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Study of anatomical and morphological features, phytochemical screening and antibacterial activity of *Cassia fistula*, Linn.

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Abstract
This study was carried out with an objective to investigate the morpholological and anatomical features, phytoconstituents and antimicrobial potentials of leaves of *Cassia fistula* Linn. In the present study, the microbial activity of ethanol and petroleum extracts of leaves of *Cassia fistula* Linn. Was evaluated for potential antimicrobial activity against medically important bacterial strains. The antimicrobial activity was determined in the extracts using agar diffusion method. The antibacterial activities of extracts (25, 50, 100, 250, 500,1000µg/ml) of *Cassia fistula* were tested against two Gram-positive—*Staphylococcus aureus, Bacillus subtilis*; two Gram-negative—*Escherichia coli, Pseudomonas aeruginosa* human pathogenic bacteria; the results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were carried out and anatomical and morphological study was done. The microbial activity of the *Cassia fistula* was due to the presence of various secondary metabolites.

Keywords: *Cassia fistula*, Linn. phytochemical analysis, antimicrobial study

Introduction
The importance of medicinal plants has been realized and well documented since ancient period. Apart from the innumerable social benefit, much emphasis has been accorded to the plants on its medicinal value.
Plants are rich in a wide variety of compounds such as tannins, terpenoids, alkaloids, flavonoids and glycosides called phytochemicals. In the present study *Cassia fistula*, Linn.; belonging to Leguminosae family has been selected. It is a semi-wild Indian Labernum also known as the Golden Shower, is distributed in various countries including Asia, Mauritius, South Africa, Mexico, China, West Indies, East Africa and Brazil as an ornamental tree for its beautiful branches of yellow flowers.
In this present study the morphological and anatomical features of *Cassia fistula, Linn.;* was characterized. Phytochemical evaluation of leaf extract is carried out to determine the various chemical compounds present in the plant and these extract tested for four different bacterial culture to analyse the antibacterial property.

Materials and Methods
Cassia fistula, Linn.; is a tree belonging to the family Fabaceae, were collected from (Balussery) Calicut district of Kerala .

I. Characterisation
Morphological and anatomical characters
The morphological characters were studied using flower and leaves of the species. The photograph showing each part of the plant were taken and documented. Anatomical studies of the leaf and the petiole were conducted

II. Phytochemical Analysis
The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. (Ramman, 2006, Karpagam *et al.*, 2008, Kokate *et al.*, 2001)

III. Antimicrobial activity assay
Antimicrobial susceptibility test: Agar disc diffusion method (Walker *et al.*, 2000) [8]
Initially the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different volumes. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted.

Results
Characterisation
1. Morphological Characterisation

Table 1: Morphology of leaf

Colour	Green
Insertion	Cauline
Phyllotaxis	Opposite
Leaf petiole	Petiolate
Composition	Compound Paripinnate
Shape	Lanceolate-Ovate
Apex	Mucronate
Margin	Entire
Base	Asymmetric
Venation	Reticulate
Texture	Papery

Table 2: Morphology of flower

Symmetry	**Zygomorphic**
Inflorescence	Terminal drooping raceme
Flower	Yellow, raceme, large showy, bisexual, complete, zygomorphic and epigynous.
Calyx	Calyx tube very short, sepals 5, imbricate, polysepalous.
Corolla	Petals 5, imbricate, polypetalous.
Androecium	Stamens-Antheriferus, 2-3 lowest anthers are larger than the others with larger filaments.
Gynoecium	Stigma-terminal, truncate, style-incurved, ovary-sessile with many ovules
Fruit	Pendulous, cylindrical, indehiscent pod, black, glabrous many seeded - embedded in a black pulp.
Seed	Flattended, teardrop shape, smooth, brown seed coat, a pulpy dark brown substance holds the in place within the pod

Table 3: Anatomical characters of *Cassia fistula*, Linn.;

Parts	**Cell type**	**Characters**
Petiole	Epidermis	Uniserate, parenchymatous
	Cortex	Parenchymatous
	Vascular bundle	Conjoint collateral open
Leaf	Cuticle	Prominent cuticle is present, sunken stomata and trichomes present.
	Mesophylls	Spongy cells -Loosely arranged, parenchymatous. Palisade-Elongated narrow columnar cells containing chloroplast
	Vascular bundle	Conjoint collateral open, xylem towards the upper epidermis and phloem towards the lower epidermis and protected by bundle sheath

Table 4: Phytochemical screening of *Cassia fistula*, Linn.;

Sl No	**Phytochemicals**	**Extracts of *Cassia fistula***	
		Ethanol	**Petroleum ether**
1	Carbohydrates	+++	+++
2	Proteins	+	-
3	Starch	-	-
4	Amino Acid	++	++
5	Steroids	-	-
6	Glycosides	+	-
7	Flavonoids	++	++
8	Alkaloids	+	-
9	Tannins	++	++
10	Saponins	+	-
11	Terpenoids	-	-

Table 5: Antimicrobial activity of ethanolic and petroleum ether extract of leaf of *Cassia fistula*,Linn.;

Microorganisms	**Zone of inhibition(mm)**											
	Concentration in μg /ml											
	Ethanol extract (μg /ml)						**Petroleum ether extract (μg /ml)**					
	1000	**500**	**250**	**100**	**50**	**25**	**1000**	**500**	**250**	**100**	**50**	**25**
Staphylococcus aureus	3	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	10	1	2	3	-	-	1	-	-	-	-	-
Escherichia coli	2	4	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	14	3	3	2	-	-	2	-	-	-	-	-

Discussion

The preliminary phytochemical analysis revealed the presence of secondary metabolites such as alkaloids, glycosides, protein, saponins, flavonoids, amino acids, tannins, carbohydrate.

The studies revealed the ethanolic extract of *Cassia fistula*, Linn.; leaves is having antibacterial potential against bacterial strains such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus.* Ethanol extract showed significant activity towards two bacterial strains such as *Pseudomonas aeruginosa* and *Bacillus subtilis*. The extract showed higher inhibition towards *Pseudomonas aeruginosa*.

Vimal Raj *et al.*, 2009 reported the activity of aqueous and alcoholic extract of stem bark of *Cassia fistula*. Alcoholic extract showed maximum inhibition against *S. aureus*. The zone of inhibition ranges 7.0-12.0 mm. Antibacterial activity of hydroalcoholic extract of *Cassia fistula* leaves against pathogenic bacteria revealed significant activity against *E. coli*. The zone of inhibition ranges 20 mm (Nayan R Balodia, 2011). The morphological and anatomical characterisations are helpful for the correct identification of the plant species. These characters are useful in pharmacognosy.

Conclusion

The morphological and anatomical features of *Cassia fistula*, Linn.; are useful for the correct identification of the plant species. These characters are useful in the pharmacognosy. Ethanolic extract revealed presence of alkaloids, glycosides, protein, saponins. Whereas in the petroleum ether extract alkaloids, glycosides, protein, saponins are absent. Ethanol extract showed significant activity towards two bacterial strains such as *Pseudomonas aeruginosa* and *Bacillus subtilis*. Petroleum ether extract

does not show any antibacterial activity towards any of the bacterial strains in any concentrations.

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