Phytochemical Screening of Cassia *fistula* Bark and Leaves Ethanolic Extracts and FTIR analysis.

[1]Rakhi Mandloi, [2]Prakash Solanki*, [3]Ravin Chouhan, [4]Mahesh Baviskar

[2,4]Assistant Professor, [1,3]Ph.D. Research Scholar.
[1-4] Department of Chemistry
[1-3]Govt. Holkar Science College, Indore India
[4] Govt. P.G. College, sendhwa India
Corresponding author *427, Shiv City behind IPS Academy, Indore Madhya Pradesh (India) 452012

Abstract

Objective: The present study was designed to characterize the phytochemical Constituents of Cassia *fistula* leaves and bark and FTIR- spectra anlysis of ethanolic extract of Cassia *fistula* bark.
Methods: The ethanolic extract of the leaves and bark of Cassia *fistula* prepared by Soxhlet Extraction method. Crude extract was analysed by phytochemical tests and FTIR-spectrum.
Findings: Phytochemical studies revealed the presence of some interesting secondary metabolites like tannins, flavonoid, carbohydrate, saponins, glycosides and phenol in the leaf extract while tannins, flavonoids, carbohydrate, saponins, glycosides, phenol and terpenoid in the stem bark during qualitative analysis. Interestingly, FTIR analysis of ethanolic stem bark extract confirm the presence of phenolic compound and flavonoid content. The FTIR signals at 3432.7, 1513.8, 1448.3, 1240, 1159 and 567cm^{-1} were considered as an indicator of the presence of biologically active functional groups alkyne, arene, alkane, alcohol, phenol, ether and halohydrocarbon in it during quantitative analysis. So the present study provide evidences that leaves and bark of Cassia *fistula* contain bioactive constituents which could be of interest for the development of new drug.

Key words: Cassia *fistula,* Phytochemicals, Solvent Extracts, FTIR- spectrum, functional groups.

Introduction:-

Medicinal plants are abundantly available all over the world and are more focused than ever because they have the ability to produce many benefits to human society, especially for the treatment of various types of human ailments. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. They are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. In most of the traditional systems of treatment, the use of medicinal plant include the fresh or dried parts, whole, chopped, powdered or an advanced form of the plant usually made through extraction with different solvents play a major role and constitute the backbone of the traditional medicine.

The therapeutic properties of medicinal plants are due to some chemical compounds they synthesize. These are regarded as secondary metabolites because the plants that synthesise them may have little need for them. They are synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, and seeds. Plant produces these chemicals to protect itself from herbivores but recent research demonstrates that many phytochemicals can protect humans against diseases. Different phytocontituents present in medicinal plants are flavonoids, carotenoids, alkaloids, anthocyanidins, phenolics and tannins, carboxylic acids, terpenes, amino acids, and inorganic acids etc.

Cassia *fistula* tree is one of the most widespread in the forests of India, usually occurring in deciduous forests. Cassia *fistula* known as Indian laburnum is a medicinal plant of immense importance. It is also known as Golden Shower. The whole plant possesses medicinal properties useful in the treatment of skin diseases, inflammatory diseases. it is distributed in various regions including Asia, South Africa, China, West Indies and Brazil[1].India, having a rich tradition of folk medicine from centuries, has provided very simple but effective remedies to various ailments using plants and plants derived compounds. There is no such risk factor to use the plant medicine as compare with the allopathic drugs [2].

Cassia *fistula* a very common plant known for its medicinal properties is a semi-wild in nature. This plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections[3]. In traditional medicine, it has been used in the treatment of diabetes, hematemesis, leucoderma, pruritis, intestinal disorder and as antipyretics, analgesic and laxative. The stem bark, and leaves of this plant contain a variety of biologically active compounds such as anthraquinones, flavonoids, alkaloid, glycosides, tannin, saponin, terpenoids, reducing sugar and steroids those have various medicinal properties. The leaves and stem bark extract shows various activities like antipyretic, antioxidant, antidiabetic, protective, antimicrobial, antitumor, antiulcer etc. Extracts relieve constipation, piles and detoxifier[4]. This article aims to provide a comprehensive review on the phytochemical and aspects of Cassia *fistula*. These results will add up to useful properties of the plant Cassia *fistula*.

Materials and Methods

Plant material collection: Cassia *fistula* Leaves and Bark was collected from Govt. Autonomous Holkar Science College, Indore (M.P.) in 20th May 2016. The plant was identified by Dr. Prakash Solanki, Assistant Professor of Chemistry and Dr. Sanjay Vyas Professor of Botany, Govt. Autonomous Holkar Science College, Indore (M.P.).

Prepartaion of Plant Material: The bark and leaves were air dried in shade at room temperature.Dried leaves and bark pieces are cut into small pieces. These pieces are then grinded in mechanical grinder. The powdered plant material was stored in an air tight container prior to extraction. The powdered sample is used for study.

Extraction of Plant Material: The extraction was performed using a Soxhlet apparatus in the normal way at the boiling point of the solvent used. Solvent used for extraction is Ethanol.Two hundred grams of the powdered leaves and bark was subjected to maceration in ethanol using a Soxhlet Extractor. The extract decanted at an interval of 72 hours.The filtrate was dried to powder by distillation and used as the crude ethanol leaf and bark extract. These crude extracts of ethanol was used for further investigation for phytochemical analysis by phytochemical tests and FTIR-spectrum analysis.

Phytochemical Analysis: The extracts were subjected to phytochemical testing to detect for the presence of different chemical groups of compounds. Air dried and powdered plant material were investigated for the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, sterols, glycosides, antraquinones, saponins, carbohydrates, starch, protein.

Phytochemical tests of extracts:

I. Test for Sterols: Salkowski test: 2 ml of extract was mixed with 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added carefully and shaken well. The chloroform layer did not appeared red and acid layer fluorescent greenish yellow. This strongly supports the absence of sterols from the extract.

II. **Test for Terpenoids: Salkowski test:** 2 ml of extract was mixed with 2ml chloroform and 2 ml concentrated H_2SO_4 was added carefully and shaken well. Positive result for the presence of terpinoides was noted by the appearance of reddish brown colour of interphase.

III. Test for flavonoides: Ammonium Test: A small quantity of the extracts heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture filtered and the filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. Waited for colouration in Ammonia layer. Yellow colouration at ammonia layer was not observed which indicates the absence of flavonoides from the extract.

IV. Test for Carbohydrate: Fehling Test: Equal quantity of Fehling solution A and Fehling solution B are mixed and few drops of extract was added and boiled. Brick red precipitate of cuprous oxide confirms the presence of carbohydrate.

V. Test for Tannins: Ferric Chloride Test: A small quantity of the extract was boiled with 5 ml of 45% ethanol for 5 minutes. The mixture was cooled and filtered. 1ml each of filtrate diluted with distilled water and two drops of ferric chloride was added and waited for colouration. A transient green to black colour did not appeared , hence giving a negative result.

VI. Test for Phenols: Ellagic Acid Test: The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. Niger brown precipitate did not occur nor the solution turned muddy. Hence the absence of Phenols were observed from the extract.

VII. Test for Glycosides: Concentrate H_2SO_4 Test: In 5ml extract, 2ml glacial acetic acid, one drop of 5% $FeCl_3$ and conc. H_2SO_4 was added. The brown ring colouration in interphase marks the presence of Glycosides.

VIII. Test for Protein: Biuret Test: 2ml of Biuret reagent was added to 2ml of extract. The mixture was shaked well and warmed on water bath. Absence of protein was noted as violate colouration was not observed.

IX. Test for Saponin: Foam Test: The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A thin foam layer was observed. This indicates that Saponin is weakly present in the extract.

Fourier Transform Infrared Spectrophotometer:

FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites. Fourier Transform Infrared spectroscopy (FTIR) is an established, time saving method to identify the structure of unknown composition or its chemical group, and the intensity of the absorption spectra associated with molecular composition or content of the chemical group. The FT-IR method measures the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample.

Dried powder of the plant extracts of Cassia *fistula* was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each extracts was loaded in FTIR spectroscope, with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Result:

Phytochemical Analysis: Phytochemical studies revealed the presence of secondary metabolites like tannins, flavonoid, carbohydrate, saponins, glycosides and phenol in the leaf extract while tannins, flavonoids, carbohydrate, saponins, glycosides, phenol and terpenoid in the stem bark.

Table – 1 Phytochemical Analysis Of Ethanolic Extract Of Cassia *fistula*:-

No.	**Phytochemicals**	**Bark**	**Leaves**
1	Sterol	–	–
2	Tannin	+	+
3	Flavonoid	+	+
4	Carbohydrate	+	–
5	Saponin	+	+
6	Protein	–	–
7	Glycoside	+	+
8	Phenol	+	–
9	Terpenoid	+	–

Where '+' = Present '-' = Absent

FTIR analysis of ethanolic bark extract

No.	**Functional Group**	**Chemical Bond**	**Position**
1	Alkynes	C≡C-H Stretching vibration	3432.7
2	Olefin	C=C Stretching vibration (Conjugate)	1614.1
3	Arene	C=C Stretching vibration	1513.8
4	Alkanes	-CH_2 Scissoring vibration	1448.3
5	Alcohol, Phenol	C-O Stretching vibration	1240
6	Ether	a-Side chain on carbon	1159
7	Ether	a-Side chain on carbon	1112
8	Alcohol, Phenol	C-OStretching vibration	1159
9	Halohydrocarbon	C- Br	567

FTIR analysis of the stem bark extracts of Cassia *fistula* has absorption bands and the wave numbers (cm^{-1}) of the prominent peaks obtained were described in Table 2. The peak at a frequency of 3432cm^{-1}, 1614 cm^{-1} and 1240 cm^{-1} were strong while the others vary from medium to weak.

Discussion:

Phytochemical studies revealed the presence of secondary metabolites like tannins, flavonoid, carbohydrate, saponins, glycosides and phenol in the leaf extract while tannins, flavonoids, carbohydrate, saponins, glycosides, phenol and terpenoid in the stem bark.

The presence of secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs[5] and therefore explains its traditional use as health remedy. Secondary metabolites in plants confers them protection against bacterial, fungal and pesticidal attacks and thus are responsible for the exertion of antimicrobial activity against some microorganisms [6]. Tannins have been reported to hasten the healing of wound, inflamed mucus membrane and to arrest

bleeding[7]. The tannin-containing plant extracts were used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals[8].

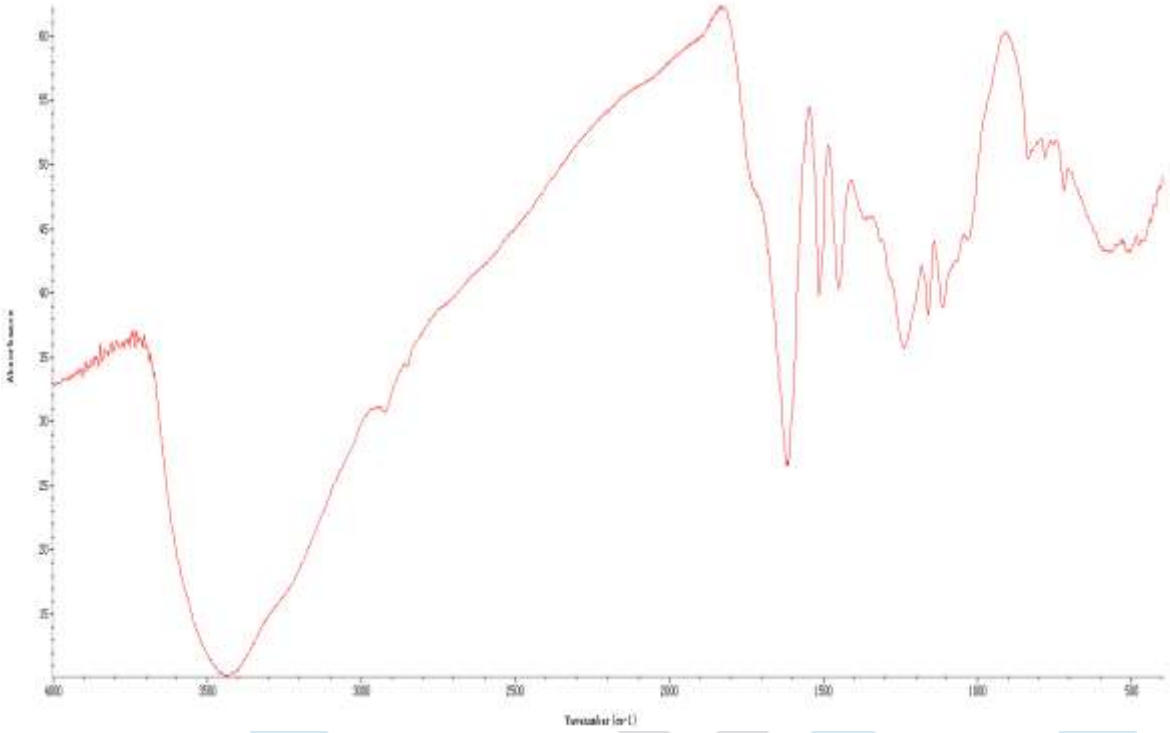

Fig. 1 FTIR spectra of ethanolic bark extract of Cassia *fistula*

The FTIR spectra analysis was utilized to identify the functional group of the active ingredients on the basis of peak value in the vicinity of infrared radiation. Results of FTIR peak values and functional groups of Cassia *fistula* ethanolic extract is represented in Fig. 1 The ethanolic bark extract has absorption bands, the wave number (cm^{-1}) of the prominent peaks obtained were described in Table 2. The IR spectrum of different extracts reveals structural information about major and minor constituents. The peak at 3432 cm^{-1} assigned to the C≡C-H stretching vibration. In addition, the peak at 1614 cm^{-1} assigned to the C=C stretching vibration(Conjugate) means that olefin existed in the bark extract. So, depending on the fingerprint characters of the peaks positions, shapes and intensities, the fundamental components may be identified [9]. The peaks at 1448 cm^{-1} Scissoring vibration belong to CH_2 (methylene) alkane; meanwhile the peak intensity at 1513 cm^{-1} is assigned to C=C Stretching vibration of arene. The peak at 1240 cm^{-1} assigned to C-O Stretching vibration of alcohol and phenol. The only weak peak observed in the stem bark was found at a frequency of 567 cm^{-1} and was assigned to C-Br stretching. The stem bark ethanol extract suggest the presence of alcohol, phenol, alkyl bromide, conjugated alkene, alkane and ether. Many workers revealed the FT-IR spectrum as an effective tool for differentiating, classifying and discriminating closely related plants and other organisms.

Conclusion:

The preliminary phytochemical screening of a plant or plant parts might be helpful in the nature of active principles and sometimes may lead to the discovery and development of new compounds. The phytochemical analysis conducted on Cassia *fistula* revealed the presence of secondary metabolites like tannins, flavonoid, carbohydrate, saponins, glycosides and phenol in the leaf extract while tannins, flavonoids, carbohydrate, saponins, glycosides, phenol and terpenoid in the stem bark. In Quantitative phytochemical analysis the characteristic absorption bands were exhibited 3432 cm^{-1} for alkyne group, 1614 cm^{-1} for olefin and 1513 cm^{-1} for arene , 1240 cm^{-1} for alcohol and phenol, 1159 and 1112 cm^{-1} for ether group, 1448 cm^{-1} for alkanes, 567 cm^{-1} for alkyl bromide.

The ethanolic extract of Cassia *fistula* contain significant amounts of phenolics and flavonoids. Phenolics and flavonoids are ubiquitously seen in most of the plant species and
reported to possess a broad spectrum of biological properties. The extensive literature survey revealed that Cassia *fistula* is an important medicinal plant with diverse pharmacological spectrum. The plant shows the presence of many chemical constituents which are responsible for varied pharmacological and medicinal property.

ACKNOWLEDGEMENTS

We thank Dr. Vijay R. Chourey, Dr. P.K. Jain for their assistance. Govt. Holkar Science College Indore is acknowledged for its financial support.

References .

[1] V. Prashanth Kumar, N.S. Chauhan, H. Padh and M. Rajani, (2006), J. Ethnopharmacol; 107 : 182-188
[2] L. Anila, N.R. Vijayalakshami, (2002), Journal of Ethnopharmacol; 79: 81-70.
[3] R. Perumal Samy, S. Ignacimuthu, and A. Sen, (1998), Journal of Ethnopharmacology; 62 :173-182.
[4] S.S. Agarwal and M. Paridhavi, (2005), Clinically useful herbal drugs, Ahuja Publishing House, 281-282.

[5] A. Mann, Y.Yahaya, A. Banso and F. John, (2008), J. Med. Plants Res., 2(5): 94-97.
[6] Marjorie, M.C. , (1999), ClinMicrobiol., 12(4) : 564-582.
[7] Mamta Saxena, Jyoti Saxena, Rajeev Nema, Dharmendra Singh and Abhishek Gupta, (2013), Journal of Pharmacognosy and Phytochemistry; 1(6) : 168-182.
[8] G. Chen, R. J. Xu, G.Y. Wang, H. Y. H. Z. Xu and Y. M. Liu, (2001), Chemistry Bulletin/HuaxueTongbao, 64 (6) : 372-374.
[9] B.O. Obdoni and P.O. Ochuko, (2001), Glob J Pure Appl Sci, 8b : 203-208.
[10] G.J. Kaur and D.S. Arora, (2009), BMC Compl Altern Med, 9: 30.
[11] V. Prasanth Kumar, N.S. Chouhan, H. Padh and M. Rajani, (2006), J. Ethanopharm; 107 : 182-188.