

Pharmacognostic and phytochemical study of the flowers of *Cordia sebestena* L.

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Abstract: The present study shows the pharmacognostic and phytochemical studies on the flowers of *Cordia sebestena* L. belongs to the family Boraginaceae. *C. sebestena* L. is found primarily in tropical and subtropical regions of the American, Asian and African continents. Though it is an important plant, until date no pharmacognostic work is found on its parts such as flowers. Various organoleptic characters were recorded by macroscopic study. Microscopic study of the flowers were also conducted which shows the presence of fibers, calcium oxalate crystals and multiple types of trichomes, along with fluorescence analysis. The present study also deals with the Fourier transform infrared spectroscopic analysis of *C. sebestena* L. FT-IR spectra revealed the presence of C-H, C=C, C-N, C-O and aromatic groups. Chemical composition of the hexane extract of the flowers of *C. sebestena* L. was detected through GC-MS and spectrum achieved through GC-MS were correlated with the database of National Institute of Standards and Technology (NIST) which comprise of beyond 62000 outlines of the mass spectrum. GC-MS analysis of n-hexane extract shown the existence of Retinoic acid, lupeol, β -sitosterol, stigmaterol, hexadecanoic acid along with fatty acids, esters, alkaloids and alcohols. These pharmacognostic and phytochemical studies can be valuable towards giving reliable proof of the quality of the plant which can benefit health professionals and herbal medicine manufacturers.

Keywords: *Cordia sebestena* L, GC-MS, standardization, microscopy.

INTRODUCTION

Plant signifies considerable extent of the worldwide medication market. In this regard globally recognized rules are fundamental for their quality evaluation. It has been evaluated that 80% of individuals living in developing nations are totally reliant on conventional herbal medicines. Thus it gets to be greatly vital to document the standardization of these plant materials that will be use as future medications (Khan *et al.*, 2015). Phytochemicals, also called phytoconstituents are, bioactive components extensively found in foodstuffs like fruits, whole-grain, leaves, roots, vegetables, nuts, seeds and legumes. Though ten of thousand phytochemicals have existed but only small numbers of them have been secluded from plants (Cao *et al.*, 2017). The foremost usual phytochemicals in food incorporate polyphenols, carotenoids, flavonoids, coumarins, indoles, isoflavones, lignans, catechins, phenolic acids, stilbenoids, isothiocyanates, saponins, procyanidins, phenylpropanoids, anthraquinones, ginsenosides, alkaoloids and others (Zhao *et al.*, 2018; Xiao, 2017). Herbal medications are safer than synthetic drugs since the phytochemicals inside the plant extract target the biochemical pathway (Nisar *et al.*, 2018).

commonly known as Geiger tree is evergreen thick, deciduous tree. The genus *Cordia* generally includes ornamental plant species. The plants which belong to the family Boraginaceae are found in the tropical, subtropical and hotter regions around the world. *Cordia sebestena* L. is perennial plant but flowers abundantly found in June and July (Adeosun *et al.*, 2015; Prakash *et al.*, 2020). It grows upto a height of 25-30 feet and spreads upto 20-25 feet, having green or white coloured fruit, orange-red 2-5 cm long flowers and leaves are ovate 4.5-10 cm. Geiger tree is local to Cuba, Northern West Indies, along with a few parts of Tropical North, Central and South America. Bloom are orangish red and gaudy and are appeared in bunches basically in spring and summer (Hanani *et al.*, 2019).

Bioassay of fractions of the ethyl acetate extract of *C. sebestena* L. have lead to the separation of sebestinoids which has restraint ability on aspartic protease (Dai *et al.*, 2010). Seed oil of *C. sebestena* L. contains palmitic acid and oleic acid (Agunbiade *et al.*, 2013). AgCuO biometallic nanomaterial from *C. sebestena* L. leaf extract synthesized through green synthesis (Ravi *et al.*, 2020). Dyeing potential of flowers of *C. sebestena* L. also reported (Kumaresan *et al.*, 2012). We herewith report the entire chemical arrangement of hexane extract and pharmacognostic features of the flowers of *C. sebestena* L.

C. sebestena L. belongs to the family Boraginaceae

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MATERIALS AND METHODS

Plant material

Flowers of *C. sebestena* L. were collected from University of Karachi, Karachi during August 2019. The plant specimen was identified by Taxonomist Dr. Muneeba Khan in the Center for Plant Conservation, University of Karachi with (GH No. 95282).

Extract preparation of flowers of *Cordia sebestena* L.

500g of flowers of *Cordia sebestena* L. were soaked in one liter of hexane in extraction flask and kept at room temperature for 3 days and had shaken three times daily. The extract was strained through Whatman filter paper No.1. The filtered extract was dried at 40°C under vacuum pressure in rotary evaporator. The resultant extract was kept in dark in amber glass bottle at ambient temperature.

Pharmacognostic study

Macroscopic evaluation

Macroscopic evaluation of the flowers of *C. sebestena* L. like color, size, shape, texture and fracture was performed (Ahmed and Hasan, 2015).

Microscopic evaluation

Powdered microscopy of the flowers of *C. sebestena* L. was conducted through light microscope. Dried flowers were pulverized to mechanical grinding and passed through sieve No. 40 (Bharthi *et al.*, 2017). Fine powder was taken on a glass slide and treated separately with water, glycerine, chloral hydrate and iodine reagents. Microscopic observations were fulfilled utilizing 4, 40 and 10 objective lenses and photomicrographs were captured (WHO, 2011; Evans, 2009).

Fluorescence analysis

Fluorescence analysis of the powder of the flowers of *C. sebestena* L. was also performed with different chemicals to check the existence of diverse fluorescent chemical compounds under visible and UV light of short (254nm) and long (365nm) wave length (Tang *et al.*, 2018; Kadam *et al.*, 2012).

Fourier transform infrared spectroscopy (FTIR) analysis

To identify the distinguishing functional groups found in the phytochemicals FTIR was used. Fine powder of the flowers of *C. sebestena* was taken for identification of functional groups. Powder was then taken on FTIR spectroscope (Nicolet avatar 330 FTIR, Thermo Electron Co. USA.) having range of wave numbers 500-4000 cm⁻¹. Data interpretation of FT-IR spectra was carried out using correlation chart (Pavia *et al.*, 2008)

Phytochemical study

GC-MS analysis

The Gas chromatography mass spectroscopy of hexane extract of the flowers of *C. sebestena* L. was carried out

by Agilent Technologies 7000A. Triple Quadrupole Acquisition Method was applied throughout the method. The instrument was packed with a non polar column stuffed with film prepared of 95% Dimethylpolysiloxane and 5% phenyl (Agilent HP-5MS-30m length × 250µm diameter × 0.25µm film thickness). For the discovery of the compounds an electron ionization source with 70eV energy was utilized. Ultra immaculate Helium gas (99.99%) was utilized as a carrier gas for mobile phase with split mode at septum purge flow rate of 3ml/min. The injection volume was 2.5µL with a split ratio of 10:1. The temperature of the injector was 250°C. The pressure was 9.05 psi and constant flow was 1.129 ml/min by average velocity of 38.724 cm/sec. Total runtime was 82.286 min. The solution was ready by taking 1gm of extract and making it dissolvable in 20ml of corresponding solvent. The arrangements were sifted through Whatman No.1 filter paper to evacuate any thick particles. All the chemicals utilized were of analytical grade. Nist spectral library was used to analyze spectra. Calculation of mole percent peak area was done according to the following formula (Ullah *et al.*, 2019). Mole % component (Peak area) = area under peak/total area of all peaks x 100

RESULTS

Macroscopic evaluation

Fresh flowers of *C. sebestena* L. were orange in color present in bunches consisted of epipetalous stamen with in a throat, salveform in shape. Flowers starts with long tube and widens into polypetalous flower, actinomorphic, gamosepalous, calyx 1.2-1.5cm, crenulate corolla, involucre bract having smooth texture, bland taste, soft fracture upon breaking and measuring 2-5cm size.

Microscopic evaluation

Orangish brown powder of the flowers of *C. sebestena* showed some significant microscopic features which are shown in fig.1.

Fluorescence analysis

Result of the fluorescence analysis of the flowers of *C. sebestena* L. has shown in table 1.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis of the flowers of *C. sebestena* L. has shown in table 2 and fig. 2.

GC-MS analysis

GCMS analysis of hexane extract of the flowers of *C. sebestena* L. are presented in table 3 and fig. 3 and structures of compounds are in table 4 which shows more compounds of fatty acid, ester groups, hydrocarbons, triterpene, alkaloids, alcohols, sterols and vitamin class.

Table 1: Fluorescence characters of the flowers of *C. sebestena* L.

Reagent	Day light	UV 254nm	UV 365nm
Chloroform	Orangish brown	Orangish brown	Grey
Ethanol	Orangish brown	Red	Bluish grey
Ferric chloride	Blackish brown	Blackish brown	Blackish brown
Glacial Acetic acid	Dark Yellow	Pink	Bluish green
Hydrochloric acid	Orangish brown	Orangish brown	Orangish brown
Methanol	Brown	Brown	Yellow
Sulphuric acid	Orangish brown	Orangish brown	Orangish brown

Table 2: FT-IR analysis of flowers of *C. sebestena* L.

Type of Vibration Assigned	Absorption Frequency (cm ⁻¹)	Intensity
C-H Aldehyde	2900	w
C=C Alkene	1600	m-w
Aromatic	1400	m-w
C-N Amines	1300	m-s
C-O Alcohols, ethers, esters, carboxylic acids, anhydrides	1025	s

(Abbreviation: S; strong W; weak M; medium)

Table 3: Phytochemical constituents of hexane extract of *C. sebestena* L.

Compound Name	Molecular Formula	Molecular Weight	Retention Time	Nist Number	ID Number	Peak Area %
Retinoic acid, 5,6-epoxy-5,6 -dihydro	C ₂₀ H ₂₈ O ₃	316	69.650	51852	5918	1.37
Lupeol	C ₃₀ H ₅₀ O	426	64.160	124852	7814	1.82
4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	C ₃₀ H ₄₈ O	424	63.668	194624	153809	2.16
β-sitosterol	C ₂₉ H ₅₀ O	414	62.620	287034	1913	5.7
Stigmasterol	C ₂₉ H ₄₈ O	412	61.612	352610	18876	1.82
Octadecanoic acid,2-propenyl ester	C ₂₁ H ₄₀ O ₂	324	60.411	36559	5707	2.25
1H-Purin-2-amine, 6-methoxy-N-methyl-	C ₇ H ₉ N ₅ O	179	59.074	34043	132450	9.77
Nonacosane	C ₂₉ H ₆₀	408	56.858	197624	5478	2.93
Heptacosane	C ₂₇ H ₅₆	380	54.605	79427	5508	1.23
Dodecanoic acid, phenyl methyl ester	C ₁₉ H ₃₀ O ₂	290	52.984	232922	11551	2.08
1,2-Benzenedicarboxylic acid , diisooctyl ester	C ₂₄ H ₃₈ O ₄	390	52.602	113206	20061	14.86
9,12-Octadecadienoic acid, ethyl Ester	C ₂₀ H ₃₆ O ₂	308	42.599	249157	28827	2.17
9-12-Octadecadienoyl chloride	C ₁₈ H ₃₁ ClO	298	37.820	76312	4450	7.66
Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	31.491	233204	49485	1.84
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	30.122	335494	6723	6.16
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	28.540	42975	9049	5.08
Rheadan-8-ol,2,2,10,11-tetramethoxy-16-methyl	C ₂₂ H ₂₇ NO ₆	401	26.505	64919	155496	0.76
9,10-Dimethyltricyclo (2,5) decane-9,10-diol	C ₁₂ H ₂₀ O ₂	196	23.505	187529	8345	3.86
Blumenol C	C ₁₃ H ₂₂ O ₂	210	22.717	108740	5952	0.86
3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo	C ₁₂ H ₁₆ O ₅	240	18.958	26532	89100	0.88
Tetradecane	C ₁₄ H ₃₀	198	17.332	113925	5511	1.76
Tridecane	C ₁₃ H ₂₈	184	15.426	229227	5468	2.73
Dodecane	C ₁₂ H ₂₆	170	13.391	291499	21869	2.64
Undecane	C ₁₁ H ₂₄	156	11.207	227975	22005	1.54
Hexylene glycol	C ₆ H ₁₄ O ₂	118	6.955	234996	26166	0.16

Table 4: Chemical structures of phytoconstituents reported in n-hexane extract of flowers of *C. sebestena* L.

Retinoic acid, 5,6-epoxy-5-6-dihydro-	
β -sitosterol	
Stigmasterol	
4,4,6a,6b,8a,11,11,14b-Octamethyl, 1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	
Lupeol	
6-methoxy-N-methyl-1H-purin-2-amine	
Rheadan-8-ol, 2,3,10,11-tetramethoxy-16-methyl-	
Hexylene glycol	
Blumenol C	
Octadecanoic acid, 2-propenyl ester	
Dodecanoic acid, phenylmethyl ester	
1,2-Benzenedicarboxylic acid, diisooctyl ester	
9,12- Octadecadienoic acid, ethyl ester	
9-12- Octadecadienoyl chloride	
Hexadecanoic acid, methyl ester	
Hexadecanoic acid, ethyl ester	
Hexadecanoic acid	
Heptacosane	
Nonacosane	
Tetradecane	
Tridecane	
Dodecane	
Undecane	
9,10-Dimethyltricyclo (2,5) decane-9,10-diol	
2-Carboxymethyl-3-n-hexylmaleic acid anhydride	

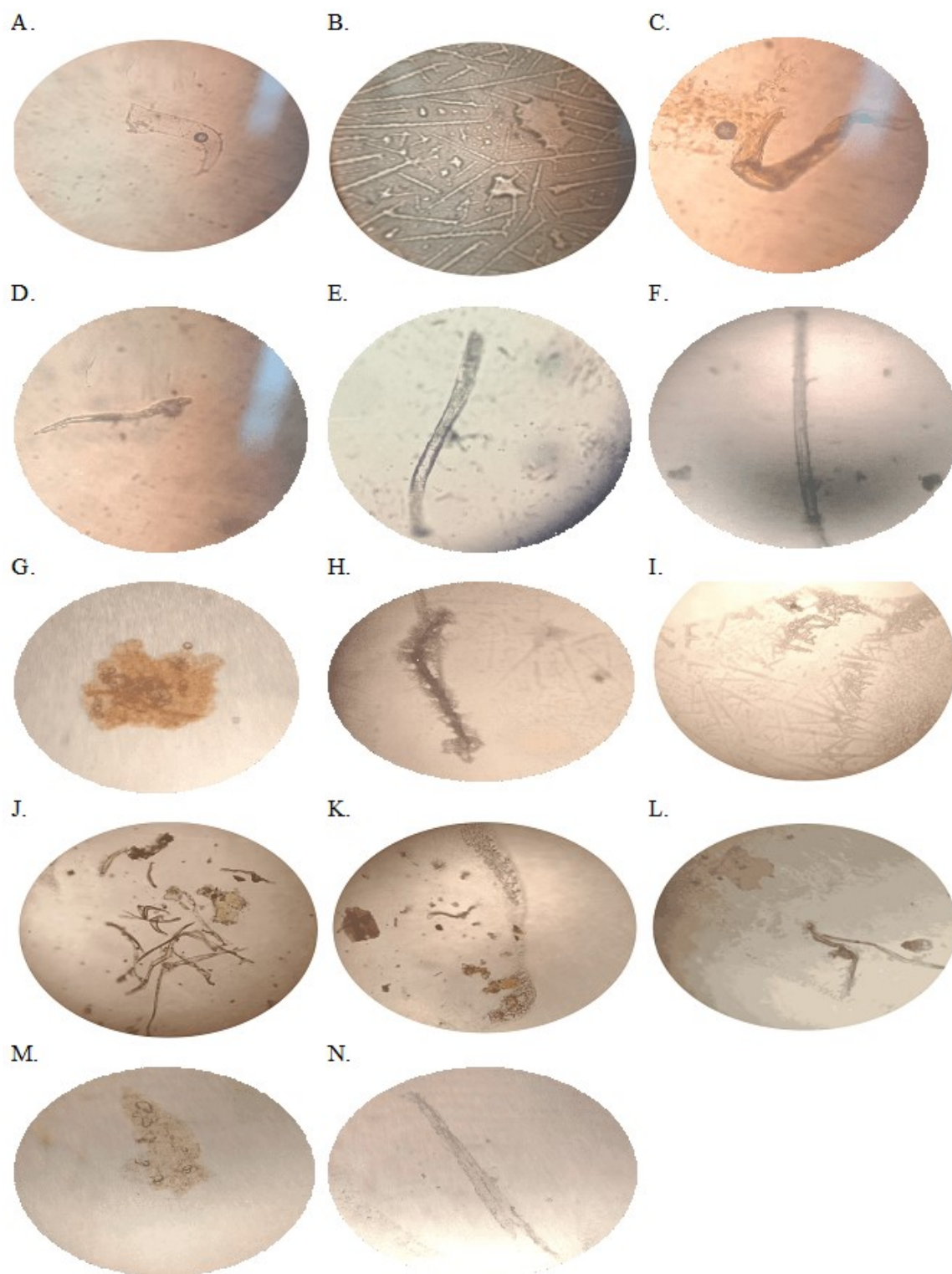


Fig. 1: Powder Microscopic features of flowers of *Cordia sebestena* L. (A) Trichome and oil globule; (B) Calcium oxalate; (C) Glandular trichomes with cicatrix and radiating pollen grains; (D) Unicellular trichomes; (E) Fibers with spiral thickening; (F) Fibers; (G) Pollen grains; (H) Lignified fibers (I) Dendritic calcium oxalate; (J) Multicellular trichomes; (K) Fragments of xylem with spiral thickening; (L) Unicellular trichomes with pollen grains; (M) Prismatic calcium oxalate crystals; (N) Pericyclic fibers

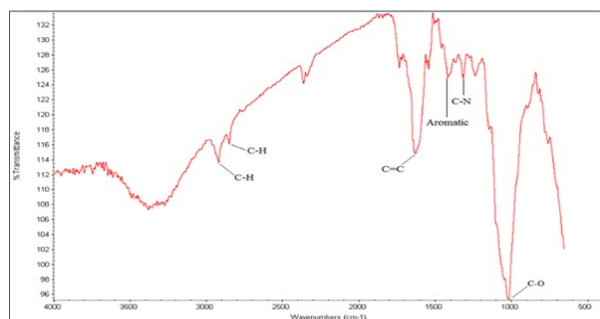


Fig. 2: FTIR spectra of flowers of *C. Sebestena* L.

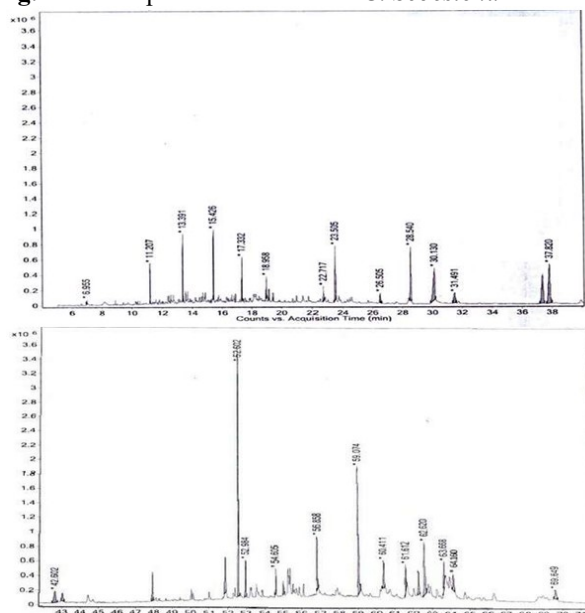


Fig. 3: Chromatograms of n-hexane extract of flowers of *C. sebestena* L.

Fatty acid esters that are present in hexane extract among them 1,2-Benzenedicarboxylic acid, diisooctyl ester was abundant in plant with a peak area percentage of 14.86 also 9-12-Octadecadienoyl chloride is present having peak area 7.66. Among alkaloids 1H-Purin-2-amine, 6-methoxy-N-methyl was abundant with a peak area percentage 9.77. β -sitosterol was present with a peak area percentage of 5.7 which is high in sterols. n-Hexadecanoic acid was present in plant extract with a peak area % age of 6.16. Similarly in fatty acid methyl esters group Hexadecanoic acid, methyl ester was present in hexane extract with a peak area percentage of 5.08. 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one which is triterpene is present with a peak area percentage of 2.16. Among hydroxy hydrocarbons 9,10-Dimethyltricyclo (2,5) decane-9,10-diol was present with peak area percentage of 3.86.

DISCUSSION

Plants with medicinal values are regarded as the source of new chemical entities which can be converted into drugs

with considerable research. A considerable number of present day drugs are synthesized either specifically or in other way from the medicinal plants. Therefore the standardization of herbal medications is of great significance in setting up its legitimate character to play a basic part in understanding its structure, science, botanical value and clinical suitability because of frequently finding of substitute or bogus herbal supplies (Zhang *et al.*, 2021). Examination of histological characters along with macroscopic evaluation are the essential tests for standardization. In view of these practicalities authors have prepared an endeavor to fig. out histological characters and macroscopic study that can be utilize for the identification and standardization of this plant, as no standard specifications for standardization has been reported so far. The projecting microscopic features of the flowers of *C. sebestena* L. are fibers, calcium oxalate crystals, multiple types of trichomes shown in fig. 1. (Bijeshmon and George, 2014; Bharthi *et al.*, 2017) reported somewhat similar types of fibers in the flowers of *Tabernaemontana divaricata* R. and *Vitex negundo* L. (Reddy *et al.*, 2015) reported similar multicellular trichomes in the flowers of *Justicia adhatoda* L. (Das *et al.*, 2021) also reported similar unicellular trichomes. (Baravalia *et al.*, 2011; Bijeshmon and George, 2014) investigated similar fragments of xylem with spiral thickening in the flowers of *Woodfordia fruticosa* Kurz. and *Tabernaemontana divaricata* R.

Fluorescence examination may be a vital parameter which demonstrates the symbol of chromophore within the drug, which is essential to perform standardization (Prasanth *et al.*, 2017). Few constituents appeared fluorescent within the ultra-violet or visible light since they may regularly be changed over into fluorescent subsidiaries by using diverse chemicals as shown in table 1 which is useful to recognize them.

Fourier-transform Infrared spectroscopy serves as a notable means for providing robust insight of various functional groups within the plant material (Selvaraju *et al.*, 2021) It also provides major information of organic and inorganic components. In our study FT-IR analysis showed types of vibrations as shown in table 2 and fig. 2 like C-H group which shows the presence of several aliphatic, aldehyde containing compounds, C=C group present confirm the presence of alkenes, presence of carboxylic acids, ethers, esters, alcohol, anhydride confirmed by the strong peak of C-O, C-N groups indicate the presence of aliphatic amines. All these functional groups present in the plant have numerous medicinal characteristics and these functional groups construct phytochemicals present in the natural product.

The identification of the chemical constituents in a plant is a vital stage because the pharmacological and biological activities of plants are dependent on these

bioactive chemical constituents. Hence, GCMS analysis was used to detect the bioactive phytochemicals present in hexane extract of flowers of *C. sebestena* L. shown in table 3, fig. 3 and table 4 which shows fatty acid, esters, sterols and alkaloids have high peak area percentage and literature shows that these phytoconstituents possess pharmacological activities like sterols have anti-atherosclerotic effects (Salehi *et al.*, 2021), fatty acids are medicinally important and have antibacterial and antifungal activity (Casillas *et al.*, 2021; Pohl *et al.*, 2011). Similarly anti-inflammatory activity of alkaloids was also reported (Souza *et al.*, 2020). GC-MS, analysis have shown the existence of n-hexadecanoic acid which is well known to have antioxidant property (Gopu *et al.*, 2021), Hexadecanoic acid, ethyl ester have antiandrogenic activity, stigmasterol and lupeol possess anticancer, anti-inflammatory, antiarthritic and diuretic activity (Rajeswari *et al.*, 2012). B-sitosterol alleviates inflammatory response (Sun *et al.*, 2020). 4,4, 6a,6b, 8a,11,11,14b-Octamethyl-1,4, 4a, 5, 6, 6a,6b,7,8,8a,9,10,11,12,12a,14, 14a, 14b-octadecahydro-2H-picen-3-one which is triterpene possess antibacterial, antioxidant, antitumour and cancer preventive activity (Duan *et al.*, 2011) Hexadecanoic acid, methyl ester may have antioxidant, hypocholesterolemic, anti androgenic, hemolytic, Alpha reductase inhibitor (Pavani and Naika, 2021). Heptacosane possess antioxidant activity (Dandekar *et al.*, 2015). Nonacosane has antibacterial activity (Ryu *et al.*, 2020). 1,2-Benzenedicarboxylic acid, diisooctyl ester known to have antifouling and antioxidant activity (Parthipan *et al.*, 2015) Hexane extract of *C. sebestena* L. also shows the presence of several hydrocarbons, these hydrocarbons contribute in chemotaxonomy of *C. sebestena* L (Adeosun *et al.*, 2013).

CONCLUSION

Standardization of herbal drugs is a topic of great concern. The pharmacognostic parameters of the flowers of *C. sebestena* L. are laid down for the first time and these findings could be helpful in the identification and authentication of these plant materials in future for further research and utilization. Phytochemical studies of hexane extract also shows different findings and therefore useful in quality control of the plant drug. In general, this study may further aid in developing standardization limits for *C. sebestena* L. but this study requires more extensive research through which it may set up as reference data and compact evidence for appropriate identification and validation of the natural product which can contribute in pharmacopeial documentation for recognition of its distinctiveness, genuineness and quality.

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