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ANALYTICAL STUDY OF CASSIA FISTULA LINN. BY ITS PHYSICOCHEMICAL AND CHROMATOGRAPHIC EVALUATION

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ABSTRACT

Background: - *There are 17-18 plant in Cassia genus out of which Cassia fistula . Linn is one of its kind in the world according to botanical data bases (plant of world online Kew) only one species worldwide found i.e Cassia fistula . Analytical study of that one species by its physicochemical and chromatographic evaluation of different part*

Method*: - Macroscopic study, powder microscopic studies, physiochemical and HPLC analysis done according to API standard and performed as per protocols*

Result: *- Macroscopic studies of different part characterized by Morphology and physicochemical studies as well as chromatographic analysis*

Conclusion*:- Standardization of plant with different parameter to see the variation*

KEYWORDS - *Aragwadha, Cassia fistula, Indian Laburnum Macroscopy, Golden Shower, Chromatographic*

INTRODUCTION

There are 17-18 plant in Cassia genus out of which Cassia fistula .Linn is one of its kind in the world according to botanical data bases (plant of world online Kew) [1] only one species worldwide found i.e Cassia fistula

Botanical Description

It is a deciduous tree with greenish grey bark, compound leaves, leaf lets are each 5-12 cm long pairs. A semi-wild tree known for its beautiful bunches of yellow flowers and also used in traditional medicine for several indications. A fruit is cylindrical pod and seeds many in black, sweet pulp separated by transverse partitions[2]. The long pods which are green, when unripe, turn black on ripening after flowers shed [3] . Pulp is dark brown in color, sticky, sweet and mucilaginous, odour characteristic, and somewhat disagreeable [4] . Drug occurs in flat or curved thick pieces; outer surface smooth to rough with warty patches; greenish grey to red; inner surface rough, reddish with parallel striations; fracture, laminate; odour, sweet and characteristic; taste, astringent [5]. A tree 6-9 m high; trunk straight; bark smooth and pale grey when young, rough and dark brown when old; branches spreading, slender. Leaves 23-40 cm long; main rhachis pubescent; stipules minute, linear-oblong, obtuse, pubescent. Leaflets 4- 8 pairs, ovate or ovate-oblong, acute, 5-12.5 by 3.8-9.5cm, bright green and glabrous above, paler and silvery-pubescent beneath when young, the midrib densely pubescent on the underside, base cuneate; main nerves numerous, close, conspicuous beneath; petiolules 6-10 mm long, pubescent or glabrous. Flowers in lax racemes 30-50 cm. long; pedicels 3.8-5.7 cm. long, slender, pubescent and glabrous. Calyx 1 cm long divided to the base, pubescent; segments oblong, obtuse. Corolla 3.8 cm across, yellow; stamens all antheriferous. The pods are pendulous, cylindric, nearly straight, smooth, shining, brown-black, indehiscent, with numerous (40-100) horizontal seeds immersed in a dark colored sweetish pulp. Seeds broadly ovate, 8mm. long, slightly less in breadth, and 5mm thick. The fruit pods are 40-70 cm long and 20-27mm in diameter, straight or slightly curved, smooth but finely striated transversely, the striations appearing as fine fissures. The rounded distal ends bear a small point marking the position of the style. The dorsal suture appears as a single vascular strand and the ventral suture as two closely applied strands. Internally the pod is divided by thin, buff colored, transverse dissepiments at intervals of about 0.5cm Chemical constituents of Cassia fistula are always of an interest. The different chemical constituents of the plant, Pulp of the pod contains anthraquinone glycosides, sennosides A & B, Rhein and its glucoside, barbaloin, aloin, formic acid, butyric acid and their ethyl esters and oxalic acid, presence of pectin and tannin is also reported[6]. Seeds give galactomannan free sugars and free amino acids; flowers gave ceryl alcohol, kaempferol, rhein and a bianthraquinone glycoside, fistulin. Leaves gave free rhein, its glycosides- sennosides A & B[7] .The pulp contains sugar, tannic matter, albuminous starch, oxalate of calcium and other important constituents. Leaves and flowers contain anthraquinone, tannin, oxyanthraquinone, rhein and volatile oils[8] . Pulp consists of sugar, gum, astringent matter, gluten, coloring matter and water [9]. Root bark besides tannins contains phlobaphenes and oxyanthraquinone substances[10] The plant contains rhein glucoside, rhein, fistulic acid, sennoside A & B [11]

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Fistucacidin, mp. 245°, from bark identified as racemic or meso 3,4,4',7,8-pentahydroxyflavan , glucoside from pod identified as rhein glucoside , lupeol, B-sitosterol and hexacosanol. leucoanthocyanidin - 5,4'-dihydroxyflavan-3,4-diol (I) - from sapwood [12]

An aqueous extract of fruit pulp exhibited laxative and antibacterial activities Rhein, glucose, sucrose and fructose isolated from fruit pulp , galactomannan isolated from seed composed of D-galactose and D-mannose , fistulic acid isolated from pods and characterized ; kaempferol and a leucopelargonidin tetramer having free glycol unit isolated from flowers; kaempferol- 3-f-glucoside, kaempferol-3-neohesperidoside and clitorin isolated.

Aurantiamide acetate (0.011), β sitosterol (0.006) and its β D glucoside (0.02%) has been isolated from flowers. The roots contain 7-methylphyscion, betulinic acid and βsitosterol [13,14,15]. The stem bark contains two flavanol glycosides, 5,7,3',4'-tetrahydroxy-6, 8- dimethoxyflavone-3-O-α-arabino pyranoside (C22H22O13, m.p.2850), 5,7,4'-trihydroxy-6,8,3'-trimethoxyflavone-3-O- α-L- rhamnosyl (1→2)-O-β-D-glucopyranoside (C30H36O18, m.p. 2100) and a xanthone glycoside, 1,8- dihydroxy-3, 7-dimethoxyxanthone-4-O- α-L-rhamnosyl(1→2)-Oβ-D-glucopyranoside(C27H32O16, m.p. 2170).

The pulp contains sucrose, 31.3; fructose, 26.2; and glucose, 42.5% and high concentration of potassium (1809mg/100g dry basis). The pods contain 5-nonatetracontanone, 2-hentriacontanone . Fruit pulp contained proteins (19.94) and carbohydrates (26.30%); arginine, leucine, methionine, phenylalanine, tryptophan, aspartic and glutamic acids isolated from fruit pulp; a new dimeric proanthocyanidin CFI isolated along with (-) epiafzelechin, (+)catechin, kaempferol, dihydrokaempferol and 1,8-dihydroxy-3-methylanthraquinone and its structure was determined [16].

N. N. Barthakur et al. (1995) reported that the fruit was a good source of Fe and Mn, and their concentrations were considerably higher than those in apple, apricot, peach, pear and orange. Aspartic acid, glutamic acid and lysine constituted 15.3, 13.0 and 7.8%, respectively, of the total amino acids in the pulp. In the seeds the same amino acids constituted, respectively, 16.6, 19.5 and 6.6%[17]. M. M. Vaishnav et al. (1996) confirmed that Rhamnetin 3-O- gentiobioside was isolated from the roots[18] . T.N. Misra et al. (1996) reported that the hexane fraction of fruits (collected from India) exhibited activity against Klebsiella sp. 5-Nonatetracontanone, 2-hentriacontanone, triacontane, 16- hentriacontanone and beta - sitosterol was isolated from the hexane fraction[19].
T. N. Misra et al. (1997) isolated a new diterpene, 3 beta - hydroxy-17-norpimar- 8(9)-en-15-one from the pods of Cassia fistula[20] . Meena Rani et al. (1998) reported that from the pods of Cassia fistula, an anthraquinone derivative, characterized as 3-formyl-1-hydroxy-8-methoxy-anthraquinone 1, was isolated. This is the first report on the isolation and characterization of this compound [21] Lee et al. (2001) reported that twenty-seven compounds including eight long- chain hydrocarbons, 1-hexacosanol, 1-octacosanol, palmitic acid, stearic acid, oleic acid, linoleic acid, heptacosyl eicosanate, glyceryl-1-tetraeicosanoate; three sterols, beta - sitosterol, stigmasterol, beta -sitosteryl-3-O-D-glucopyranoside; one triterpene, lupeol; eight anthraquinones, chrysophanol, emodin, physcion, citreorosein, rhein ,rhein methyl ester, ziganein, 1,4,5- trihydroxyanthraquinone; two coumarins, isoscopoletin, scopoletin; two chromones, 2,5-dimethyl-7- hydroxychromone,2,5-dimethyl-7-methoxychromone,three
aromatic compounds, isovanillic acid, vanillic acid and 2,4-dihydroxybenzaldehyde were isolated and identified from the aril of Cassia fistula. Their structures were determined on the basis of spectral data [22]

MATERIAL AND METHOD

Plant Material

Leaves of Cassia fistula is been collected from Maharashtra state Thane district and for authentication these been sent to Agarkar research institute Pune in dry herbarium form.

Macroscopic and Organoleptic Analysis

Macroscopic and organoleptic character of Aragwadha leaves , pods, flower , stem were observed and noted

Powder Microscopy and Physicochemical Analysis

Powder microscopic as well as physiochemical analysis done by Alarsin Research institute . It includes color, odour taste texture ash value , acid soluble extract, water soluble extract , acid insoluble extract these parameter were carried as per the method described by Ayurvedic pharmacopeia of India

Chromatographic Analysis

Chromatographic analysis (HPLC) of Aragwadha leaves done by Ultra pure analytics pvt .ltd. Std of Rhein and churna sample were individually dissolved in 50:50 Methanol:Water to prepare the solution of concentration 1000 ppm for std and 2500ppm for the sample and injected into HPLC . The std peak of Rhein was observed at RT around 13.9 mins

Hplc method details

Column: Inertsil ODS 3v, 150 x 4.6, 5 micron
Flow: 1 mL/min
Mobile Phase: 0.05% TFA: ACN Gradient Wavelength: 230 nm
Gradient Program:

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Time.	% ACN
0-20 MIN	0-100% ACN
20-24 MIN.	100% ACN
24-25 MIN	100-0% ACN
25-30 MIN	0%ACN

OBSERVATION AND RESULT

Macroscopic and organoleptic evaluation

- **Leaves :-** Paripinnately compound , Alternate-spiral arrangement along the branches. **Leaflets-**Opposite, typically 3-8 pairs, large (7–21 cm long, 4-9 cm broad), ovate to oblong-ovate, with a rounded to subacute base**. Texture-** Glabrous (smooth) on the upper surface, often pubescent (hairy) below. With smooth margins **,** Reticulate venation , **Color-** Bright green when young, becoming darker green with age, Petioles are short, 2–6 mm long.
- **Bark-** Greenish-grey to pale grey when young; turns dark brown to red- brown when mature. , **Texture-** Smooth in younger trees, becoming rough, brittle, and cracked (rhytidome formation) in older trees, **Surface-** horizontal lenticular markings and warty patches. **Inner Surface-** Reddish-brown or pale red with prominent parallel striations. **Fracture-** Laminate (layered) and fibrous.
- Flower -Inflorescence- Long, lax, pendulous racemes, axillary and range from 30–60 cm in length, Corolla (Petals)- Five bright yellow, obovate petals often measuring up to 3.5 cm across , Calyx (Sepals): Five yellow-greenish, pubescent and parted nearly to the base. Androecium (Stamens): 10 stamens in total, complex arrangement (heteranthery), Gynoecium (Pistil)- monocarpellary, stalked, pubescent (hairy) ovary with a curved style and terminal stigma, Pedicel- The flower hangs from a long, slender pedicel (stalk).

All the macroscopic character are mention in figure 1&2

ORGANOLEPTIC EVALUATION

Organoleptic character of powder product of Aragwadha leaves done by Alarsin pharmaceutical labs and the powder microscopic image of part of Aragwadha patra is shown in figure 3

Aragwadha leave

Test	Result
Appearance	Fine powder
Taste	Slightly bitter
Colour	Dark brown
Odour	Characteristics
Texture	Rough

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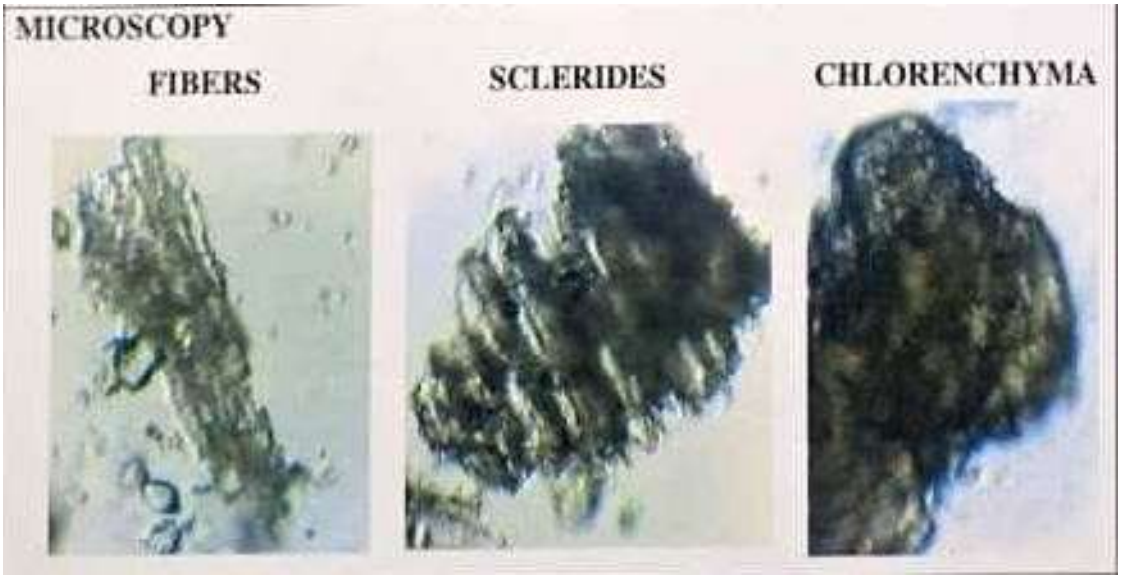

Microscopic of powder of Aragwadha leaf contains fibers , sclerids and collenchyma

PHYSICOCHEMICAL ANALYSIS

Result obtained from physicochemical content such as moisture contain, total ash value, acid insoluble ash , alcohol soluble extract, water soluble extract of the sample are depicted in table.
Done as per API protocol

Physicochemical Parameter

Sr no	Parameters	Aragwadha Patra result
1	Moisture content	3.11%
2	Total ash value	1.96%
3	Acid insoluble ash	0.76%
4	Alcohol soluble extract	17.22%
5	Water soluble extract	47.65%

CHROMATOGRAPHIC EVALUATION

Rationale : To Identify Rhein from Aragwadha leaf churna
Procedure: Aragwadha leaf churna was received for the identification of Rhein by HPLC.

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Hplc method details:

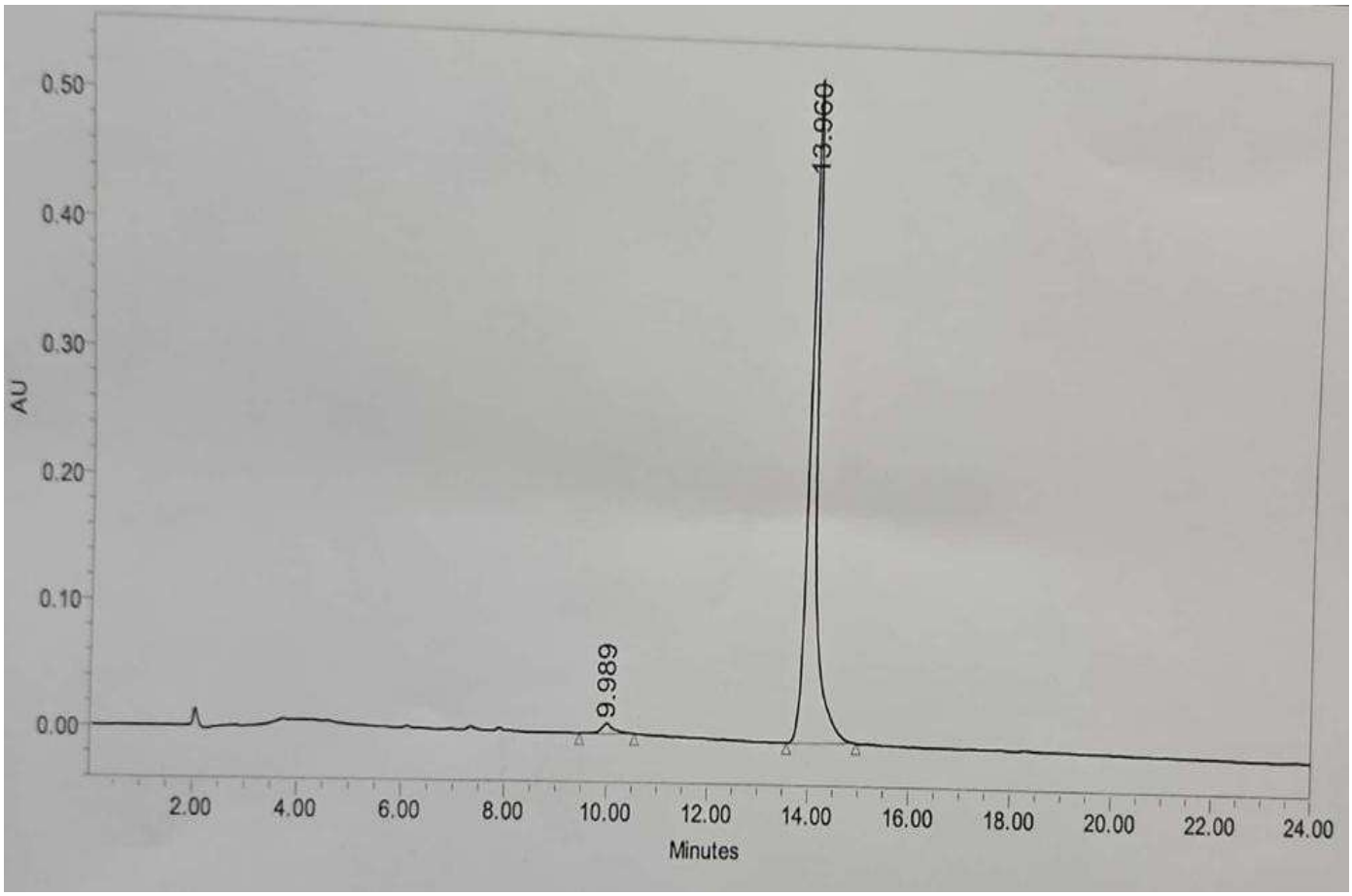
Column: Inertsil ODS 3v, 150 x 4.6, 5 micron

Flow: 1 mL/min

Mobile Phase: 0.05% TFA: ACN Gradient Wavelength : 230 nm Gradient Program:

Time	% CAN
0-20 MIN	0-100% ACN
20-24 MIN	100% ACN
24-25 MIN	100-0% ACN
25-30 MIN	0% ACN

Observation: Std Rhein and the churna sample were individually dissolved in 50:50Methanol:Water to prepare the solution of concentration 1000 ppm for std and 2500 ppm for the samples and injected into HPLC.
The std peak of Rhein was observed at RT around 13.9 mins. The extract showed Peak at 13.9 mins.
Conclusion: Since the std peak RT Matches with the sample peak RT, the extracts may contain Rhein



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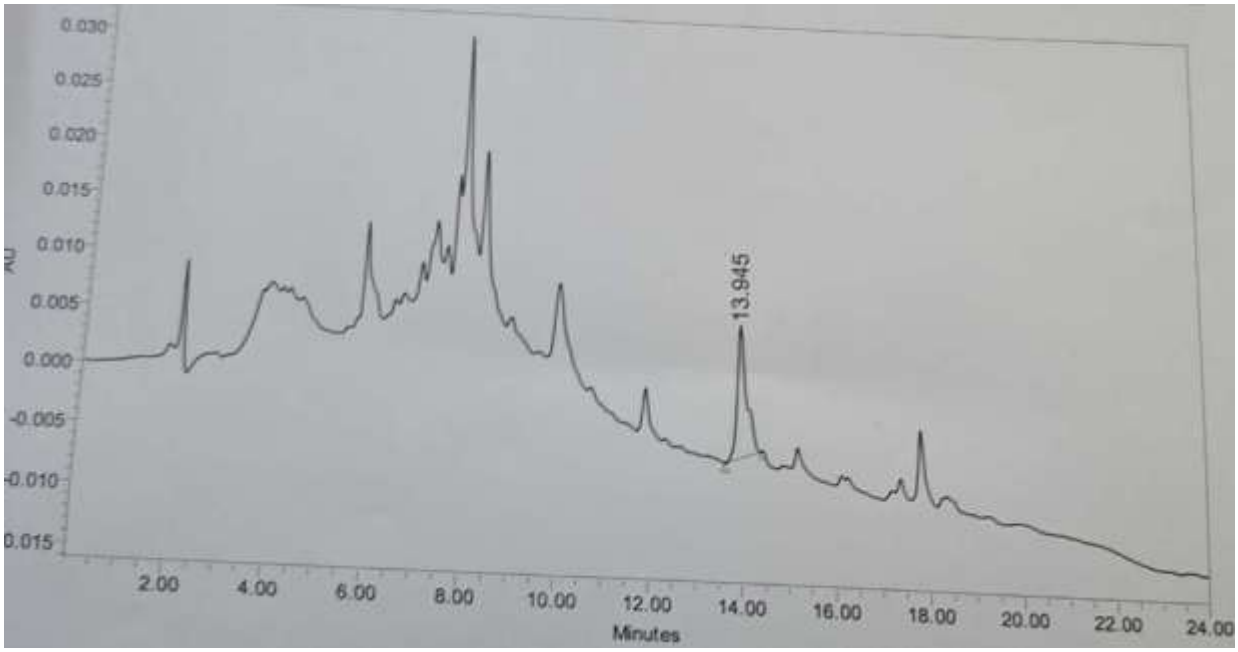
	RT	% Area	Area (µV*sec)	Height (µV)
1	9.989	2.24	138125	7865
2	13.960	97.76	6030596	535157



RT	% Area	Area (µV*sec)	Height (µV)
13.945	100.00	155212	11339

DISCUSSION

Outcome of this drug research firstly macroscopic evolution of Aragwadha i.e Cassia fistula its physical appearance like leaves venation, color, petiole, bark character color , flower phyllotaxy its arrangement fragrance and organoleptic character .

Organoleptic as well as physicochemical character of leaf part of the plant are like presence of acid soluble extract, water soluble extract, ash value, etc. as mention in this research article. By chromatographic evaluation came to know that Rhein which is most important chemical present in Aragwadha in anthraquinone compound is found in the research study

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