

**Phytochemical Profile Of *Cassia Javanica* and *Cassia Renigera*
In Maharashtra by HRLC-MS Techniques**

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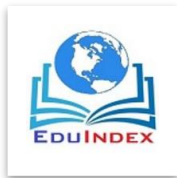
Abstract:

Cassia javanica (Java cassia, pink shower, apple blossom tree and rainbow shower tree) and *Cassia renigera* (Burmese pink cassia, Burmese Senna, common pink cassia, deciduous cassia, pink shower, and velvet cassia) are [tree](#) species in the family [Caesalpiniaceae](#). Both plants are used medicinally for treating constipation, colic, chlorosis and urinary disorders. A wide range of chemical compounds including benzene, alkylamines, flavone, dihydropyridine, uricourics, sulfonylureas, alkaloid, steroid, fatty acyls, limonoide, polyketide, sulphonamide, triterpenoids, vitamin B1, lipids, amino acids etc. have been isolated in various parts of *Cassia javanica* and *Cassia renigera*. These bioactive components have been evaluated using high resonance liquid chromatography technique (HRLC-MS). These bioactive compounds can be employed to delimit the species and also to standardize the drug.

Keywords: Phytochemical, Cassia, metabolites, HRLC-MS.

Introduction:

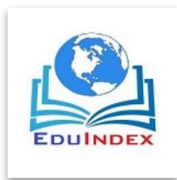
Phytochemicals literally mean “plant chemicals.” Scientists have identified thousands of different phytochemicals, found in vegetables, fruits, beans, whole grains, nuts and seeds. Eating lots of plant foods rich in phytochemicals may help to prevent at least one in every five cases of cancer, as well as other serious ailments such as heart disease. Stimulate the immune system, the body’s defense against viruses, bacteria and other disease-causing agents. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005). Phytochemicals defined in the strictest sense, as chemicals produced by plants. However, the term is generally used to describe chemicals from plants that may enhance health status of organisms, but are not essential nutrients (Srivastava et al., 2011).



There is ample evidence to support the health benefits of the diet in the form of fruits, vegetable, legumes, whole grains and nuts (Mojab et al., 2003). Because plant based foods are complex mixtures of bioactive compounds, information on the potential health of individual phytochemical is linked to information on the health effects of foods that contain those phytochemicals (Manjula et al., 2009). The phytochemical constituents of the medicinal plants were recorded by a number of workers (Joshi, 2000, Syed and Usha, 2005 and Ramasubbu 14). In India thousands of plant especially the angiosperms that all being exploited by the natives in tribal in variety of ways.

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plants colour, aroma and flavour. In general, the plant chemicals that protect plant cells from environmental hazardous such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Gibson and Wardle, 1998). In wide ranging dietary phytochemicals are found in fruits, vegetables, legumes, grains, nuts, seeds, fungi, herbs and spices (Mathai k., 2000). Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flower, fruits and seeds (Costa and Zia, 1999). These phytochemical compounds are known as secondary metabolites.

The separation procedure for the phytochemicals is mainly depends on the some physical characteristics of the compound. It is relatively easy to separate compounds that have significantly different physical characteristics by simple techniques such as solvent extraction. The separation and purification of plant constituents is mainly carried out by paper chromatography (PC), thin layer chromatography (TLC), high performance thin layer chromatography (HPLC) and high resonance liquid chromatography (HRLC) (N. Raman, 2006). The genus *Cassia* L. comprises of 580 species of herbs, shrubs and trees which are widely distributed throughout the world. Out of these there are only twenty species indigenous to India. (Anonymous, 1950). Many of cassia species possesses a good amount of medicinal properties



(Kritikar and Basu, 1918). In present investigation two species of genus *Cassia* have selected, i.e., *Cassia javanica* and *Cassia renigera*.

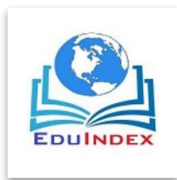
The different parts of the plants are collected from campus of DR. BAMU Aurangabad (MS) India. The identity of plant was confirmed by DR. A.S. Dhabe, Department of Botany, BAMU Herbarium, Dr. BAMU, Aurangabad, (MS) India.

Materials and Methods

The stem, leaves, bark and seeds of *Cassia Jawanica* and *Cassia renigera*, were collected from forest, kept separately, dried under shade and prepared powder of leaves, stem, root and seeds with grinder simultaneously. The dried and powdered leaves, stem, seeds and bark of *Cassia Jawanica* and *Cassia renigera* (5gm) were extracted with 95% ethanol (300 ml). Powdered samples were run for Soxhlet extraction for 76 hours. Phytochemical analysis done by using chromatographic HPLC-MS techniques. The sample was prepared according to the procedure. 3 μ l of plant extract of *Cassia Jawanica* and *Cassia renigera* was sent for qualitative analysis of its chemical constituents at SAIF, IIT, Bombay by HPLC-MS technique. For this the instrument used is of Agilent technologies G6550A- ifunnel, Q-TOF, LC/MS. Column type is ZORBAX RRHDSBC18, with 100 mm length, 2.1 mm diameter and 1.8 pore sizes. It was carried out with mass spectrometry mainly for the classes of compounds which are non- volatile like higher terpenoids, phenolic compounds, alkaloids, flavonoids, lipids, sugar, amino acids etc. so as to provide an accurate molecular weight of relevant compounds. Isolated compound were identified by spectrometric method such as MS as well as the comparison of data reported in literature. The most relevant compounds along with its medicinal properties also reported

Result:

The present work reports the isolation and identification of Benzene, Alkylamines, Flavone, Dihydropyridine, Uricourics, Sulfonylureas, Antiarrhythmic, Benzyl sulphonamides, Sulfone, Carbonyl phosphate synthetase, Alkaloid, SteroidFatty acyls, Limonoide, Tetranortriterpenoids, Polyketides, Triterpenoids, Vitamin B1, Lipid, Sulfon amide in various



parts of *Cassia Jawanica* and *Cassia renigeraplants*. All above mentioned compounds are isolated and reported here for the first time of these plants.

Benzyl benzoate is an effective against a treatment for human scabies. It also has effects against asthma and cough. It is used as medications for treating hypogonadism. Nefopam used in the prevention of shivering. It is also used to treat severe hiccups. Epiatzelechin used as an folk medicine for rheumatoid arthritis. Diflunisal is used to relief mild to moderate pain from various conditions. It also reduce pain, swelling and joint stiffness caused by arthritis. This medication is known as a non-steroidal anti-inflammatory drug (NSAID). Probenecid is used to prevent gouty arthritis. it lowers high uric acid in body by helping the kidneys to get rid of uric acid. Flecaïnide is a potent anti-arrhythmia agent, effective in a wide range of ventricular and atrial arrhythmias. Terfenadine is an anti-histamine, used for the treatment of allergic conditions. Benfotiamine is used as a natural remedy for the anxiety, back pain, depression, diabetes, thyroid disease. Valdecoxib is a drug used in the treatment of osteoarthritis, rheumatoid arthritis and menstrual symptoms. Clobetasol propionate is used to treat a variety of skin conditions like eczema, psoriasis, dermatitis, allergies, and rashes. It reduces the swelling, itching and redness. Chlorambucil's uses mainly in chronic lymphocytic leukaemia. It also has been used as an immunosuppressive drug for various inflammatory conditions. Flecaïnide is a potent anti-arrhythmia agent, effective in ventricular and atrial arrhythmias. Amikacin is used for number of bacterial infections. This include joint infections, intra-abdominal infections, meningitis, pneumonia, sepsis and urinary tract infections. It is also used for the treatment of tuberculosis. Candesartan is used to treat high blood pressure. Rescinnamine is used for the treatment, control, prevention and improvement of hypertension. Sulfamethazine is used for the treatment of bacterial infections.

Table No 1: Phytochemical analysis of leaves of *Cassia jawanica* L.

Sr no	Compound name	Group	Formula	Mass	Rt	DB Diff (ppm)
1	Benzyl Benzoate	Benzene	C ₁₄ H ₁₂ O ₂	197.074	0.975	92798.04
2	7- [2-Triflromethyl-4(2 Hydroxyphenyl)-1,3-Dioxan-Cis-5-Yl]-Hept-5z		C ₁₈ H ₂₁ F ₃ O ₅	374.1325	0.977	4.37

	Enoic Acid					
3	Nefopam	Alkylamines	C17 H19 N O	253.1463	1	1.51
4.	EpiafzelechinTrimethyl Ether	Flavone	C18 H20 O5	316.1291	1.017	6.35
5	3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-, carboxymethyl methyl ester	Dihydropyridine	C18 H18 N2 O8	390.1059	1.019	1.14
6	Probenecid	Uricourics	C13 H19 N O4 S	285.1005	2.101	10.37
7	Glibornuride M2 (p-carboxyglibornuride)	Sulfonylureas	C18 H24 N2 O6 S	396.1314	4.866	10.26
8	Phenytoin-N-glucuronide	Antiarrhythmic	C35 H49 N3 O3	428.1209	6.323	2.55
9	3-Hydroxyzafirlukast	Benzyl sulfonamides	C31 H33 N3 O7 S	591.203	6.376	1.51
10	Monoacetyldapsone	Sulfone	C14 H14 N2 O3 S	290.0725	6.929	0.06
11	N-Carbamylglutamate	Carbonyl phosphate synthetase	C6 H10 N2 O5	190.0581	7.746	4.59
12	Ethyl phenothiazine-2-carbamate		C15 H14 N2 O2 S	286.0771	8.12	1.7
13	Cepharanthine	Alkaloid	C37 H38 N2 O6	606.2695	18.649	5.78
14	Sulfoglycolithocholate	Steroid	C26 H42 N O7 S	512.2676	18.908	1.2

Table No 2: Phytochemical analysis of stem of *Cassia javanica* L.

Sr no	Compound name	Group	Formula	Mass	Rt	DB Diff (ppm)
1	Probenecid	Uricourics	C13 H19 N O4 S	285.1008	2.085	9.47
2	Glibornuride M2 (p-carboxyglibornuride)	Sulfonylureas	C18 H24 N2 O6 S	396.1323	5.264	7.98
3	Formoterolglucuronide		C24 H30 N2 O10	506.1874	7.396	5.15
4	Cepharanthine	Alkaloid	C37 H38 N2 O6	606.2697	18.655	5.44

Table No 3:Phytochemical analysis of seeds of *Cassia jawanica* L.

Sr no	Compound name	Group	Formula	Mass	Rt	DB Diff (ppm)
1	12S-Acetoxy-Punaglandin 1	Fatty acyls	C29 H39 Cl O11	598.2133	4.205	8.06
2	4,4-Bis(P-fluorophenyl) Butyric Acid		C16 H14 F2 O2	276.095	6.991	4.43
3	Dihydrofissinolide	Limonoide	C29 H38 O8	514.2529	7.872	7.29
4	N-formylmethionyl Phenyl Alanine		C15 H20 N2 O4 S	324.1127	8.779	5.04
5	Carapin-8 (9)-Ene	Limonoide	C27 H30 O7	466.1964	9.057	5.98
6	Swietenine	Tetranortriterpenoids	C32 H40 O9	568.2628	9.865	7.88
7	Protorifamycin I	Polyketides	C35 H45 N O10	639.2988	10.323	8.62
8	Protoveratrine A	Alkaloid	C41 H63 N O14	793.4185	10.505	7.98
9	Sulfamethazine	Sulphonamide	C12 H14 N4 O2 S	278.0813	10.78	8.66

Table No 4: Phytochemical analysis of bark of *Cassia jawanica* L.

Sr no	Compound name	Group	Formula	Mass	Rt	DB Diff (ppm)
1	N-Formylmethionyl alanine	Amino acid	C9 H16 N2 O4 S	248.0823	1.406	2.94
2	Probenecid	Uricourics	C13 H19 N O4 S	285.1017	2.075	6.38
3	EstradiolDiacetate	Steroid esters	C22 H28 O4	356.1965	4.561	6.3
4	Gedunin	Triterpenoids	C28 H34 O7	482.2262	6.266	8.83
5	Glibornuride M2 (P-carboxyglibornuride)	Sulfonylureas	C18 H24 N2 O6 S	396.1339	6.407	4.17
6	26,26,26,27,27,27-Hexafluoro-1alpha,25-Dihydroxy-16,17,23,23,24,24-Hexadehydrovitamin D3 / 26,26,26,	vitamin D3	C27 H32 F6 O3	518.2225	8.048	6.01

Table No 5: Phytochemical analysis of leaves of *Cassia renigera* Wall.exBenth.

Sr no	Compound name	Group	Formula	Mass	Rt	DB Diff (ppm)
1	Probenecid	Uricourics	C13 H19 N O4 S	285.1025	2.006	3.28
2	Glucosamine	Amino sugar	C6 H13 N O5	179.0786	3.788	4.07
3	Irigenin, 7-Benzyl Ether		C25 H22 O8	450.1294	5.15	4.57
4	Fluconazole glucuronide		C19 H20 F2 N6 O7	482.1356	5.828	1.16
5	Benfotiamine	Vitamin B1	C19 H23 N4 O6 P S	466.1031	6.938	9.62
6	Carapin-8 (9)-Ene	Limonoide	C27 H30 O7	466.1965	8.991	5.73
7	Swietenine	Tetranortriterpenoids	C32 H40 O9	568.2623	9.866	8.69
8	Protorifamycin I	Polyketides	C35 H45 N O10	639.2988	10.326	8.62
9	Sulfamethazine	Sulphonamide	C12 H14 N4 O2 S	278.0811	10.783	9.58
10	RamiprilGlucuronide	Angiotensin converting enzyme	C29 H40 N2 O11	592.2575	18.178	9.58
11	Cepharanthine	Alkaloid	C37 H38 N2 O6	606.2727	18.546	0.4
12	C16 Sphinganine	Lipid	C16 H35 N O2	273.2641	26.672	9.65

Table No 6: Phytochemical analysis of stem of *Cassia renigera* Wall. exBenth.

Sr no	Compound name	Group	Formula	Mass	Rt	DB Diff (ppm)
1	Probenecid	Uricourics	C13 H19 N O4 S	285.1009	2.077	8.9
2	Monoacetyldapsone	Sulfon amide	C14 H14 N2 O3 S	290.0724	5.453	0.41
3	Carvedilolglucuronide		C30 H34 N2 O10	582.2169	5.521	7.57
4	10-Deoxymethymycin	Organooxygen	C25 H43 N O6	453.3059	5.957	6.88

Table No 7: Phytochemical analysis of seeds of *Cassia renigera* Wall. exBenth.

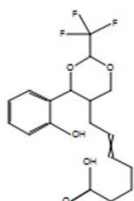
Sr no	Compound name	Group	Formula	Mass	Rt	DB Diff (ppm)
1	3,5-Pyridinedicarboxylic	Dihydropyridine	C17 H16 N2	376.0905	0.989	0.35

	acid, 2-(hydroxymethyl)-6- methyl- 4-(3-nitrophenyl)-, 5-(2- hydroxyethyl) ester		O8			
2	Benzyl benzoate	Benzene	C14 H12 O2	212.0837	1.003	0.33
3	4-Phospho- Npantothenoylcysteine		C12 H23 N2 O9 P S	402.0848	2.857	3.36
4	CMP-N-acetylneuraminic acid	Pyrimidine	C20 H31 N4 O16 P	614.1473	4.653	0.01
5	Atractyloside	Toxic Glycoside	C30 H46 O16 S2	726.2182	5.154	6.24
6	Glibornuride M2 (p- carboxyglibornuride)	Sulfonylureas	C18 H24 N2 O6 S	396.1319	5.271	9.17
7	10-Deoxymethymycin	Organooxygen	C25 H43 N O6	453.3056	5.968	7.65
8	Atorvastatin	Statins	C33 H35 F N2 O5	558.2516	6.178	2.45
9	L-Phosphatidic acid	Phospholipids	C29 H57 O10 P	596.3681	16.866	1.4

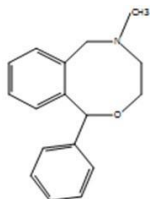
Table no 8: Phytochemical analysis of bark of *Cassia renigera* Wall. exBenth.

Sr no	Compound name	Group	Formula	Mass	Rt	DB Diff (ppm)
1	Bisnorbiotin	Delta amino acid	C8 H12 N2 O3 S	216.0559	1.019	4.59
2	Monoacetyldapsone	Sulfon amide	C14 H14 N2 O3 S	290.0724	5.455	0.24
3	Carvedilolglucuronide		C30 H34 N2 O10	582.2167	5.516	8.02
4	10-Deoxymethymycin	Organooxygen	C25 H43 N O6	453.3059	5.955	6.91

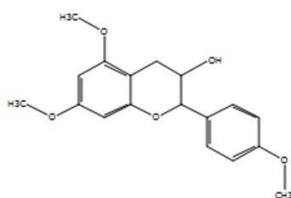
1. 7- [2-Triflromethyl-4(2 Hydroxyphenyl)-1,3-Dioxan-Cis-5-Yl]-Hept-5z
Enoic Acid.



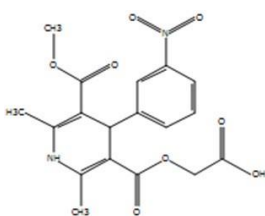
2. Nefopam



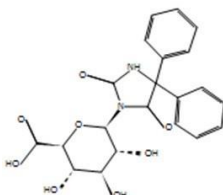
3. EpiafzelechinTrimethyl Ether



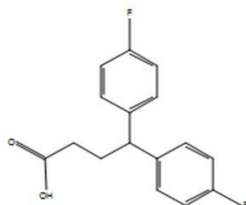
4. 3, 5 - Pyridinedicarboxylic acid, 1, 4 - dihydro- 2, 6 - dimethyl - 4 - (3-nitrophenyl)-
carboxymethylmethyl ester



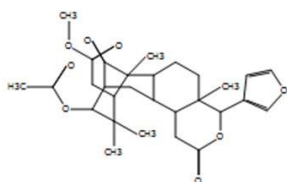
5. Phenytoin-N-glucuronide



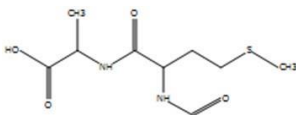
12. 4,4-Bis(P-fluorophenyl) Butyric Acid



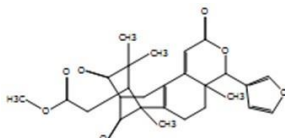
13. Dihydrofissinolide



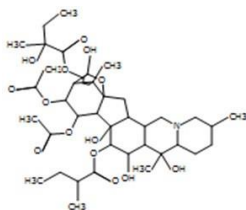
14. N-formylmethionyl Phenyl Alanine



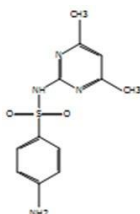
15. Carapin-8 (9)-Ene



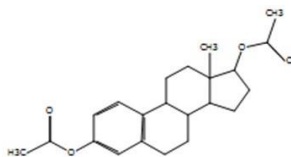
16. Protoveratrine A



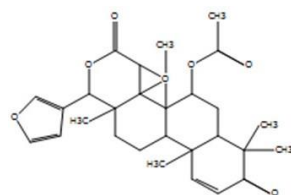
17. Sulfamethazine



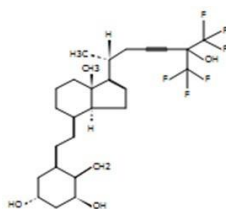
18. Estradiol Diacetate



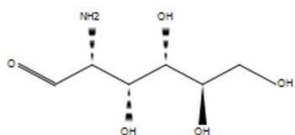
19. Gedunin



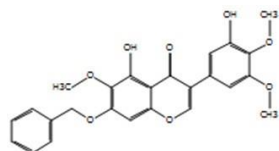
20. 26, 26, 26, 27, 27, 27-Hexafluoro-1 α , 25 Dihydroxy-16, 17, 23, 23, 24, 24 - Hexadehydrovitamin D₃ /26, 26, 26



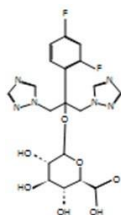
21. Glucosamine



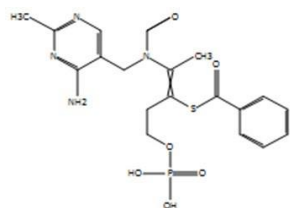
22. Iriogenin, 7-Benzyl Ether



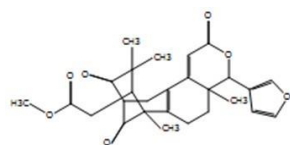
23. Fluconazole glucuronide



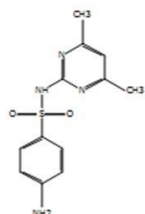
24. Benfotiamine



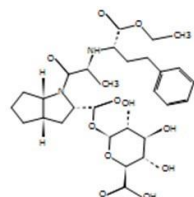
25. Carapin-8 (9)-Ene



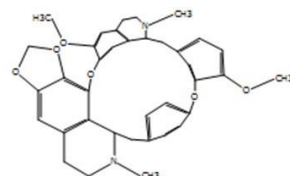
26. Sulfamethazine



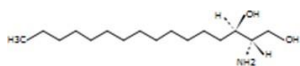
27. RamiprilGlucuronide



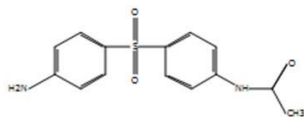
28. Cepharanthine



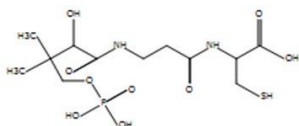
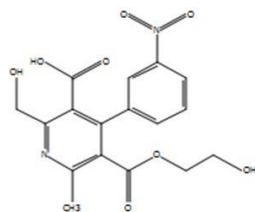
29. C16 Sphinganine



30. Monoacetyldapsone

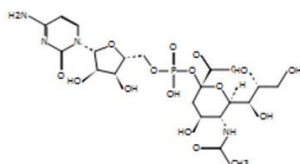


31. 3, 5 -Pyridinedicarboxylic acid-2 - (hydroxymethyl)-6-methyl-4-(3-nitrophenyl) - 5 - (2-hydroxyethyl) ester

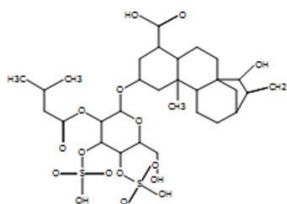


32. 4-Phospho-Npantothenoyl cysteine

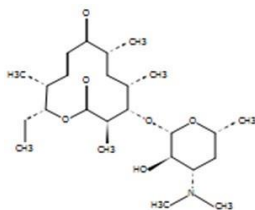
33. CMP-N-acetylneuraminic acid



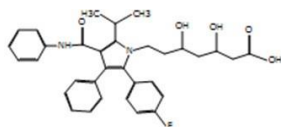
34. Atractyloside



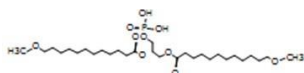
35. 10-Deoxymethymycin



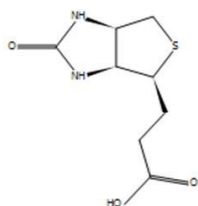
36. Atorvastatin



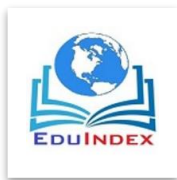
37. L-Phosphatidic acid



38. Bisnorbiotin



39. Carvedilolglucuronide

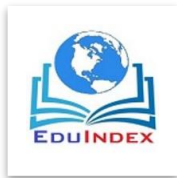


1. Conclusions:

The isolated compounds like 7-[2-Trifluoromethyl-4-(2-Hydroxyphenyl)-1,3-Dioxan-Cis-5-Yl]-Hept-5z; Enoic Acid; Nefopam; Epiafzelechin Trimethyl Ether; 3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-carboxymethylmethyl ester; Phenytoin-N-glucuronide; 3-Hydroxyzafirlukast; N-Carbamylglutamate; Ethyl phenothiazine-2-carbamate; Cepharranthine Sulfoglycolithocholate; Formoterol glucuronide; 12S-Acetoxy-Punaglandin 1; 4,4-Bis(P-fluorophenyl)Butyric Acid; Dihydrofissinolid; N-formylmethionyl Phenyl Alanine; Carapin-8 (9)-Ene; Protoveratrine A; Sulfamethazine; Estradiol Diacetate; Gedunarene found to be species specific to *Cassia javanica* and compounds like Glucosamine; Iridin, 7-Benzyl Ether; Fluconazole glucuronide; Benfotiamine; Carapin-8 (9)-Ene; Sulfamethazine; Ramipril Glucuronide; Cepharranthine and C16 Sphinganine; Monoacetyldapsone; 3,5-Pyridinedicarboxylic acid; 2-(hydroxymethyl)-6-methyl-4-(3-nitrophenyl)-,5-(2-hydroxyethyl) ester; 4-Phospho-N-pantothenoyl cysteine; CMP-N-acetylneuraminic acid; Atractyloside; 10-Deoxymethymycin; Atorvastatin; L-Phosphatidic acid; Bisnorbiotin; Carvedilol glucuronide are species specific to *Cassia renigera*. These compounds can be employed to delimit the species and also to standardize the herbal drugs.

References:

1. Anonymous (1950), the wealth of India. Raw materials, Vol II. CSIR: Delhi; 93-98.
2. Cosata M.A., Zia Z.Q., Davin L.B., Lewis N.G., (1999). Chapter four: towards engineering the metabolic pathways of cancer-preventing Lignans in cereal grains and other crops. In recent advances in phytochemistry, vol. 33, phytochemicals in Human Health protection, Nutrition and plant Defenses, ed. JT Romeo, New York; 67-87.
3. Edeoga O.H., Okwu D.E. (2005) Phytochemical constituents of some Nigerian Medicinal Plants African Journal of Biotechnology 4(7) :685-688
4. Gibson E.L., Wardel J., Watts C.J., (1998). Fruits and vegetable consumption, Nutritional knowledge and benefits in mothers and children. Appetite; 205-228.3.



5. Hasler C.M., Blumberg J.B., (1999). Symposium on phytochemicals: Biochemistry and Physiology. *Journal of Nutrition*; 129: 756S-757S.2.
6. Joshi, S.G. (2000) *Medicinal Plants*. Oxford & IBH Publishing, Co. Pvt. Ltd. New Delhi.
7. Kritkar K.R., Basu B.C., (1918). *Indian medicinal plants*, vol II ed. Lalit Mohan Basu: Allahabad, India; 870.
8. Mathai K., (2000). Nutrition in the adult years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed. L.K. Mohan and S. Escott-Stump; 271: 274-275.
9. Manjula M. , Indira V and Dhasarathan Pallvarnanathasamy (2009) Phytochemical characterization and antibacterial activity screening of *Achyranthes aspera* Asian Journal of Microbiology, Biotechnology and Environmental Sciences 11(2):365-368
10. [Mojab Faraz](#) , Mohammad Kamalinejad , [Ghaderi Naysaneh](#) and [Hamid Reza Vahidipour](#) (2003) Phytochemical Screening of Some Species of Iranian Plants *Iranian journal of pharmaceutical research (IJPR)* 2(2) : 77-82
11. Nudrut, Z. Sayed and Usha, M.(2005) *Medicinal and aromatic plants Part – 1*. In: (eds. Khan and Khanum) Ukazz publication, Hyderabad.
12. Ramasubbu, R. and Chandra Prabha, A.(2009) Medicinal plant diversity of Virudhunagar district, Tamilnadu. *Current Biotica*. 3(3): 373 – 385
13. Shrivastava Bhanu, Shrivastava Vandana and Shrivastava Archana (2011) In vitro study of *Entamoeba histolytica* causative agent of Amoebiasis with Lemon juice at different concentration showed antiameobic properties. *International Research Journal of Pharmacy* 2 (9): 88-90