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RESEARCH ARTICLE

Plant Drug Analysis - A Comparative Analysis of Cassia Fistula

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ABSTRACT

Medicinal plants are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. This article aims to provide a Comparative analysis of different characteristics of bark and pulp of Cassia fistula. It is obtained from deciduous and mixed-monsoon forests throughout greater parts of India, is a fast growing, medium sized tree in *Caesalpinacenae* family with a common name of Golden Shower Tree, is widely used in traditional medicinal system of India has been reported to possess hepatoprotective, anti-inflammatory, antitussive, antifungal and also used to check wound healing and antibacterial. It is known as a rich source of anthraquinone derivatives, tannins, flavonoids, β–sitosterol, fistulic acid and glycosides. The innumerable medicinal properties and therapeutic uses of Cassia Fistula as well as its phytochemical investigations prove its importance as a valuable medicinal plant.

Keywords- Cassia fistula, pharmacological activities, phytochemistry, Anthraquinone derivatives, Sennosides, Rhein and traditional uses.

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INTRODUCTION

Nature has gifted us with abundant herbs and plants that have the ability to cure a wide range of diseases in natural way. The aim of herbal product is to cure the root cause of the problem and offer lasting cures for diseases and do not have harmful side effects. Ayurveda is considered by many scholars to be the oldest healing science. Ayurvedic knowledge originated in India more than 500 years ago and is often called "mother of all healing". Ayurveda has become an internationally acclaimed form of healing, rejuvenation and healthy living. The *plant drug analysis* is a course which introduces the chemical techniques used to extract and identify medicinal drug derived from plants. The analysis which includes the herbal collection and processing, sample preparation and chromatographic analysis of extract, fraction and compounds isolated from herbs and medicinal plants. Natural products isolated from the higher plants and microorganisms have been providing clinically active drug. The key to the success of discovering naturally occurring therapeutic agent rest on bioassay guided fractionation and purification procedure. Screening of both synthetic organic compounds and extracts of natural product has an impressive history of identifying active agents.

Cassia fistula Linn, Which is popularly known as *'Indian laburnum'* in English. It comes under the family of *caesalpiniaceae*. It is well known as golden shower flower tree. The drug is majorly used in ayurvedic medication for various ailments. It is a tree known for its beautiful bunches of flower; it is a monsoon plant all over the greater parts of India is mostly used in traditional medicine of Indian system for its liver protective, antimycotic and wound healing. Various parts are used for medicinal purpose such as root, bark, pulp, leaves and flower (Modi, V.K, Rao, T.V.P and Venkataeswaraln, V). Nowadays the Research works are going on to identify, isolate and validate the various medicinal components from cassia fistula (Agrawal, G.D, Rizvi.A.I, 1972. Gupta, P.C and Tewari. J.D). The speciesis native to the Indian Subcontinent and adjacent regions of Southeast Asia. Cassia fistula is the national tree of Thailand, and cassia fistula flower is national flower of Thailand. In India Cassia fistula is state flower of Kerala and having great importance amongst Malayali population. Cassia fistula is a popular ornamental plant and having great importance in herbal medicine (Aiyer, K.K, et al and Kolammal, M et al, 1969).

Taxonomic Classification

Kingdom:	*Plantae*
Subkingdom:	*Tracheobinota*
SuperDivision:	*Spermatophyta*
Division :	*Mangoliophyta*
Class:	*Magnoliopsida*
Sub Class:	*Rosidae*
Order:	*Fabales*
Family:	*Fabaceae*
Genus:	*Cassia*
Species:	*Fistula*

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Geographical Source

Cassia fistula is abundant in different parts of India. It is cultivated in South Asia, Rajasthan, Gujarat, Thailand, Southern Pakistan and east through India to Myanmar and South to Srilanka. It grows in valleys up to 1300 m in Himalaya and Alps.

Morphology

Cassia fistula is a fast growing, medium sized, deciduous tree in the *caesalpinacenae* family, which grows to about 9 meters in height. Leaves are pinnately compound with 4-8 pairs of opposite leaf lets, ovate, acute, bright green, glabrous above, paler and silvery-pubescent beneath when young. It produces main numerous, flowers which are golden yellow in lax pendulous racemes and hang in showering bunches of up to 40 cm long earning its common name of 'golden shower tree'. It has pendulous, dark brown fruits cylindrical, woody, seed pods, 30-60 cm long, shortly stipulate nearly straight, smooth, shiny, brownish black, seeds broadly ovate, horizontally immersed in dark coloured sweetish pulp and which persist on the tree throughout the winter before falling to the ground. Bark of cassia fistula is a medium sized one with 8-15 cm in height and a greenish grey smooth in nature. Cassia fistula blooms late spring. Flowering is profuse, with trees being covered with yellow flora, with almost no leaf being seen. Not recommended for dry climates. Growth is best in full sun on well-drained soil. It will be damaged by even short spells of freezing weather. It can be subjected to mildew, leaf spot and root diseases.

Traditional Medicinal Uses

Medicinal uses of cassia fistula are very popular in Ayurveda. Therapeutically, cassia fistula is a purgative and laxative in nature. The drugs made by the use of pulp and bark of cassia fistula are very important and popular in medical field (Esposito Avella, M., Diaz, A., De Gracia, I., De Tello, R. and Gupta, M.P, 1991). The bark is used as tonic and it act against dysentery, various skin diseases, jaundice, syphilis and heart diseases. The bark of fistula is laxative, anthelmintic, febrifuge, diuretic, and depurative and is useful in pustules, leprosy, ring worm, colic, dyspepsia, constipation, fever, and diabetes (Ilavarasan Raju, Mallika Moni and Venkataraman Subramanian, 2005). The fruit pulp is sweet, cooling, emollient, anodyne, anti-inflammatory, depurative, antipyretic, diuretic and ophthalmic in nature (Lilly Kutty, L and Santhakumari, G.1969). So it is very much useful in vitiated conditions of leprosy, skin diseases, pruritus, rheumatism, anorexia, hepatomegaly, jaundice, inflammations, anthrax, dysentery and diabetes (Yokozawa, T., Chen, C.P. Dong, E., Tanaka, T., Nonaka, G.I. and Nishioka, I., 1998). It is a safe purgative for children and pregnant women. The purgative activity is

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due to the presence of anthraquinone derivatives (Kaji, N.N, Khorana, M.L and Sunghavi, M.M). Both bark and pulp has significant antibacterial activity due to the presence of flavonoids. The important medicinal action of the drug is to purify the blood by promoting the elimination of toxins. The drug act as a helpful adjuvant for healthy skin by curing the infected area.

Phytochemistry

The important chemical constituent present in all parts of *cassia fistula* is anthraquinone derivatives (Kapadia, G.J. and Khorana, M.L 1962). Cassia fistula bark contain flavonoids (Yadava, R.N, and Verma, V, 2003), fistulic acid, rhein, fistucacidine, kaempferol, procyanidine, tannin, oxyanthraquinone, emodin, steroids like luperol, β-sitosterol (Murty, V.K, Rao, T.V.P and Venkataeswaralan, V., 1967).
Cassia fistula pulp contains proteins, carbohydrates, sennoside A & B, rhein, its glucoside barbaloin, aloin, pectin, flavonoids (Venkitaraman, S and Radhakrishnan, N. 1972), tannin, fistulic acid, oxyanthraquinone, emodine and chrysophanol (William Pelletie, S. et al, Khanna, K.L., Takido, M., Rusenberg, H., and Paul, A.G, 1970).

Pharmacological Activities

Cassia fistula is highly energetic and powerful drug. The drug provides a positive energy to the human body. The anti-inflammatory and anti-infective action reduces inflammation and promotes healing (Lilly Kutty, L. 1968, Prajapati, Purohit, Sharma and Kumar). The laxative property of the drug corrects chronic constipation associated with haemorrhoids. The anti hemorrhoidal and anti-inflammatory properties of the drug, helps to shrink the pile mass, control bleeding and hasten the healing process in inflamed skin and mucous membranes. The analgesic action of drug relieves pain, and the soothing action facilitates the smooth evacuation of feces. The anti-allergic property is beneficial in the control of pruritus associated with skin infection (Bhakta, M. and Mukherjee, P.K, Mukherjee, K., Banerjee, S., Mandal, S.C., Maity, T.K., Pal, M. and Saha, B.P.1999). The hepatic stimulant property helps in the improvement of liver functions and removal of toxic metabolic product in various systemic and skin infections. The anthelmintic action is useful in management of cutaneous manifestations of worm infestations. The various constituents of cassia fistula provides antiseptic, antibacterial, antiparasiti, antioxidant, antitumor diuretic, antipyretic (Patel, D.G, Karbhari, S.S, Gulati and Gokhale, S.D), antifungal, antiviral and wound healing (Patel, R.P and Patel, K.C.1957).

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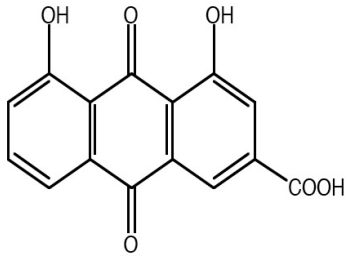
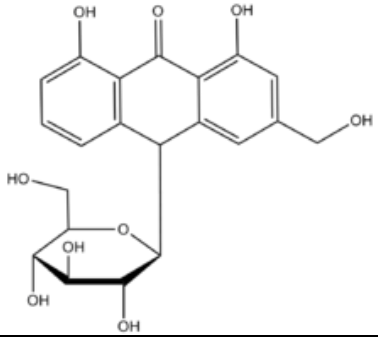
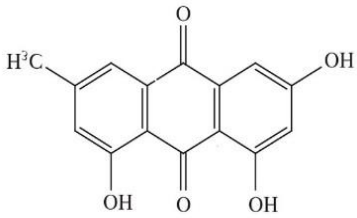
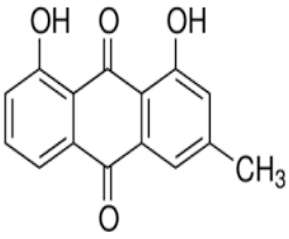
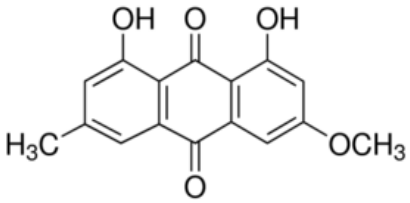
<table>
<tr><th colspan="2">Anthraquinone Derivatives</th></tr>
<tr><td>Rhein ($C_{15}H_8O_6$)

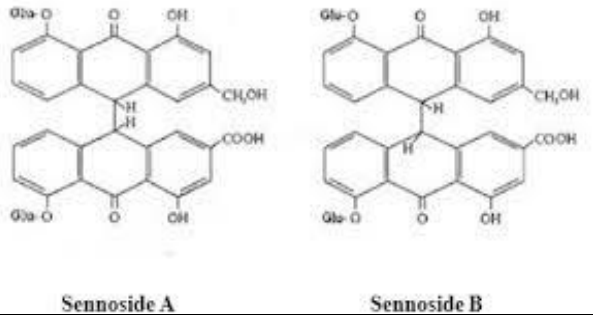
Molecular weight, melting point and absorption are 284.22 g/mol, 321.32°C and 260 respectively. It is insoluble in water and soluble in most of the solvents. It act as an enzyme inhibitor.
  </td><td>Barbaloin ($C_{21}H_{22}O_9$)

Yellow-brown coloured crystalline substance with molecular weight and melting point are 418.4 g/mol and 148°C respectively. It act as a part of peripheral defence strategy.
  </td></tr>
<tr><td>Emodin ($C_{15}H_{10}O_5$)

Orange crystalline powder with molecular weight and melting point are 270.24 g/mol and 224°C respectively. It is soluble in solvents with absorption at 435 nm.
  </td><td>Chrysophanol ($C_{15}H_{10}O_4$)

Golden yellow powder with the molecular weight and melting point are 254.24 g/mol and 356°C respectively.
  </td></tr>
<tr><td>Physcion ($C_{16}H_{12}O_5$)

Orange – yellow pigment with molecular weight is 284.263 g/mol.
  </td><td>Senniside A & B

Dianthrones with crystalline structure, having high molecular weight and gives absorption at 350 nm.
 
Sennoside A Sennoside B</td></tr>
</table>

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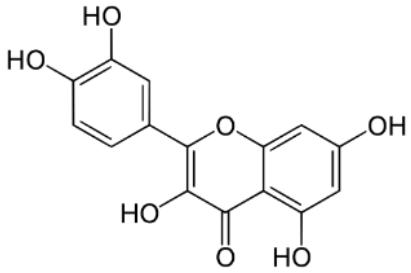
Flavanoids

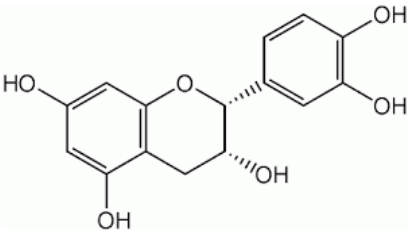
Quercetin ($C_{15}H_{10}O_7$)

Yellow crystalline powder with molecular weight is 302.236 g/mol. It is more specific aglycone form. It helps to prevent some types of cancer.



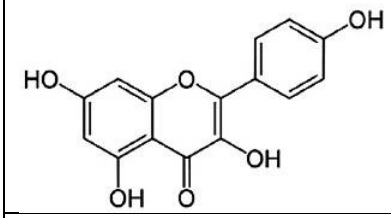
Epicatechin ($C_{15}H_{14}O_6$)

It is a natural flavonoid. It improves blood flow, good cardiac health.



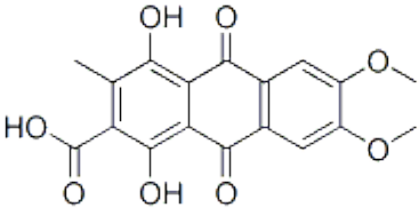
Kaempferol ($C_{15}H_{10}O_6$)

Yellow crystalline solid with molecular weight and melting point are 286.23g /mol and 276-278^{o}C respectively. It is slightly soluble in water and soluble in hot ethanol and diethyl ether. It reduces the risk of heart diseases.



Fistucacidin ($C_{15}H_{14}O_6$)

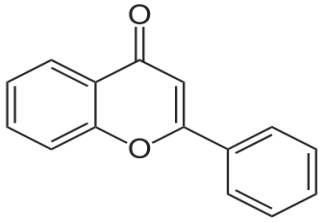
Its molecular weight is zero with a melting point of 245^{o}C.



Tannin

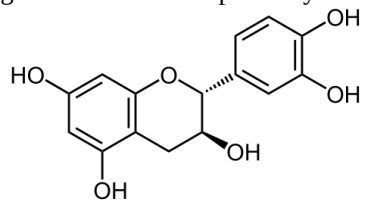
Flavone ($C_{15}H_{10}O_2$)

Condenced tannin, soluble in water.It is a colourless crystalline, water soluble compound.



Catechin ($C_{15}H_{14}O_6$)

Condenced tannin, flavan- 3 - ol. It is a colourless solid with molecular weight and melting point are 290.26 g/mol and 175^{o}C respectively.



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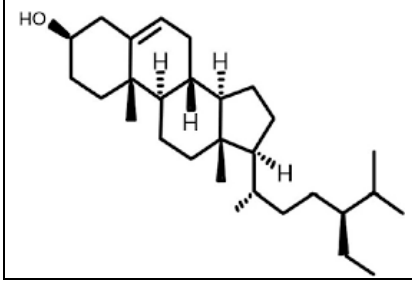
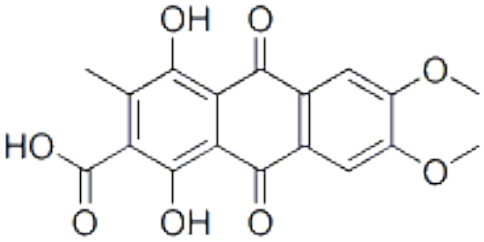
Sterol	Acid
β-Sitosterol ($C_{29}H_{50}O$) White colour and waxy in nature. Molecular weight is 414.71 g/mol. It is used in treating cholesterolemia, breast cancer and herbal therapy. 	**Fistulic acid** It is a colouring matter from the pods of cassia fistula. It also reduces the lipid level in blood and liver. 

MATERIALS AND METHODS

Collection of Plant Materials

The fresh and healthy bark and pulp of the plant *Cassia fistula* were collected from the various areas of Thrissur district, Kerala, India. Plant parts were collected on the basis of the information provided in the ethno botanical survey of India (Sen, A.B., and Shukla, Y.N, 1968).

Preliminary Phytochemical Screening

Preliminary phytochemical screening is the successive solvent extraction. The dried powdered plant material is used for extraction. About 100g of the pulp of cassia fistula is accurately weighed and made up to 1L by using water. Rotary vacuum evaporator is used for extraction. Alkaloid extraction takes place at a temperature of 48^{o}C. Collect the extract in clean airtight bottles and stored at 4^{o}C. The same procedure is also done for the bark of cassia fistula. The extracts obtained are then subjected to qualitative tests separately for the identification of various plant constituents (Trease, G.E and Evans, W.C, 1997).

Determination of Ash

Accurately weighed ground drug is taken in a platinum or silica dish which is ignited and weighed. Scatter the ground drug in a fine even layer on the bottom of the dish. Incinerate gradually at a temperature of 500-600^{o}C until free from carbon, cool and weighed. Calculate the percentage of ash with reference to the air dried drug (Kirtikar, K.R, and Basu, B. A., 1991).

Determination of Acid insoluble ash

Ash is boiled with 25 ml of dilute HCl for 5 minutes, collects the insoluble matter in a crucible or in a ash less filter paper washed with hot water. Ignited to 600^{o}C in an oven for 4 hours and cool and weighed, calculate the percentage of acid insoluble ash with reference to the air dried drug.

Determination of Water soluble ash

Ash is boiled with 25ml of water for 5 minutes, collects the insoluble matter in a crucible or in a ash less filter paper washed with hot water. Ignited to 600^{o}C in an oven for 4 hours and cool and weighed, calculate the percentage of water soluble ash with reference to the air dried drug.

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Extracts
The extracts obtained by exhausting drug are indicative of approximate measure of their chemical constituents. Its percentage is taken as a standard for an herb (Sathyavathi, G.V., Raina, M.K and Sharma, M, 1987).

Determination of Alcohol soluble extract
Accurately weighed ground drug is taken in a filter paper thimble, soxhlet extractor. It is connected to a previously weighed RB flask. About 50 ml of methanol is added through the filter paper containing substance and boil gently for 3 hours and the RB flask containing the extracted liquid can be distilled to remove methanol and the dried material present in the RB flask can be cooled and weighed, its percentage can be calculated with reference to the air dried drug.

Determination of water soluble extract
Accurately weighed ground drug is mixed with 100 ml of water, taken in a RB flask. Condensing the mixture for 4 hours and cool. Filter rapidly, evaporate 10 ml of the filtrate to dryness in a flat bottomed shallow dish and its percentage is calculated with reference to the air dried drug.

Analysis of extracts
The extract prepared is subjected to analysis to find out its complexity. For the analysis ofan organic solvent extract or water extract of plant material, the simplest as well as reliable method is TLC. The hydrolysed water extract and solvent extract contains more polar constituents are directly used for TLC and UV analysis (Iyengar, M. A., Pendse, G.S and Narayanan, 1960).

Thin Layer Chromatography
TLC fingerprints were performed on a precoated aluminium plate of silica gel $60F_{254}$ (10 x 20 cm) using suitable solvents as the mobile phase.

TLC of Hydrolysed water extract of pulp and bark of cassia fistula
Hydrolysed water extract of pulp and bark are spotted in an activated plate separately. It is placed in a glass jar containing the solvents light petroleum, ethyl acetate and formic acid in the ratio 1 : 5.2 : 5, jar was covered with another glass plate and placed until solvent front reach the top of the silica gel plate. The dried plate is sprayed with 5% ethanolic KOH, a pink spot develops. R_f values are determined by comparing the pulp and bark extracts of cassia fistula.

TLC ofMethanol extract of pulp and bark of cassia fistula
Methanol extract of pulp and bark are spotted in an activated plate separately. It is placed in a glass jar containing the solvents ethyl acetate, methanol and water in the ratio 10 : 1.35 : 1, jar was covered with another glass plate and placed until solvent front reach the top of the silica gel plate. The dried plate is sprayed with con.HNO_3 and heated for 10 minutes at 120^oC, a spot develops, it can be identified by spraying the plate with 5% ethanolic KOH, gives brown-red spot. R_f values are determined by comparing the pulp and bark extracts of cassia fistula.

Spectrophotometric method
All the extracts were measured by UV/VIS spectrophotometric method at 515 nm (Lambda 35 UV/VIS spectrophotometer, Perkin Elmer, USA).

UV Spectrophotometry
UV Spectrum is taken for both hydrolyzed water and methanol extracts of pulp and bark, by dissolving the corresponding extracts in 10 ml of methanol, and compared the absorbance of both parts of cassia fistula.

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A Synthetic evidence for the preparation of AragwadhamruthadiChoornam

All the ingredients such as Cassia fistula, Tinosporacordifolia, Terminalia chebula, Acacia catechu are sliced in to small pieces and allowed to undergo drying in an oven for 2 days. The dried ingredients are pulverized in to fine powder separately. For each 10 g of choornam is prepared by mixing 2.5 g of each ingredient.

RESULTS AND DISCUSSION

Phytochemical Screening

The preliminary phytochemical screening of plant material shows that the various constituents present in the respective parts of Cassia Fistula are given in the Table-1.

Table-1: Parts of Cassia Fistula.

Indian Cassia Fistula		
Constituent	**Bark**	**Pulp**
Anthraquinone	present	present
Tannin	present	present
Flavanoids	present	present
Glycosides	present	present
Alkaloids	present	present
Carbohydrate	absent	present
Polyphenol	present	present
Saponins	absent	absent

Characterization

The ash value will give the idea of mineral strength present in the raw herb. The extracts obtained by exhausting drug are indicative of approximate measure of chemical constituents. Its percentage is taken as the standard for an herb. The data is given in Table-2 and Table-3.

TABLE-2: IDENTITY, PURITY AND STRENGTH – CASSIA FISTULA BARK

Contents	Standard Value	Calculated Value
Total ash	Not more than 10	7.04
Acid insoluble ash	Not more than 0.1	0.012
Water soluble ash	Not more than 2	1.03
Water soluble extractive	Not less than 20	29.39
Alcohol soluble extractive	Not less than 15	25.52

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TABLE-3: IDENTITY, PURITY AND STRENGTH – CASSIA FISTULA PULP

Contents	Standard Value	Calculated Value
Total ash	Not more than 6	5.58
Acid insoluble ash	Not more than 1	0.54

Extract Analysis

The analysis of extract gives the correct idea about its complexity. The chemically active constituents of the drug is determined and confirmed by Thin Layer Chromatographic analysis. For both bark and pulp of cassia fistula, anthraquinone derivatives are the active constituents present (Gupta, V., Agrawal, A., Tiwari, H.P., 1989). The R_f values are also very much agrees with each other. These active constituent makes cassia fistula is very in drug manufacturing especially for skin diseases (Luximon Ramma, A, Bahorun, T., Soobrattee, M.A and Aruoma, 2002). The TLC data is given in Table-4.

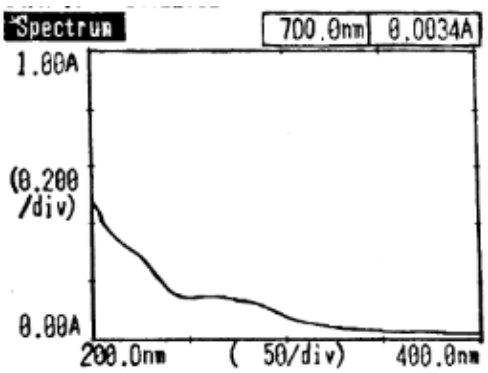
Table-4: Data of TLC.

Extract	Plant Part	R_f Value
Hydrolysed water extract	Bark	0.5
	Pulp	0.5
Methanol extract	Bark	0.805
	Pulp	0.6017

The Ultra Violet Spectrum of hydrolysed water extracts and methanol extracts of both bark and pulp were taken which shows that the absorptions at 216 nm, 281 – 285 nm, and 261 nm corresponds to the existence of anthraquinone skeleton, especially the absorption at 261 nm is given by the anthraquinone derivative, rhein (Kuo, Y.H, Lee, P.H and Wein, Y.S, 2002) . UV Spectrual data is given below.

UV Spectrums of Absorbance (A) verses Wavelength (nm)

Hydrolysed Water Extract of Pulp



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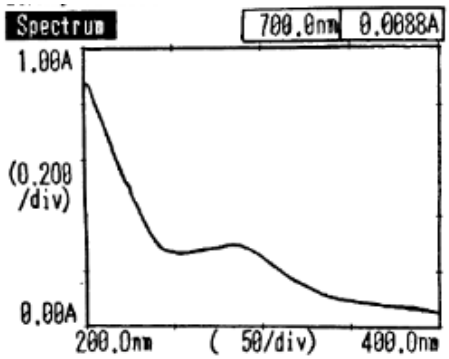
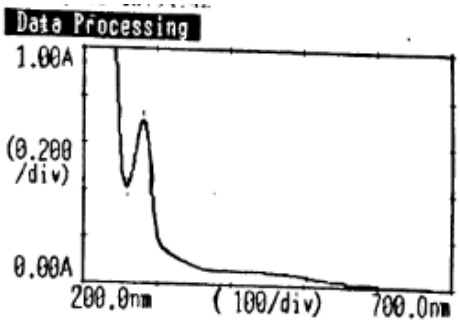
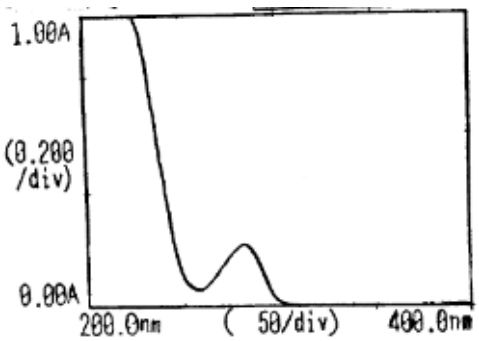
Alcohol Extract of Pulp



Hydrolysed Water Extract of Bark



Alcohol Extract of Bark



Peak Detection

Hydrolysed Water Extract		Alcohol Extract	
Pulp	Bark	Pulp	Bark
261nm/ 0.1519A	281nm / 0.7031A 216 nm/ 3.7628A	284nm / 0.2961A	282nm / 0.2174A

Physico Chemical Properties of Aragwadhamruthadi Choornam Product Specification

Description		Parameters	
Nature	Powder	Moisture	4
Texture	Smooth, Shining	Total ash	2
Colour	Pale green brown	Acid insoluble ash	0.1
Taste	Bitter	Water soluble ash	3
Smell	Pleasant		

CONCLUSION

Cassis fistula Linn is an important source of naturally occurring bioactive compounds. It is becoming clear that traditional systems of medicine have become a topic of global importance. Current estimates from the World Health Organization suggest that, in many developing countries, a large proportion of populations rely heavily on traditional practices. Herbal medicines or phytomedicines have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries are turning to alternative or

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complementary therapies, including medicinal herbs. Many of these plants are tropical plants emanating from less developed countries. Cassia fistula could be one of them particularly because of its less toxicity and its widespread use for its multiple medicinal effects. Although traditional medicines help to fill the gaps in modern health care, it is of upmost importance to evaluate the safety and bioefficacy of the extracts used. Thispaper also highlights the importance of Cassia fistula in medical field, especially for the curing skin disorders, due to the presence of wide variety of chemical constituents like rhein, barbaloin, sennosides etc.

Preliminary phytochemical analysis of all the evaporated solvent extracts shows that, active constituents present in both pulp and bark of cassia fistula is anthraquinone derivatives. Identity, purity and strength of the drug are determined by ash values and extractives. The results of these are agreed well with the standard pharmacological values. The chemically active constituents of the drug is determined and confirmed by chromatographic analysis. For both pulp and bark of cassia fistula, anthraquinone derivatives are the active constituents present. The R_f values are also very much agrees with each other. Because of the presence of these active constituents in both pulp and bark of cassia fistula, these are verymuch important in drug manufacturing especially for skin diseases. The antibacterial and antifungal activities of the active constituents are the main reason for their action against skin infections. UV absorbance method is used for making a comparative study of the extracts of pulp and bark of cassia fistula. UV absorptions at 216 nm, 281-285 nm and 261nm corresponds to the existence of anthraquinone skeleton, especially the absorption at 261 nm is given by the anthraquinone derivative, rhein. On the basis of the above concept "Aragwadhamruthadi Choornam" was prepared for the treatment of allergic skin disorders and acid peptic diseases. It is one of the evidence for the uses of cassia fistula in medical field.

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