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Research Article
Phytochemical Analysis of Flower extracts of Different *Cassia* Species by Using Gas Chromatography-Mass Spectrometry

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Abstract

Background and Objective: Most of the studies carried out with leaves, barks and stems of *Cassia* species. So, the objective of this study was to characterize the chemical constituents of flowers of *Cassia alata*, *Cassia auriculata*, *Cassia fistula* and *Cassia occidentalis* by using gas chromatography-mass spectrometry (GC-MS). **Materials and Methods:** The GC-MS analysis of flower extracts was performed using a GC and MS JEOL GC mate system and Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). **Results:** From the GCMS study, 12 phytocompounds were identified from *C. alata* flowers, 9 compounds from *C. auriculata* flowers, 12 compounds from *C. fistula* flowers and 13 phytocompounds identified from the *C. occidentalis* flower extract. A compound namely, Pregna-5, 8, 16-triene-3à-ol-20-one acetate (its chemical name is 16-Dehydropregnenolone acetate) found first time in *C. occidentalis* flowers, which is used in the preparation of anti-cancer agents. **Conclusion:** From the results obtained it was concluded that *Cassia* sp. contain various phytochemicals, justifying their use for various human ailments.

Key words: Phytochemicals, GCMS, *Cassia alata*, 16-Dehydropregnenolone acetate, *Cassia auriculata*, *Cassia fistula* and *Cassia occidentalis*, Pregna-5, 8, 16-triene-3à-ol-20-one acetate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant metabolism is mainly classified as primary or secondary. Primary metabolites are required for maintenance of plant cells[1], while secondary metabolites are essential to the normal growth, development and defence of plants. To date, thousands of different types of secondary metabolites have been identified in plants. Chemically, these compounds are either nitrogen-containing (alkaloids) or nitrogen-deficient (terpenoids and phenolics)[2]. Nearly 20% of plant species accumulate alkaloids, which mainly include terpenoid indole alkaloids, tropane alkaloids and purine alkaloids[3]. However, under *in vitro* anti-oxidant measurement assay conditions, the radical scavenging potential of alkaloids is reportedly moderate to non existent. Terpenoids comprise another large family of secondary metabolites, consisting of over 40,000 different compounds[4]. Monoterpenes, sesquiterpenes and diterpenes have been found to possess notable anti-oxidant activity in different *in vitro* assays. However, most of these activities have no physiological relevance[5]. Tetraterpenes and carotenoids have been shown to possess potent anti-oxidant activity within both *in vivo* and *in vitro* studies[6], however, some valuable carotenoids such as beta-carotene showed prooxidant effects at high concentration and oxygen pressure[7]. Among all secondary metabolites, phenolic anti-oxidants appear to be the most important since they have shown promising anti-oxidant activity in both *in vivo* and *in vitro* investigations. Plant phenolics are mainly classified into 5 major groups, phenolic acids, flavonoids, lignans, stilbenes and tannins[8-10]. Phenolic compounds generally possess one or more aromatic rings with one or more hydroxyl groups. It has commonly been assumed that the anti-oxidant capacity of phenolics will increase with the number of free hydroxyls and conjugation of side chains to the aromatic rings[11]. Flavonoids and phenolic acids, the largest classes of plant phenolics, are biosynthetically derived from the acetate and shikimate pathways, as well as the shikimate pathway from phenylalanine or tyrosine[12]. Phytochemicals from these classes were found to have excellent anti-oxidant activity in both *in vitro* and *in vivo* investigations. Moreover, they are known to interact with other physiological anti-oxidants such as ascorbate or tocopherol and to synergistically amplify their biological effects[13]. Flavonoids and phenylpropanoids are also oxidized by peroxidase and act as H_2O_2 scavengers[14]. Under experimental conditions, the anti-oxidant potential of plant phenolics is always linked to their electron donation, reducing power and metal ion chelating ability[15].

The genus *Cassia* comprises of 580 species of herbs, shrubs and trees. Many of the *Cassia* sp. shown a good amount of medicinal properties and a few among them supply tanning materials, which are of great economic importance[16]. *Cassia* sp. are already reported in literature indicated its use against various skin diseases such as ringworm, eczema and scabies. Since the high incidence of skin diseases, especially among the weaker section of the Indian population, it was felt worthwhile undertaking research on this plant[17]. The phytochemical studies of the medicinal plants have provided some biochemical basis for their ethnopharmacological uses in the treatment and prevention of various diseases and disorders[18]. This work carried out to screen the presence of phytochemicals of flowers of *Cassia alata*, *C. auriculata*, *C. fistula* and *C. occidentalis* using GCMS analysis.

MATERIALS AND METHODS

Plant materials: The flowers of *C. alata*, *C. auriculata*, *C. fistula* and *C. occidentalis* were collected from Thanjavur District, Tamil Nadu, India from January-April, 2018. About 1.0 kg of shade-dried coarse powders of the flower material was extracted with 80% v/v aqueous ethanol by maceration at room temperature for 72 h. After the completion of each extraction, the extracts were filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50°C). The residues were stored in a vacuum desiccator for further use.

GCMS: The ethanolic flower extracts were subjected to GC-MS analysis on the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier. The JEOL GC MATE II GC-MS with data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI) (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m×0.25 mm I.D. Analysis conditions were 20 min at 100°C, 3 min at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µL) was evaporated in a split less injector at 300°C. Run time was 22 min.

Chemical composition by GCMS analysis: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library.

RESULTS

GCMS analysis of *C. alata* flowers: The chromatogram (Fig. 1) shows 12 prominent peaks. The largest peak (RT 18.55) is due to the presence of Oleic acid. From the GCMS study, 12 phytocompounds were identified from *C. alata* flowers, shown in Table 1.

GCMS analysis of *C. auriculata* flowers: The chromatogram (Fig. 2) shows 9 prominent peaks. The largest peak (RT 16.55) is due to the presence of n-Hexadecanoic acid. The Second less prominent peak (RT 18.13) is due to the presence of Oleic acid. Flavone also found with less intense peak (RT 14.85). The identified phyto constituents from *C. auriculata* flowers were shown in Table 2.

GCMS analysis of *C. fistula* flowers: The GC-MS analysis of *C. fistula* flowers showed the largest peak (RT 19.2) is due to the presence of Octadec-9-enoic acid, classified as a member of the Long-chain fatty acids (Fig. 3). The identified components are shown in Table 3.

GCMS analysis of *C. occidentalis* flowers: Chromatogram shows 13 phytocompounds identified from the *C. occidentalis* flower extract (Fig. 4). The largest peak (RT 18.6) is due to the presence of Oleic acid. Oleic acid is classified as a monounsaturated omega-9 fatty acid. The identified components are shown in Table 4.

DISCUSSION

In the present research, an attempt has been made to identify phytochemical studies in flowers of some *Cassia* sp. namely *C. alata*, *C. auriculata*, *C. fistula* and *C. occidentalis* by GCMS. In the previous study, 3 species of *Cassia* namely *Cassia alata* Linn., *Cassia occidentalis* Linn. and *Cassia sieberiana* DC were carried out to identify and quantify the bioactive components of these ornamental shrubs. The alkaloid, flavonoid, saponin, steroid, phenol and tannin contents of the vegetative and reproductive parts of these plants viz. the leaves, stems and roots were screened and compared while the flowers were not analyzed[19]. Deshpande and Bhalsing[19] presented

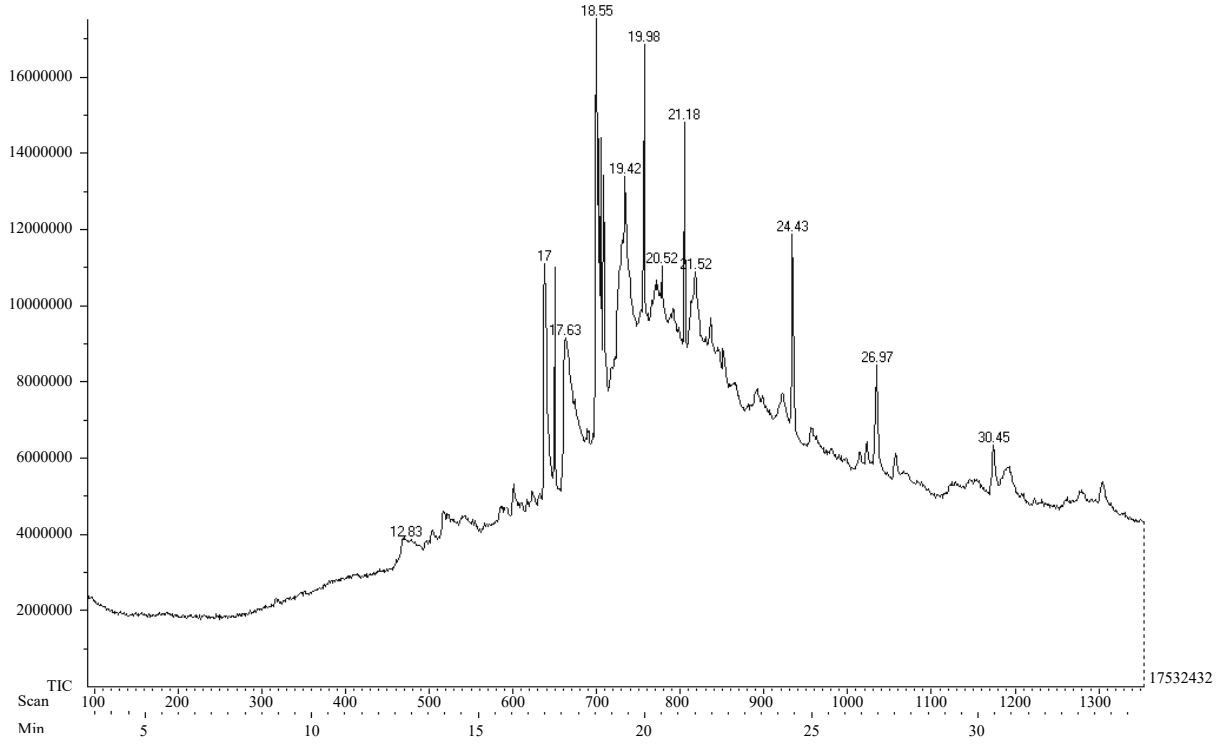


Fig. 1: Chromatogram of *Cassia alata* flowers

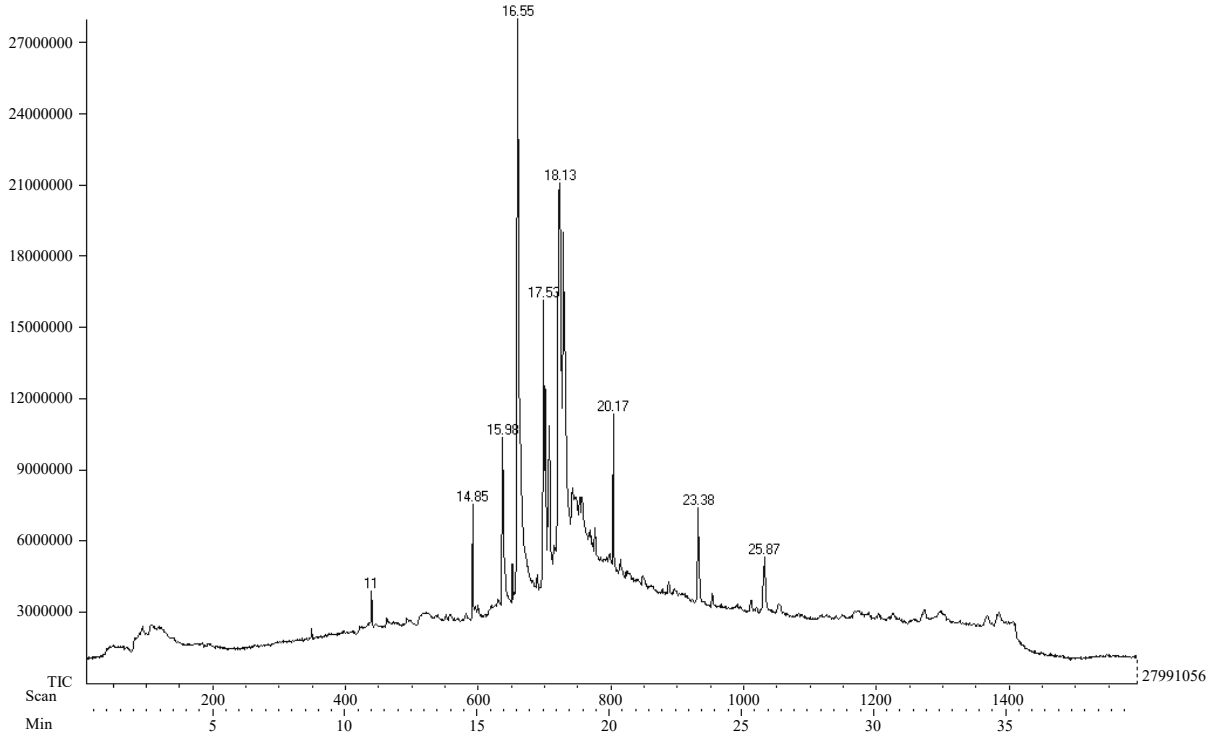


Fig. 2: Chromatogram of *Cassia auriculata* flowers

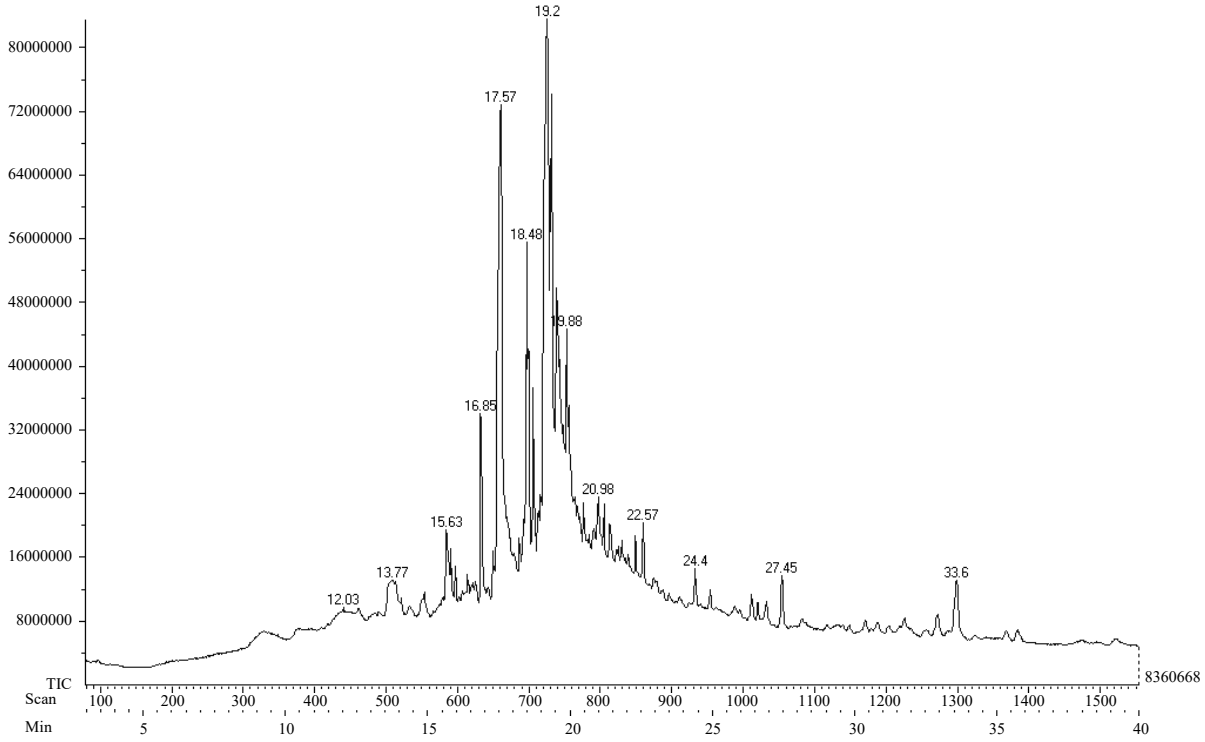


Fig. 3: Chromatogram of *Cassia fistula* flowers

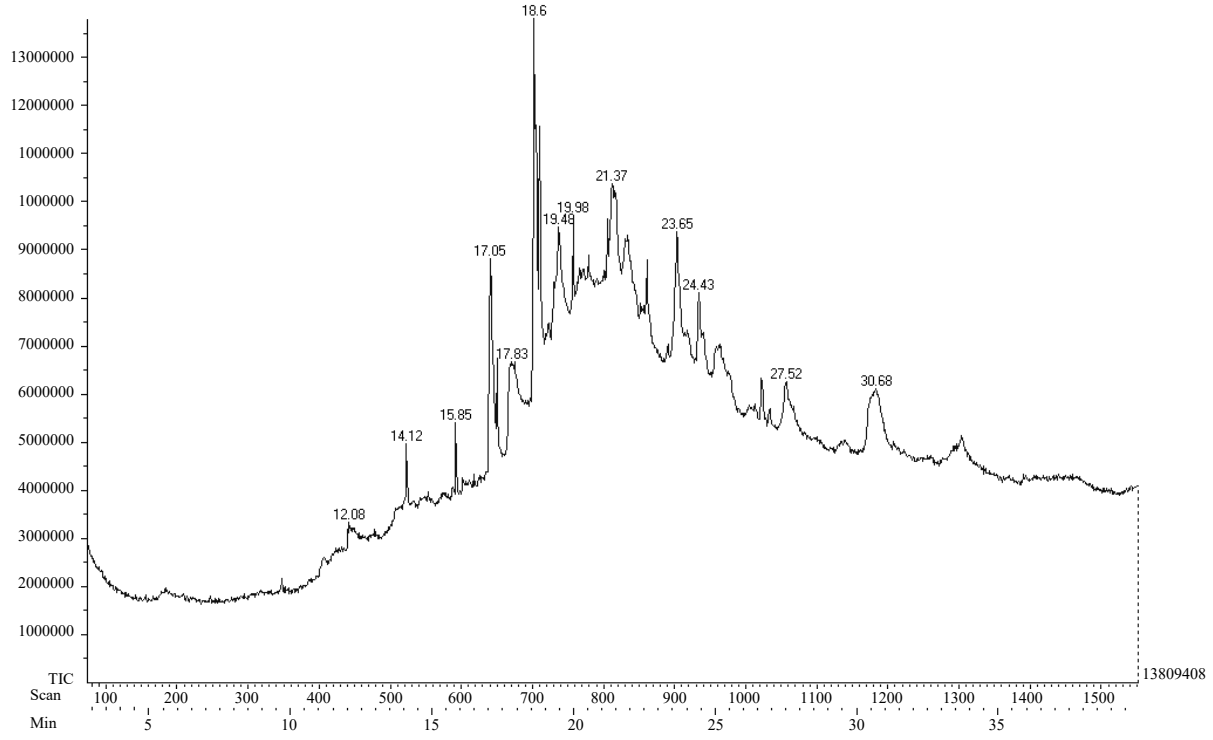
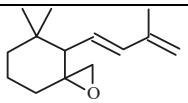
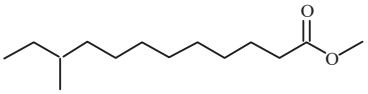
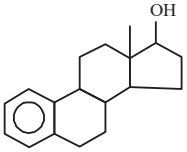
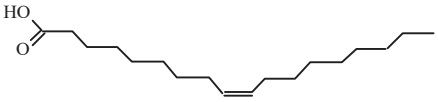
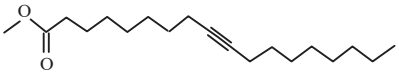
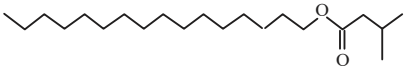
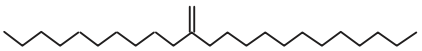
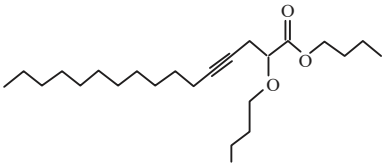
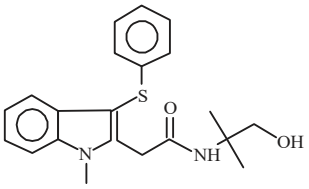
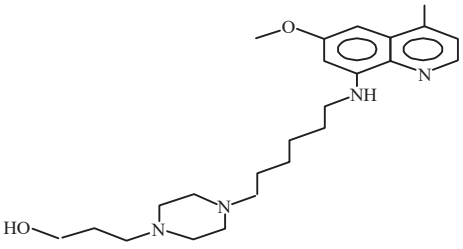
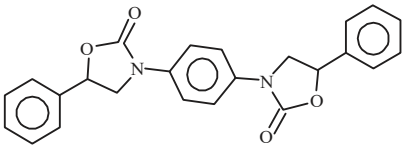


Fig. 4: Chromatogram of flowers of *Cassia occidentalis*

a review of the phytochemistry of some medicinally important *Cassia* sp., because there are about 580 species of this genus "*Cassia*" scattered all around the world. Only 46 species have been phytochemically studied. Most of the studies carried out with leaves, barks and stems[20]. In the present study, flower extracts were used and compared, most of the identified compounds were hydrocarbons, fatty acids, fatty esters, flavones and coumarin derivatives. Many of these identified compounds have already been reported to be pharmacologically active. For example, hexadecanoic acid is known to have potential anti-bacterial and anti-fungal activities, long-chain unsaturated fatty acids, such as oleic acid, also show anti-bacterial activity and are the key ingredients of anti-microbial food additives and some anti-bacterial herbs[21], unsaturated fatty acids are also suggested to be responsible for the anti-inflammatory activity[22] and hexadecanoic acid, methyl ester, phytol have shown anti-oxidant and anti-cancer properties, respectively[23,24]. Oleic acid identified as the major phytocompound in *C. alata*, *C. auriculata* and *C. occidentalis*. The principal use of oleic acid is as a component in many foods, in the form of its triglycerides. It is a component of the normal human diet as a part of animal fats and vegetable oils. Oleic acid as its sodium salt is a major component of soap as an emulsifying agent and it is used as an emulsifying or solubilising agent in aerosol products[25]. Oleic acid also found in *Daniellia oliveri* stem bark extract[25]. Flavone found in *C. auriculata* and *C. Occidentalis* flowers. Flavones, are a class of flavonoids based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). Flavones represent one of the largest groups and possess a functional C2=3 double bond in the basic structure[26]. Flavones are important anti-oxidants and promote several health effects. Aside from anti-oxidant activity, these molecules provide the following beneficial effects: Anti-viral, anti-cancer, anti-inflammatory, anti-allergic[27]. A steroid, estra-1,3,5(10)-trien-17á-ol present in *C. alata* and *C. occidentalis* it may be responsible for the observed estrogenic and/or anti-estrogenic activity[28]. Pregna-5,8,16-triene-3à-ol-20-one acetate present in *C. occidentalis* flowers, its chemical name is 16-Dehydropregnenolone acetate. A 16-Dehydropregnenolone acetate is the dehydration product of pregnenolone acetate. 16-Dehydropregnenolone acetate is used in the preparation of pregnane derivatives and its glycosides as potential anticancer agents[29]. Isopropyl stearate is found in *C. fistula* flowers. It is used in cosmetics and personal care products, most frequently in the formulation of eye makeup, skin makeup, skin conditioning agent, lipstick

Table 1: Compounds identified in *Cassia alata* flowers

No.	RT	Name of the compound	Structures
1	12.83	1-Oxaspiro[2,5]octane,5,5-dimethyl-4-[3-methyl-1,3-butadienyl]-	
2	17.0	Dodecanoic acid, 10-methyl-, methyl ester	
3	17.63	Estra-1,3,5(10)-trien-17a'-ol	
4	18.55	Oleic acid	
5	19.42	9-Octadecynoic acid, methyl ester	
6	20.52	Butanoic acid, 3-methyl-, hexadecyl ester-	
7	21.18	1-Tetra decene,2-decyl-	
8	21.52	4-Hexadecyanoic acid, 2-butoxy-, butyl ester	
9	24.43	Acetamide, N-[2-hydroxy-1,1-dimethyethyl]-2-[1-methyl-3-phenylthio-2-indolyl]-	
10	26.97	2-Oxazolidinone,3,3'-[1,4-phenylene]bis[5-phenyl-	
11	30.45	4-[6-[[6-Methoxy-4-methyl-8-quinolinyl]amino]hexyl]-1-piperazinepropanol	

and skincare products. Phytol is found in *C. fistula* flower extract with RT 18.48. Phytol is known to be anti-microbial, anti-cancer, anti-inflammatory, hepatoprotective[30]. Phytol also found in *Amaranthus caudatus*[31], *Broussonetia luzonica*[32]. Palmitic acid or n-Hexadecanoic acid was found in *C. auriculata* flower extract. It is a saturated fatty acid that has anti-androgenic, anti-fibrinolytic, hemolytic 5-alpha reductase inhibitor, antioxidant, hypo-cholesterolemic, anti-inflammatory, phospholipase inhibitor, anti-cancerous, anti-microbial, nematicide and mosquito larvicidal properties[33-38]. Recently, n-Hexadecanoic acid was found in different plant parts of *Leptadenia reticulate*[39]. Biological activity of some of the identified compounds listed in Table 5. This study summarized phytoconstituents of some genus *Cassia*, which can be further investigated for the improvement of new novel herbal drugs. Due to medicinal properties there is enormous scope for future research on *Cassia* sp. and further clinical and pharmacological investigation should be conducted to investigate unexploited potential of this plant.

Table 2: Compounds identified in *Cassia auriculata* flowers

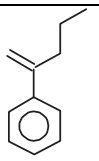
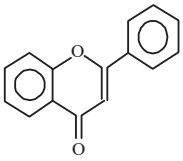
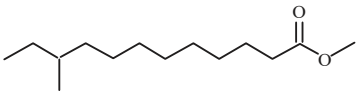
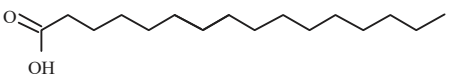
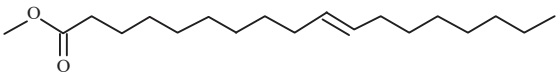
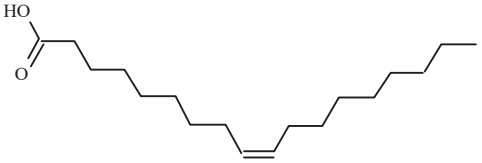
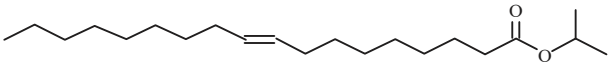
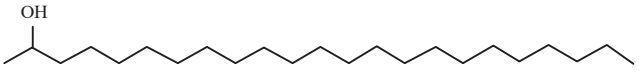
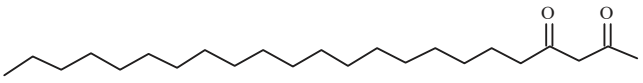
No.	RT	Name of the compound	Structures
1	11	Benzene, [1-methylenebutyl]-	
2	14.85	Flavone	
3	15.98	Dodecanoic acid, 10-methyl-, methyl ester	
4	16.55	n-Hexadecanoic acid	
5	17.53	10-Octadecanoic acid, methyl ester	
6	18.13	Oleic acid	
7	20.17	Elaidic acid, isopropyl ester	
8	23.38	Tricosan-2-ol	
9	25.87	Tricosane-2,4-dione	

Table 3: Compounds identified in flowers of *Cassia fistula*

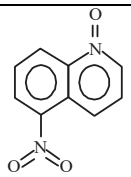
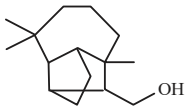
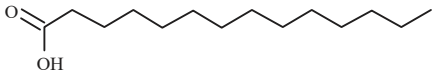
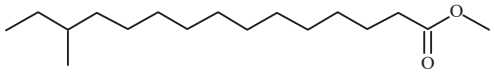
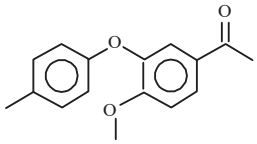
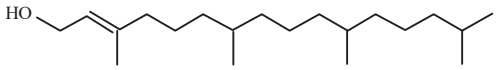
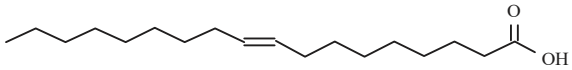
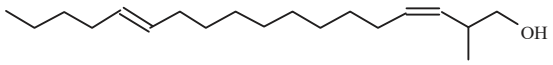
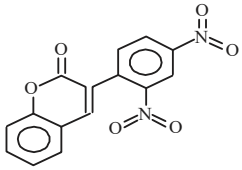
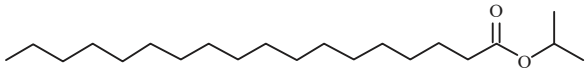
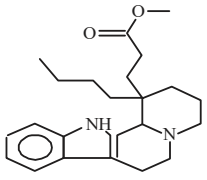
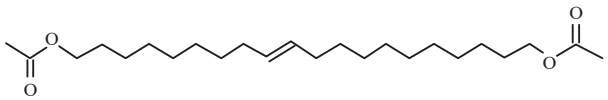
No.	RT	Name of the compound	Structures
1	12.03	Quinoline, 5-nitro-,1-oxide	
2	13.77	1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-[1s-(1à,3aà,4à,8aà,9R")]-	
3	15.63	Tetradecanoic acid	
4	16.85	Pentadecanoic acid, 13-methyl-, methyl ester	
5	17.57	Ethanone, 1-[4-methoxy-3-[4methylphenoxy]phenyl,-	
6	18.48	Phytol	
7	19.2	Octadec-9-enoic acid	
8	19.88	Z,E-2-Methyl,3,13-Octadecadien-1-ol	
9	20.98	Coumarine, 3-[2,4-dinitrophenyl]-	
10	22.57	Isopropyl stearate	
11	27.5	1à-n-butyl-1à[2-methoxycarbonyl-ethyl]-1,2,3,4,6,7,12,12bà-octahydroindolo[2,3-a]quinolizine	
12	33.6	Eicos-9-ene-1,20-diacetate	

Table 4: Compounds identified in the flowers of *Cassia occidentalis*

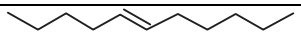
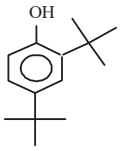
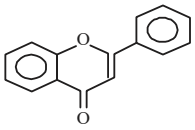
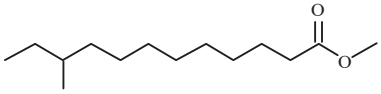
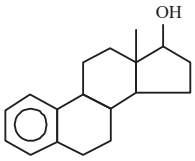
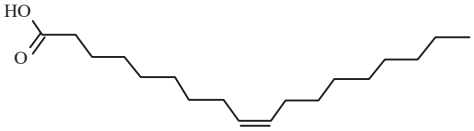
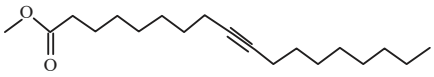
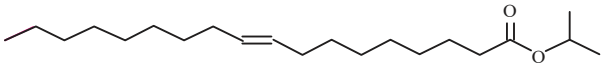
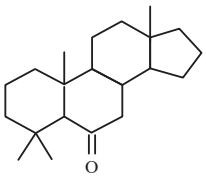
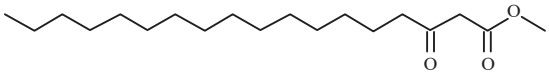
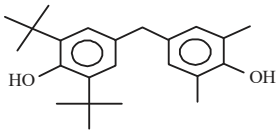
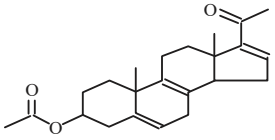
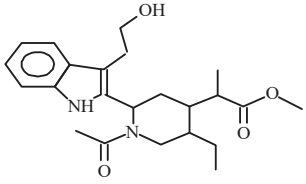
No.	RT	Name of the compound	Structures
1	12.08	5-Undecene, [E]-	
2	14.12	Phenol,2,4-bis[1,1-dimethylethyl]-	
3	15.85	Flavone	
4	17.05	Dodecanoic acid, 10-methyl-, methyl ester	
5	17.83	Estra-1,3,5(10)-trien-17a'-ol	
6	18.6	Oleic acid	
7	19.48	9-Octadecynoic acid, methyl ester	
8	19.98	Elaidic acid, isopropyl ester	
9	21.37	5à-Androstan-6-one,4,4-dimethyl-	
10	23.65	Octadecanoic acid, 3-oxo-, methyl ester	
11	24.43	Phenol,2,6-bis[1,1-dimethylethyl]-4-[[4-hydroxy-3,5-dimethylphenyl]methyl]-	
12	27.52	Pregna-5,8,16-triene-3à-ol-20-one acetate	
13	30.68	4-Piperidine acetic acid, 1-acetyl-5-ethyl-2-[3-[2-hydroxyethyl]-1H-indol-2-yl]-à-methyl-,methyl ester	

Table 5: Biological activity of some identified compounds

Compounds	Biological activity
Oleic acid	5-α reductase inhibitor, allergenic, α-reductase inhibitor, anti inflammatory, anti androgenic, cancer preventive, anemiagenic, anti alopecic, anti leukotriene-D4, choleretic, dermatitigenic, hypocholestrolemic, insectifuge, perfumery, propecic and flavour[25]
Flavone	Anti-viral, anti-cancer, anti-inflammatory and anti-allergic[27]
Pregna-5,8,16-triene-3à-ol-20-one acetate	Anti-cancer activity[29]
Phytol	Anti-microbial, anti-cancer, anti-inflammatory and hepatoprotective[31]
Hexadecanoic acid (Palmitic acid)	Anti-oxidant, hypocholesterolemic, nematicide, pesticide, lubricant, anti androgenic, flavour and hemolytic-5-α reductase inhibitor[39]
Isopropyl stearate	An emollient, skin conditioning agent, binder and humectant activities[40]

CONCLUSION

The *Cassia* sp. have been studied for their phytoconstituents. Flavone was widely distributed throughout this genus which suggests that these compounds may be chemotaxonomic markers of the genus *Cassia.* The genus *Cassia* definitely holds the promise of providing potent drugs for both chronic and acute diseases like diabetes mellitus and others. It is also clear that much needs to be discovered, both as to the active ingredients and their biological effects. The results presented here are intended to serve as a reference tool to researchers in the fields of ethnopharmacology.

SIGNIFICANT STATEMENT

Over the years, many scientists have performed research on different plant families to be able to identify secondary metabolites. Present study discover many secondary metabolites that can be beneficial for the researchers in the field of ethnopharmacology This paper summarizes GCMS studies on some flowers of *Cassia* sp., which can be further investigated for the improvement of new novel herbal drugs. The detailed information regarding the phytoconstituents of *Cassia* sp. can be useful for the development of new traditional medicine and for the benefit of the mankind. This study will help the researchers in identifying active components of *Cassia* sp. in future. Thus a new theory on it may be arrived at.

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