properties therefore some plants are also classified as herbs.The pharmacological activities of any plant sample are due to the presence of metabolites. Secondary metabolites and secretary products in it (Gupta *et al*., 2012). These usually consist of the phenolic compounds, alkaloids, tannins, saponin, carbohydrates, glycosides, flavonoids, steroids etc. Most phenolic compounds such as flavonoids, glycosides, triperinoids, flavones, carbohydrates and antraquinone are found and distributed throughout the plant kingdom (Harbone J B., 1973). Similarly the polyphenolic compounds most commonly found in plants extracts are the phenolic acids, flavonoids and tannins (Naik *et al.,* 2006; Sati *etal*., 2010). Plants form the *Cassia* family are identified as a potential source for herbal medicine. These plants contain anthrquinone, glycosides and their derivatives and the derivatives are potentially known for their laxative

property and skin and respiratory diseases. The main objective of present studywas to determine the chromatograms of standards phenolic chemical compounds which are commonly found in medicinal plants samples by HPLC using methanol and water (5:95) mobile phasewith different elution gradients and run times.

II. MATERIAL AND METHODS

Collection and extraction of medicinal plant material:

The raw material of medicinal plant *C. auriculata*was collected from different regions of Parbhani district India. The voucher specimen was deposited at Department Botany D.S.M College, Jintur. The dried powdered of plant material a flower was extractedseparately with methanol using soxhlet apparatus for 48 hrs. The solvent was distilled off at lower temperature under reduced pressure in rotary flash evaporator and concentrated on water bath to get the crude extract which is stored indesiccator for future use.

III. PREPARATION OF SAMPLE SOLUTION

The phenolic acids were extracted as per the method of (Singh *et al.*, 2002). The extract was dissolved in HPLC grade methanol having approximate concentration 500mg/L in stock solution. Prior to use, the mobile phase was filtered through 0.45µm filter paper with filtration assembly followed by sonication for 10 min. For the complete removal of air bubble/dissolve oxygen. Sample analysis was carried out by using same conditions and results were compared with authentic standards.

High Performance Liquid Chromatography Analysis

HPLCanalysis was performed on isocratic system with UV detector for the detection phenolic compounds. The instrument specification and analysis conditions were optimized. Isocratic system from shimadzu (Asia pacific) Pvt. Ltd. Model No. LC-10 Advp with UV detector was used. The sample volume was kept 20µ with 20µL peodyne injector system. The analytical column with 250×4.6 mm id 5µm was used. The mobile phase prepared from the HPLC grades. The flow rate was kept at 0.6 ml/min and detection was carried out at 270 nm.

Table 1 HPLC analysis of *Cassia auriculata L.* extract

Peak	Retention time	Area	Area percent
1	2.165	123170	14.81
2	2.859	524215	63.05
3	3.723	60767	7.31
4	4.416	47485	5.71
5	4.853	21699	2.61
6	21.781	54108	6.51

IV. RESULT

In the present studythe HPLC finger prints of the crude extracts of *C.auriculata* flowers shows the anthrquinone and flavonoids. Although a primary objective of carrying out HPLC may be to standardize dosage, more information may be obtained during the course of a run, if appropriate detection hardware and software are used. The fig.1 shows the chromatogram of fraction collected form flowers of *Cassia auriculata*. The fig are clearly

show the presence of desired components in the fraction collected through column with C-18 column are detected 270 nm in UV detector. The retention time of all components are matching with the retention time of standards.

The HPLC finger print of the methanolic extract of *Cassia auriculata* shows major peaks at the retention times of 2.165, 2.859, 3.723, 4.416, 4.853 and 21.781 at a wavelength of 270 nm. The retention time for flavonoid is found the value is compare with the standard deviation. The retention time for anthrquinone is (2.165) found. Linearity curves for standards and samples are carried out for method is used to confirm the linearity of all components. The table.1 represents the HPLC data of linearity for these standards.

According to Bouer and Tittel 1996 and spring field *et al* (2005), they reported HPLC finger printing is the best was for chemical characterization and therefore this study also established HPLC finger print for the active phenolic acids that can act as antioxidant, antifungal, antibacterial and antiflammatory. The diverse pharmacological activities have been accredited to phenolic acids for instance, Gallic acids is reported to be anti-inflammatory (Kores *et al.*, 1992) and antibacterial (Raven *et al.*, 1989). Recent researches indicate that the polyphenols being secondary metabolites are present in rich amount in several plants.

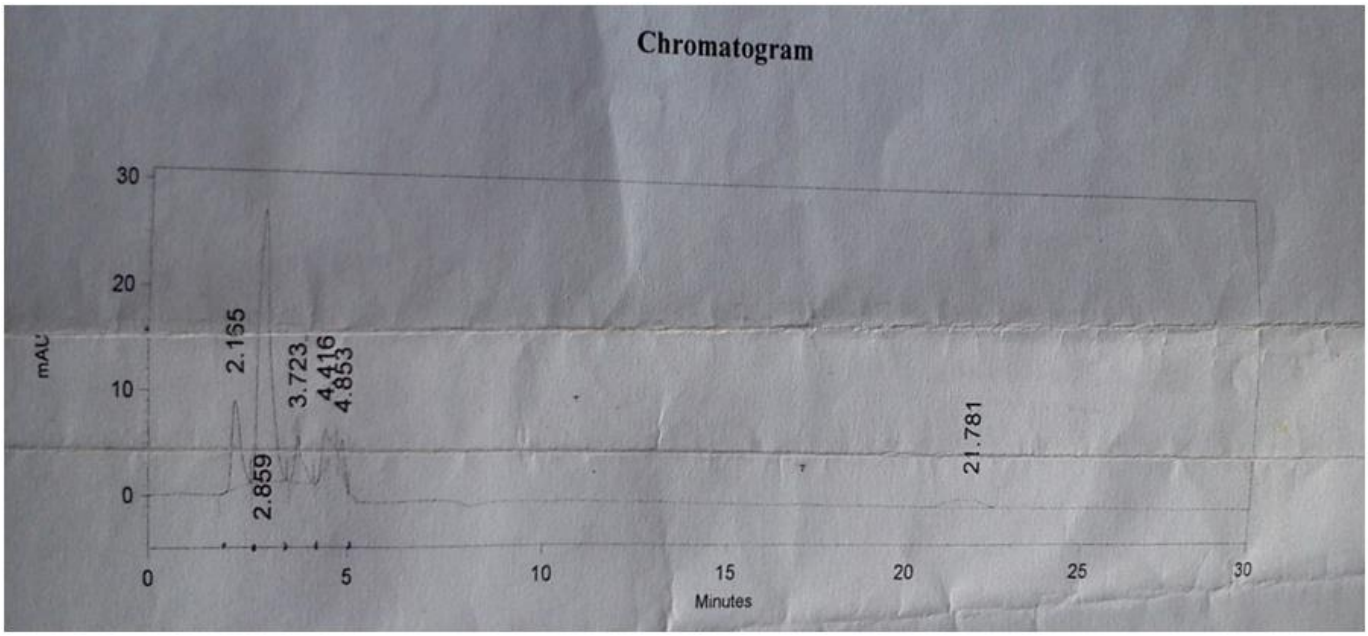

Figure 1 HPLC analysis of methanolic extract of *Cassia auriculata L.*

V. DISCUSSION

The HPLC finger prints of these standards phenolic compounds obtained using the methods. Described above would serve the purpose of established benchmarks for future plant research. The qualitative and quantitative analysis of the actual phenolic compounds present in any unknown plant sample would be facilitated by means of comparison with such standard chromatograms enabling identification and confirmation of presence of these phenolic compound in the research sample. The use of multiple methods involving different mobile gradient phase would increase the validity and reliability of the obtained results. Alkaloids like berberine, palmatine temberaine, choline, tinosporin and magnoflorine have been isolated form the non-polar fraction of extracts of stem and root of *T. Cordifolia* (Jagetia and Rao. 2006). A simple method was developed, optimizes and validated by ultra-fast liquid chromatography for the analysis of quercetin3-O-rutinoside & quercetin in *Cassia auriculata* extracts. (Girme AS *et al.*, 2018). From the GCMS study, 12 phytocompounds were identified from *C. alata* flowers,

9 compounds from *C. auriculata* flowers, 12 compounds from *C. fistula* flowers and 13 phytocompounds identified from the *C. occidentalis* flower extract. A compound namely, Pregna-5, 8, 16-triene-3à-ol-20-one acetate (its chemical name is 16-Dehydropregnenolone acetate) found first time in *C. occidentalis* flowers, which is used in the preparation of anti-cancer agents. Savarinayagam H. Socrates and Shanmugavadivelu C. Mohan, (2019) Studies on the plants of *Acacia pennata*, *Cassia auriculata*, *Glycomis pentaphylla* and *Tadehagi triquetrum* have reported the presence of several bioactive compounds whose biological and pharmacological applications can further be investigated. Phytochemical analysis showed that the antimicrobial activity of *C. auriculata* was due to the presence of Phytochemical compounds like alkaloids, carbohydrates, fixed oils & fats, tannins, gum & mucilage, flavonoids, saponin, terpenoids, lignin and sterols(Raja *et al.*, 2013).It is quite evident from this review that *Cassia auriculata* contains a number of phytoconstistiuents which reveals its uses for various therapeutic purposes. The Plant or its individual parts can be used as Antidiabetic, anthelmintic, hepatoprotective, antifungal and antimicrobial, anti-inflammatory, antipyretic, antioxidant, antihyperlipidemic activity (Guru Prasad C. Nille and K.R.C.Reddy2015). It is quite evident from this is *Cassia auriculata* contains a number of phytoconstistiuents which reveals its use for various therapeutic purposes. Our results suggest that *Cassia auriculata* is a very good potential source of antioxidant and antimicrobial agents, anti-inflammatory(M. Monisha*et al.*, 2017).It was evident from the previous studies that polyphenols exert remarkable antioxidant activity and also inhibit the damaging effect on DNA occurring due to harmful UV radiation. Also it substantially nullifies various adverse biochemical events occurring due to solar UV radiation (Krantisinha Hanumant Randive*et al.*, 2019).The observed effect may be due to the presence of biologically active ingredients in the flower extract. Hence, from the results obtained it can be concluded that *C.auriculata* flower extract can be used for the treatment of diabetes mellitus (G. Sriram Prasath*et al.*, 2019).The studies cited in this review suggest that this plant and its extracts may be of therapeutic value with regard to several pathologies (Vandana Meena*et al.*, 2019). Studies have be done on its bioactive principles of *Cassia auriculata*, which are responsible for the health befits offered by these plants so that the bioactive compounds could give some leads for new drug discovery to various chronic diseases (Salma B., *et al* 2020).From above literature it is concluded that *Cassia auriculata Linn*. Is responsible for the various therapeutic potentials especially in diabeties. It contains a number of phytoconstistiuents and amino acids(Dr. Pranam Suresh Khar*che et al.*, 2020).

VI. CONCULSION

Conventional column chromatography and sample HPLC technique are used to separate therapeutic agents like rhein, embodin, and chrysophonic acid, flavonoids from *Cassia auricualata.*Many therapeutic bioactive compounds found in flower extract and showed antifungal and antioxidant activity.*Cassia auriculata L.* has been examined scrupulously for its phytochemical and pharmacological activities In future, this flower extract could be used for a novel wide-spectrum antimicrobial formulation.. From the above review, it is concluded that *Cassia* has been used as an important curative agent for patients. It is a very useful herbal plant and needs to explore more to know the exact mechanism. In both in vivo and in vitro studies, *Cassia auriculata L.* has various pharmacological properties.

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