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ISOLATION, CHARACTERIZATION AND FORMULATION OF *CASSIA FISTULA* .Linn (Caesalpiniaceae)

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ABSTRACT

Aim: To perform the isolation, identify the isolated compound and spectral study by using a plant *cassia fistula* Linn. Objective: The *cassia fistula* was collected, authentified and performed the phytochemical evaluations. Then ethyl acetate fraction isolated by using column chromatography. The isolated compound was found using characterisation. The formulation of micro-emulsion was prepared and evaluated by FT-IR study. Conclusion: Isolated and characterization of the compound which has to be performed for the exploration of the drug. Evaluation of the micro-emulsion form by using FT-IR study for its structural elucidation. The formulation used to Cross the BBB (Blood Brain Barrier) in the treatment CNS disorder.

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INTRODUCTION

Cassia fistula Linn, family Caesalpiniaceae known as Amulthus/indian laburnum1. It is one of the drugs present in tropical regions of Asia. Cassia is naturally distributed across India. The study primarily based on phytochemical, Pharmacognostical and instrumental techniques such FT-IR and others.

COLLECTION AND AUTHENTIFICATION;

The barks of *cassia fistula* were collected from Ariyalur district, Tamil Nadu and it was Taxonomic identified and authenticated by Prof. Dr.P. Jayaraman, Ph.D, Director, Retd, Plant Anatomy Research Centre (PARC) West Tambaram, Chennai. An authentification certificate number PARC/2015/3025. The bark of *cassia fistula* was separated and shade dried. The dried material was reduced to a coarse powder and was successively extracted in soxhlet apparatus using ethyl acetate for 24 hrs). The solvents were redistilled and ethyl acetate extract was concentrated under reduced pressure and air dried. The yield of ethyl acetate extract was found to be 36%w/w.

EXTRACTION PROCESS:

MATERIALS REQUIRED:

- Bark
- Ethyl acetate
- Distilled water
- Hammer mill
- Soxhlet apparatus
- Heating mantle
- Electronic balance

SOXHLET APPARATUS

PROCEDURE:

A solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded in to the main chamber of the soxhlet extractor.

The extraction solvent to be used is taken in to a distillation flask and the soxhlet extractor is now placed on to this flask. The soxhlet is then equipped with a condenser .The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid.

The condenser ensures that any solvent vapour cools, and drips back down in to the chamber housing the solid material. The chamber containing the solid material is slowly filled with warm solvent. Some of the desired compound will then dissolve in the warm solvent.

When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask; Then the thimble ensures that the raped motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hours or days.

PHYTOCHEMICAL STUDY

Phytochemical study is based on the extract which is collected from the soxhlet apparatus and the results are tabulated below.

COLUMN CHROMATOGRAPHY

The column chromatography is taken and washed with water then with acetone. Again washed with ethyl acetate solution then the column is packed with required components which includes cotton, sand, glass wool and asbestos. Then the slurry of silica gel G is poured into the column and set aside for some time. Now the sample packed in ashless filter paper previously soaked with mobile phase. Then column is again closed with cotton layer. Now the column is made to run for 7 to 8 hours and the elution is made to take part.

The fractions separate as colour bands each band is collected slowly in a separate beaker carefully. The desired colour band is chosen for the further studies.

PHYTOCHEMICAL STUDY IS BASED ON THE ETHYL ACETATE FRACTION OBTAIN FROM COLUMN CHROMATOGRAPHY.

Various phytochemical tests are performed for the fractionated compound and the result table is given below.

PREPARATION OF MICROEMULSION7:

The procedure involved in the preparation of micro emulsion formulation using ethyl acetate fraction is as follows:

Micro emulsion are liquid dispersion of water and oil that are made homogeneous transparent and stable by addition of relative large amount of surfactant. Micro emulsion is immediate in preparations between micelle containing solubilized oil and water. Emulsion is lyophobic and unstable micro emulsion onto borderline between lyophobic and lyophilic colloid. Therefore they are formed spontaneously when oil, water surfactant and co-surfactant are mixed together.

When oil is solubilied by micelle in water it blends into micelle are formed by the hydrocarbon tails of surfactant molecules and swells and micelle.

All the ingredient are sonicated homogenous using the ultrasonication high speed homogenizer till oil, water and surfactant was mixed spontaneously to form micro emulsion.

YIELD OF MICROEMULSION

The micro emulsion prepared from the ethyl acetate fraction was checked for the % yield. The values are given below in result and disscussion.

MICROEMULSION STABILITY TEST

CRACKING

The micro emulsion is checked for the cracking effect, no such effects was seen.

CREAMING

The formed micro emulsion checked for creaming and it was seen no creaming occurred.

PHASE INVERSION

The formulation remained unchanged in w/o/w form it doesn't undergone any phase inversion.

SEM ANALYSIS

The prepared micro emulsion is analysed using scanning electron microscopy (SEM) and the size and shape are found, the results are tabulated below**.**

CONDUCTIVITY TEST

The emulsion is made to conduct the current by immersing to wires which connected to a bulb.

FT-IR STUDY OF MICROEMULSION FORM

To predict the concentration of the ethyl acetate fraction in the micro emulsion form. It was found that from the ft-ir studies the concentration of secondary metabolite in the micro emulsion form was very low when

SPECTRAL STUDIES

The spectral studies are performed for the ethyl acetate fraction to determine the molecular structure and its composition. The following studies are performed.

UV spectral studies8

The ethyl acetate fraction is used for uv spectral study and it is performed in BRUKER UV VIS- LAMBDA 25 SPECTROMETER using hexane as solvent and results are given below

FT-IR studies9

The ethyl acetate fraction is used for the FT-IR studies and it is performed in OPUS-65-BRUKER-FT-IR SPECTROMETER by pelleting technique, the results are given below

NMR studies10

The ethyl acetate fraction is used for the NMR studies and it is performed in CHENOMX NMR SUITE SPECTROMETER using methanol as solvent results are given below

MASS studies11

The ethyl acetate fraction is used in the mass spectra study and it is performed in BRUKER – MASS-MAXIS Q-TOF SPECTROMETER results are given below

RESULT AND DISCUSSION

TABLE SHOWING RESULTS OF THE TEST.

Component	Name of the test	PT	CH	EA	MT	AQ
CARBOHYDRATES	Molisch Test	-	-	-	-	-
TANNINS AND PHENOL	ferric chloride Test	+	+	+	+	+
GLYCOSIDES	Keller-kiliani Test	-	-	+	+	-
PROTEIN	Biurets Test	-	-	+	+	+
GUM AND MUCILAGE	Molischs Test	-	-	-	-	-
FLAVANOIDS	Sodium hydroxide test	+	+	+	+	+
SAPONIN	Foam test	-	-	+	+	+
OILS AND FATS	Filter paper	-	-	-	-	-
ALKALOIDS	Mayer test	-	-	+	+	+
STEROIDS AND STEROLS	Salkowskis test	-	-	-	-	-
TRITERPENES	Thionyl chloride test	+	-	+	+	+

YIELD OF EXTRACTION

$$\text{PERCENTAGE YIELD} = \frac{\text{Amount of extract formed}}{\text{Amount of crude drug taken}} \text{ X } 100$$

$$= \frac{8.33}{10} \text{ X } 100$$

=83.3 g % w/w

RESULT OF PHYTOCHEMICAL ANALYSIS

By using the extract following test are performed and the results are given below in the tabulation

RESULT OF PHYTOCHEMICAL STUDY OF EXTRACTOF *CASSIA FISTULA* LINN.

S.NO	COMPOUND	TEST METHOD	TEST RESULT
1	FLAVONOID	SHINODA TEST13	+
2	GLYCOSIDE	KILLER-KILLIANI TEST	+
3	ALKALOID	MAYER'S TEST	+
4	TANNIN	FERRIC CHLORIDE	+
5	SAPONIN	FOAM TEST	-
6	ANTHROQUINONE	BORNTRAGER'S TEST	-
7	STEROID	LIBERMANN- BURCHARD TEST	-
8	TERPENOIDS	LIBERMANN- BURCHARD TEST	+
9	REDUCING SUGAR	FEHLIN'S TEST	-

RESULTS OF VARIOUS FRACTIONS OBTAINED FROM THE COLUMN CHROMATOGRAPHY.

S. no	Fraction obtained	Colour	Yield
1.	Pet.ether	Deep yelllowish green	0.4mg
2.	Chloroform	Yellow colour	0.5mg
3.	Ethyl acetate	Brown colour	5mg
4.	Methanol	Brownish red	0.7 mg
5.	Aqueous	brownish yellow	1mg

CHEMICAL TESTS FOR THE VARIOUS FRACTIONS OBTAINED FROM THE COLUMN:

Component	Name of the test	PT	CH	EA	MT	AQ
ALKALOIDS	Mayers Test	-	-	+	+	+
	Dragandoff`s Test	-	-	+	-	-
	Hagers Test	-	-	+	-	+
CARBOHYDRATES	Molisch Test	-	-	-	-	-
	Fehlings Test	-	-	-	+	+
	Benedicts Test	-	-	--	+	
TANNINS AND PHENOL	ferric chloride Test	+	+	+	+	+
GLYCOSIDES	Keller-kiliani Test	-	-	+	+	-
	Legals Test	-	-	-	-	-
PROTEINS AND AMINO ACID	Borntragers Test	-	-	-	-	-
	Biurets Test	-	-	+	+	+
	Ninhydrin Test	_	_	+	+	+
	Xanthoprotein Test	-	-	-	-	-
GUM AND MUCILAGE	Molischs Test	-	-	-	-	-
FLAVANOIDS	NaoH	+	+	+	+	+
	Sulphuric acid	-	+	+	+	+
	Magnesium hydrochloride13	-	-	+	+	+
SAPONINS	Honey comb foam	-	-	-	-	-
	Foam test	-	-	+	+	+
STEROIDS AND STEROLS	Salkowskis test	-	-	-	-	-
	Libermann –bruchard test	-	-	-	-	-
TRITERPENES	Thionyl chloride test	+	-	+	+	+
OILS AND FATS	Filter paper		-	-	-	-
	Alkalaine koH	-	-	-	-	-

PT-Pet. Ether, CH-chloroform, EA-ethyl acetate, MT-methyl alcohol, AQ-aqueous

By performing the chemical test we found that ethyl acetate extract contains major constituents hence column chromatography is performed to elute the desired compound by using the ethyl acetate.

TLC OF FRACTIONS OBTAINED FROM THE COLUMN CHROMATOGRAPHY:

The fraction obtained from the column chromatography is used for the further TLC separation.

S.no elute	TLC RF value	Compound seperated	Chemical test
1 chloroform (50)	Single Spot 0.7	Tannins	ferric chloride tests
2 chloroform: Ethyl acetate (10:40)	Single Spot 0.5	Tannins	ferric chloride test
3 chloroform: Ethyl acetate (5:45)	Single Spot 0.47	Triterpnes	thionyl chloride test
4 Ethyl acetate (500	Single Spot 0.29	Flavanoid	keller- killani test
5 Ethanol (35:15)	Single Spot 0.23	Flavanoid	keller- killani test

YIELD OF MICROEMULSION

$$\text{PERCENTAGE YIELD} = \frac{\text{Amount of micro emulsion formed}}{\text{Amount of ethylacetatefraction taken}} \text{ X } 100$$

$$= \frac{9.32}{10} \text{ X } 100$$

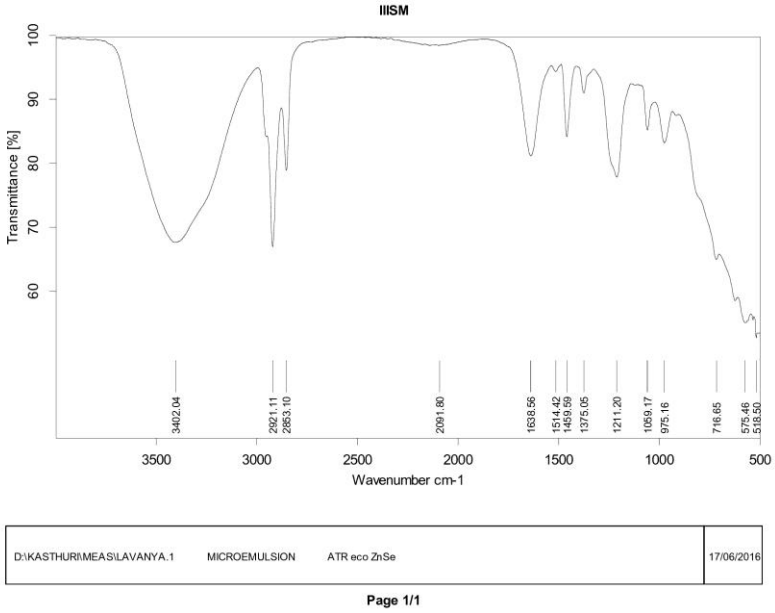
$$= 93.2 \text{ g \% w/w}$$

MICROEMULSION STABILITY TEST.

S.no	Test performed	Result of the test
1	Cracking	No cracking found
2	Creaming	No creaming seen
3	Phase inversion	No phase inversion seen
4	SEM analysis	Size and shape complies standard
5	Conductivity test	W/O emulsion formed.

FTIR STUDY OF MICROEMULSION FORM OF ETHYL ACETATE FRACTION:

From the FTIR studies of the micremulsion form and the ethyl acetate fraction the comparison gives the follwing predictions:

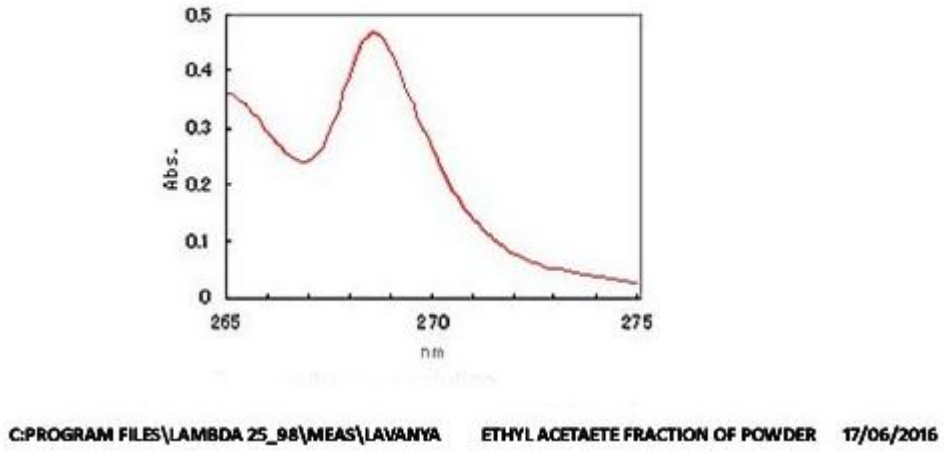


The baseline matching technique is done to check the quantity of the extract in micro emulsion form. It says that there is variation like:

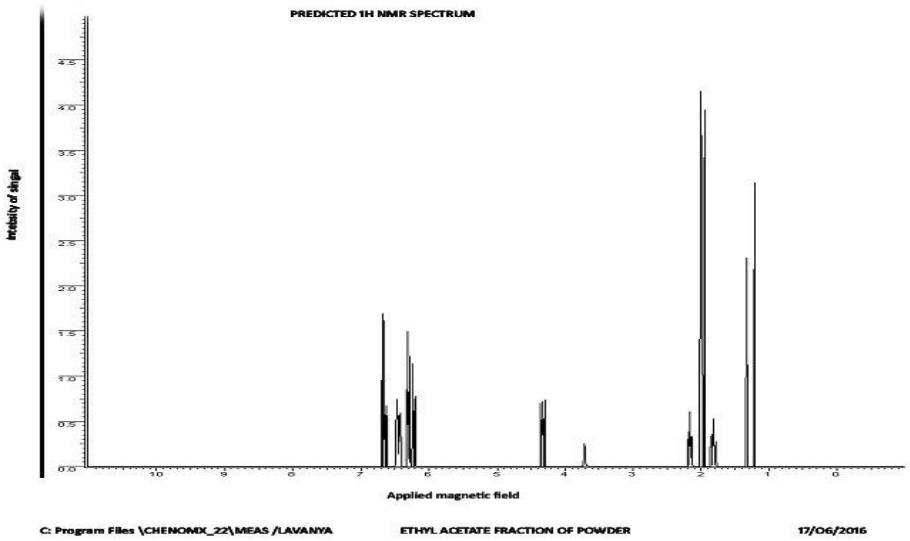
- Peak region between 1500 to 1800 region,
- There is widening of the peaks when compared with that of the ethyl acetate fraction and
- Thus concentaration of the extract is found to be low in the micro emulsion form.

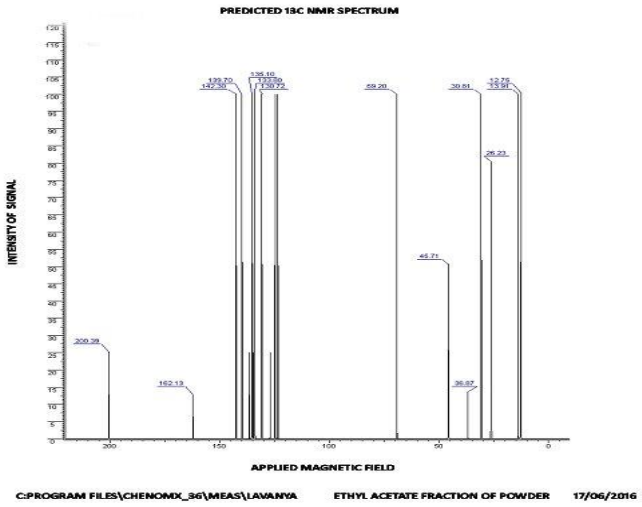
SPECTRAL STUDIES REPORT:

UV SPECTROSCOPY

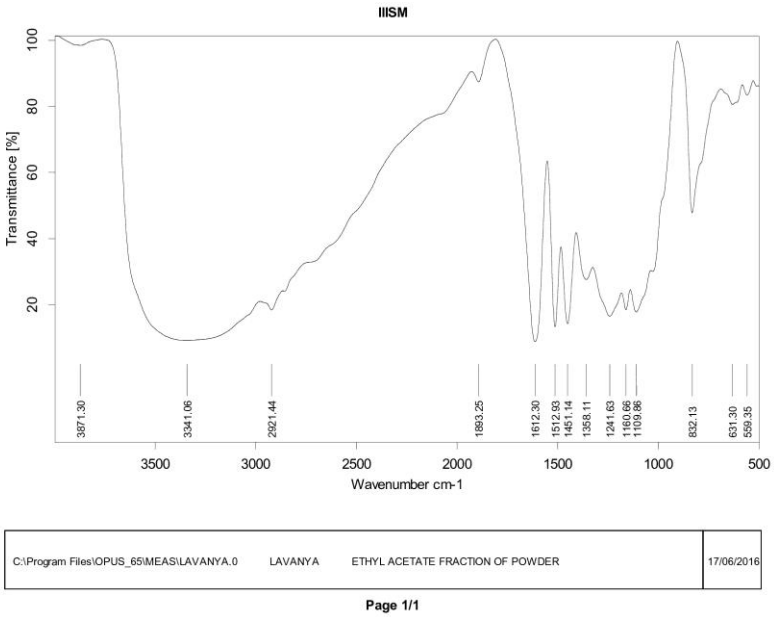


NMR SPECTROSCOPY

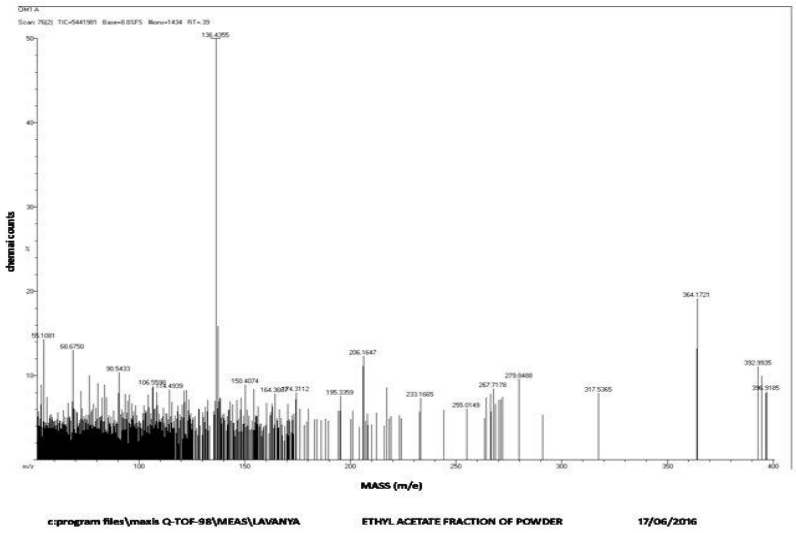




FT-IR SPECTROSCOPY



MASS SPECTROSCOPY



CHARACTERIZATION OF ISOLATED COMPOUNDS

The Triterpenoid compounds were subjected to spectral analysis. After characterization of compounds by spectral analysis the report was found that compound-Hopane. The reports of compounds mentioned below as follows

Compound

UV spectrum

The UV spectrum of the compound exhibited major absorption peaks at 260nm. The UV data obtained using various shift reagents represents the presence of conjugated compound.

IR spectrum

The IR spectra of the compound 1 shows the following groups.1451.14, 1512.93 - cm-1 (CH group), 1269.14, 1358.11cm-1- (CH group), 832.13cm-1 - (CH stretching), 562.75- cm-1 (saturation)

MASS spectrum

The electron impact mass spectrum showed the molecular base peak at m/z- 136.43 and M+ peak at m/z -364.17.

NMR spectrum

The 1H NMR spectrum and 13 C NMR spectra shown in the

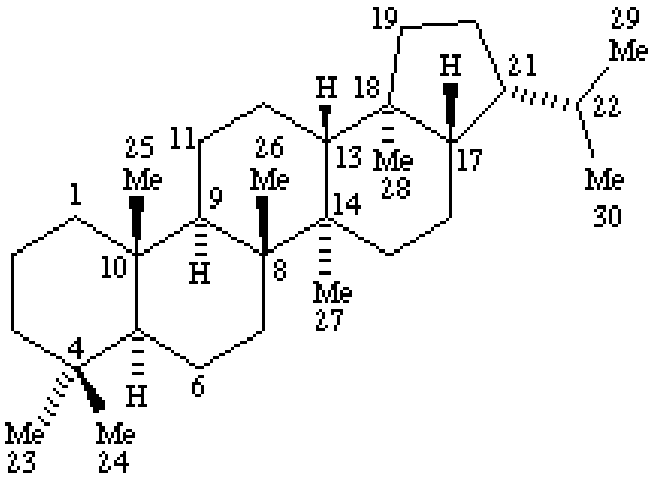
CHARACTERIZATION OF COMPOUND.

S.No.	Signals in 13C NMR	Signals in 1H NMR	Assignment
1	0.9 .1.3 , 1.5	4.6-5.9	Aromatic ring
2	2-3 , 6-8.5	5.5-7.5	Hydrogen
3	9-10 , 4-12	-	Methyl
4	15-17	10.0-13.2	Five member ring

Properties of the isolated compound.

S.no	Compound	Mol.formula	Mol.weight	boiling point
1.	Hopane	C30H52	412.73388g/mol	459.460 c

HOPANE:



IUPAC:

(1R, 2R, 5S, 6R, 9S, 10R, 13R, 14S, 19S)-1, 2, 9, 14, 18, 18- hexamethyl -6 propylpentacyclo [11.8.0.0^{2,10}.0^{5,9}.0^{14,19}] henicosane.

CONCLUSION

- *Cassia fistula* linn belonging to family caesalpiniaceae, popularly known as Indian laburnum is highly valued medicinal plant.
- We were performed isolation and characterization of pentacyclic triterpenoid compound namely HOPANE which gives the medicinally important used for further pharmacological studies
- We were formulated the ethyl acetate extracted plant as microemulsion form which is used to Cross the BBB (Blood Brain Barrier) in the treatment CNS disorder.
- Micro emulsion form of plant was standardised by the Stability studies and FT-IR study.
- The present review summarizes some important phytochemical study on cassia fistula and phytochemical investigation, spectral studies which can be investigated further to achieve lead molecules in the search novel herbal drug.
- Since the global scenario is now changing towards the use of nontoxic plant product having traditional medicine use, development of modern drug from *C. fistula* should be emphasized for the control of various diseases.

Hence, the active principles needs to be isolated and can formulate to treat various ailments by performing clinical trial studies to understand the molecular mechanism of action.

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