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HPLC Analysis of Methanolic Extract of *Cassia Fistula Linn*. Plant Leaves

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ABSTRACT

In the present study, we have applied easiest and quicker method for extraction of methanol soluble phytochemicals. Crude extract of plant leaves of *Cassia fistula Linn.* Was analysed the soluble fraction of phenols using HPLC coupled to UV-Vis detector at 254 nm. Which gives different peaks at this region, it may be phenolic compounds.

Keywords – *Cassia fistula Linn*., Phytochemicals, Phenols, Extraction, HPLC

Introduction

HPLC analytical technique is used for the isolation of various natural products. HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture [1]. Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize its properties. The resolving power of HPLC is ideally suited to the rapid processing of such multi component samples on both an analytical and preparative scale. Several authors describe the use of HPLC for characterization and quantification of secondary metabolites in plant extracts [2-3]. Natural products and secondary metabolites formed by living systems, notably from plant origin, have shown great potential in treating human diseases such as cancer, coronary heart diseases, diabetes and infectious diseases [4].

In the present study we are analysing the phytochemical present in *Cassia Fistula Linn*. Leaves through the chromatographic technique. Plants are always used for curing the disease from the ancient time hence it is important to analysis of chemical present in plants parts.

Herbal medicine and omics systems science offer significant synergy to aid drug discovery and development. *Cassia Fistula Linn.*, a *Caesalpinaceae* shrub, is native to India and Sri Lanka, present in Indo-Malaysia, and cultivated in Myanmar. In Ayurvedic medicine, *C. fistula* is one of the notable medicinal herbs. The individual parts of the *C. Fistula* plant, including the flowers, flower buds, root, leaves, seeds, and bark, are used in traditional herbal medicine practices with various indications for each (5). On the other hand, while *C. fistula* has been used as a medicinal herb in a context of diabetes, its mechanisms of action and the evidence base for

its ant diabetic medicinal potentials and components need to be deciphered. Moreover, the photocomposition of the various plant parts is not fully known. In Ayurvedic medicine, Golden Shower Tree is known as "disease killer". Its fruit pulp is used as mild laxative. As well as cardiac conditions and stomach problems such as acid reflux. Flowers used for fever, root as a diuretic. The bark and leaves are used for skin diseases. The seeds are recognised as ant bilious, aperitif, carminative, and laxative while the root is used for curing adenopathy, burning sensations, leprosy, skin diseases, syphilis, and tubercular glands. [8]. the use of Plants with pharmaceutical properties has received increased interest nowadays from both homeopathic and allopathic branches. These medicinal plants play an important role in public health, especially in developing countries, where it is believed that the intense utilization of plants with therapeutic action does not lead to intoxication [9].

Material and Methods

Sample Collection:- The plant leaves of *Cassia Fistula Linn.*. were collected and identified in March 2019 from the campus of Vasantrao Naik Agari University Parbhani (M.S.). Plant leaves was washed with fresh water and dried under the shade at room temperature. The leaves were powdered and stored in sterile container for further use.

Preparation of Plant Extracts

1 gm. of fine powdered sample of plant leaves were extracted with 10 ml HPLC grade methanol through open reflux process at 40 °C for 30 min. Then the extracts were filtered through filter paper (Whatman No. 1) to remove free unextractable substances. The filtrates of plant extracts were preserved 4-5°C for further process.

Experimental Methods

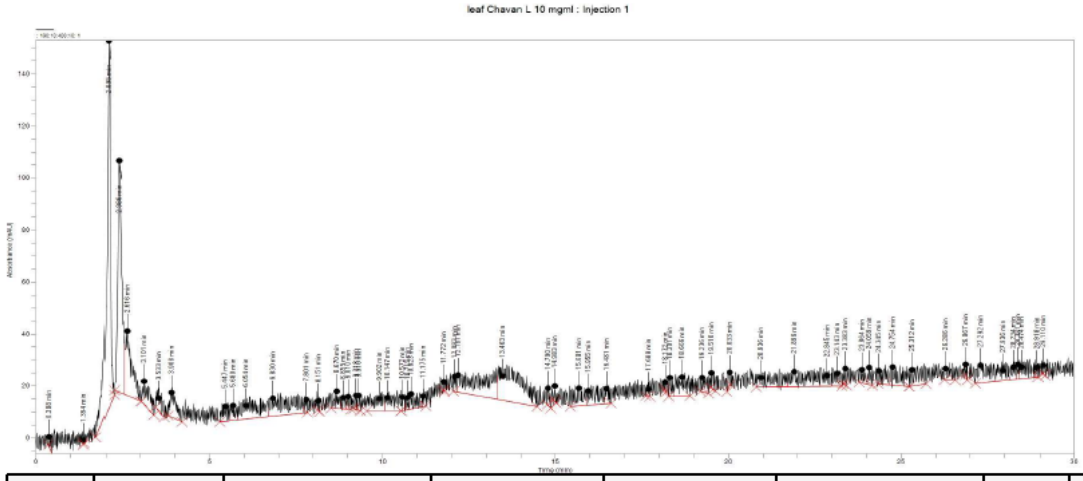
HPLC Analysis

The HPLC analysis of Methanolic extract was carried out with Chromatographic system (YL 9100, Korea) consist of auto sampler (YL 9150) with 100 µl fixed loop and an YL9120 UV-Visible detector. The separation was performed on a SGE Protecol PC18GP120 (250mm×4.6 mm, 5µm) column at ambient temperature. The mobile phase consists of methanol to water (70:30 v/v) and the separations were performed by using isocratic mode, elution performed at a flow rate of 1 ml/min. The samples were run for 15min. and detection was done at 254 nm by UV detector. All chromatographic data were recorded and processed using autochro-3000 software.

Results and Discussion

Two spots (brown, Rf 2.095, Rf 2.386) coincided with that of the standard reference compound Saponins, Alkaloids and Tannin were marked in *Cassia Fistula Linn.*. For standardization of Methanolic extracts of plant leaves, HPLC is a sensitive and accurate of plant extract and its derived product. HPLC fingerprints of *Cassia fistula Linn.* are given in figure no.1. Results of HPLC analysis of *Cassia fistula Linn.* Methanolic extract at 254 nm, shows presence of various constituents as evidenced by the chromatogram obtained at various retention times are the constituents found in *Cassia fistula Linn.* leaves mainly. Leaf of *Cassia fistula* mainly contains Oxalic Acids, Tannins, Oxyanthra-quinones, Anthraquinones derivatives. Fruit of Cassia fistula contains Rhein Glycosides Fistulic Acids, Sennosides A B, Anthraquinones, and Flavanoid-3-ol-derivatives. Ceryl Alcohol, Kaempferol, Bianthraquinone Glycosides, Fistulin, Essential Oils, Volatile

Components, Phytol (16.1%), 2-Hexadecanone (12%), Crystals, 4- Hydroxy Benzoic Acids Hydrate have been reported from the plant. Standardization and characterization of herbal drugs is a topic of continuous scientific interest in the herbal drug industry. With the advent of modern chromatographic systems there is an ever increasing intent to produce and develop easy, rapid, convenient and cost effective methods for standardization [6]. For standardization of Methanolic extract of plant leaves, HPLC is a sensitive and accurate tool that widely used for the quality assessment of plant extract and its derived product/formulation [7]. The plant extract yield percentage on the usage of methanol agreed with the earlier reported [10] obtained in Hypochaerisradicata L. The plant extract obtained using soxhlet is varied among the herbal plants to plant. In a plant, different parts having differently yielded [11]. The plant extract yield percentage on the usage of methanol agreed with the earlier reported obtained in Brassica oleracea [12]. The results pertaining to the HPLC profile of the leaves of *Cassia fistula Linn.* Revealed their highest peaks for the secondary metabolites as protein source. Similar kind of earlier experiments demonstrates the processing of a crude source material to provide a sample suitable for HPLC analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation. The source material, e.g., dried powdered plant, will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. In the case of dried plant material, an organic solvent (e.g. methanol, chloroform) may be used as the initial extracting and following a period of maceration, solid material is then removed by decanting off the extract by filtration. The filtrate is then concentrated and injected into HPLC for separation. The usage of guard columns is necessary in the analysis of crude extract. From this present investigation, we observed wide variation within the biochemical aspects of *Cassia Fistula Linn.* which is further exploited to popularize the useful accessions for the extraction an invention of latest new drugs in future.



Peak #	Time	Component Name	Area	Height	Final Amount	Units	Area %
1	0.388		18,436.4	3,815.5			0.28
2	1.394		2,538.6	3,028.6			0.04
3	2.095		984,322.1	141,417.7			15.20
4	2.386		679,242.5	87,194.2			10.49
5	2.616		346,561.5	24,357.8			5.35
6	3.101		116,740.0	8,188.6			1.80
7	3.533		66,427.3	6,300.4			1.03
8	3.908		128,643.7	10,449.3			1.99
9	5.447		62,667.5	4,444.1			0.97
10	5.680		47,689.0	4,741.4			0.74
11	6.054		231,775.6	6,213.7			3.58
12	6.830		278,597.7	5,849.6			4.30
13	7.801		21,075.6	5,405.0			0.33
14	8.151		9,947.3	4,013.2			0.15
15	8.670		52,954.7	5,165.5			0.82
16	8.855		25,314.6	4,649.5			0.39
17	9.010		23,947.8	4,414.3			0.37
18	9.218		13,886.2	4,718.9			0.21
19	9.310		16,970.4	5,150.3			0.26

Peak #	Time	Component Name	Area	Height	Final Amount	Units	Area %
20	9.902		129,035.0	5,379.5			1.99
21	10.147		111,379.3	6,414.0			1.72
22	10.572		28,770.9	5,353.1			0.44
23	10.715		17,345.9	4,079.7			0.27
24	10.823		59,036.2	4,675.9			0.91
25	11.175		25,384.6	4,381.7			0.39
26	11.772		3,953.6	3,780.9			0.06
27	12.083		20,694.0	4,542.2			0.32
28	12.187		387,046.9	5,816.0			5.98
29	13.463		578,442.1	10,790.3			8.93
30	14.790		21,382.4	6,343.0			0.33
31	14.983		9,985.2	4,528.9			0.15
32	15.681		109,985.1	6,375.7			1.70
33	15.955		130,943.2	6,083.6			2.02
34	16.481		44,964.6	5,812.3			0.69
35	17.688		17,318.2	3,441.2			0.27
36	18.172		4,721.5	3,141.1			0.07
37	18.301		31,194.1	6,370.6			0.48
38	18.666		130,904.7	7,009.4			2.02
39	19.236		50,486.7	5,079.7			0.78
40	19.510		23,661.3	4,851.1			0.37
41	20.033		15,941.3	3,877.0			0.25
42	20.936		161,360.7	3,993.1			2.49
43	21.896		189,011.8	5,573.6			2.92
44	22.845		74,491.0	4,204.8			1.15
45	23.143		62,922.4	4,270.0			0.97
46	23.383		18,196.4	6,274.8			0.28
47	23.864		37,730.2	4,858.2			0.58
48	24.059		33,679.6	5,222.9			0.52
49	24.345		66,617.6	5,619.3			1.03
50	24.754		137,645.7	6,412.3			2.13
51	25.312		115,418.7	7,127.6			1.78
52	26.286		56,908.2	4,238.2			0.88
53	26.867		7,239.1	3,473.0			0.11

Peak #	Time	Component Name	Area	Height	Final Amount	Units	Area %
54	27.292		154,718.5	6,353.0			2.39
55	27.936		96,468.4	5,119.9			1.49
56	28.234		41,514.9	5,043.3			0.64
57	28.381		19,568.7	4,902.2			0.30
58	28.474		96,803.2	5,841.4			1.49
59	28.918		17,658.9	3,596.9			0.27
60	29.110		6,949.8	3,135.7			0.11
Total			6,475,219.1				100.00

Conclusion

The results above showed, therefore, that *Cassia fistula Linn.* are rich in the presence of the important biologically active phenolic compounds. The described HPLC procedure could be useful for the qualitative and quantitative analysis of saponins in plant materials. Plants produce saponins to fight infections by parasites. When ingested by humans, saponins also seem to help our immune system and to protect against pathogens. The non-sugar part of saponins have also a direct antioxidant activity, which may result in other benefits such as reduced risk of cancer and heart diseases. Saponins have beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system. Saponins bind with bile salt and cholesterol in the intestinal tract and cause a reduction of blood cholesterol by preventing its re-absorption. Therefore, determination of saponins is very important related to the quality of medicinal plants. The described HPLC procedure could be useful for the qualitative and quantitative analysis of saponins in plant materials. It can also be used in the quality control of phytopreparations containing saponins

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