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Pharmacognostic and phytochemical studies of *Hibiscus rosa-sinensis* L

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Abstract
Hibiscus rosa-sinensis L. is a cosmopolitan shrub of family Malvaceae. This plant is considered very essential in traditional medicine, and it contains a variety of chemical substances including flavonoids, coumarins, tannins, and terpenes. The present study was aimed to standardize and authenticate the flower drugs of *Hibiscus rosa-sinensis* L. by using Maceration, Palynology and GC-MS analysis. The maceration of flowers reveals vessels, tracheids, fibres, and parenchyma cells. The palynological investigation of *Hibiscus rosa-sinensis* L. was carried out by using scanning electron microscopy. Pollen grains of *Hibiscus* were, Pantoporate, spherical to globose, isopolar, radial symmetry in polar view and bilateral in equatorial view, circular to oval. The GC-MS analysis of flower revealed twenty-five distinct peaks, indicating the presence of twenty-five different compounds. These compounds are biologically active. It is conclude that *Hibiscus* flower extract contains 25 phytocompounds.

Keywords: *Hibiscus rosa-sinensis* L. flower, maceration, palynology and GC-MS, chemical constituents

Introduction
Hibiscus rosa-sinensis L. the perennial shrub belongs to the family Malvaceae. The leaves are 3.5 to 12 cm in length and 2-5 to 5 cm in width. Simple oval or ovate-lanceolate leaves. At the base, the leaves are entire and at the tip they are coarsely serrated. It has a mucilaginous flavour. Pedicillate, actinomorphic, pentamerous and entire flowers. The corolla has five petals, is red in colour and measures about 3 inches in diameter. It is widely available across its hardiness zone (Sarje *et al.*, 2019) [24]. *Hibiscus rosa-sinensis* L. is a native to south eastern Asia (China) however it can be found in the tropical and subtropical and as a houseplant all over the world. Most of the ornamental varieties are hybrids (Rao *et al*., 2014) [23].
Hibiscus rosa-sinensis L. is a flowering plant that is used to treat a variety of ailments, including alopecia (Upadhyay and Upadhyay 2011) [31]. In India, an infusion of the petal is usually used as a demulcent refrigerant drink in fever and a decoction is administered in bronchial catarrh (Kumar and Singh, 2012) [18]. The flower has anti-spermatogenic, anti-androgenic and anti-tumor properties (Scalbert, 1991; Dey *et al*., 2010) [26, 9].
The flower of *Hibiscus rosa-sinensis* L. has anti-diabetic activity (Venkatesh and Thilagavathi, 2008) [34].
Several articles and ancient literature revealed that the flowers of this plant possess antifertility activity, like antimplantation, abortifacient, in rodents (Gilani *et al*., 2005) [12].
Apart from that, it is used as an anti-dandruff ingredient for centuries (Vyjayanthi *et al*., 2004) [35]. It also increases hair growth by activating hair follicles and increasing blood supply to the hair follicles. The activity of hair growth promoters was examined in a mouse study, and the results were positive (Chakraborty, 2016) [7]. The alcoholic extract of *Hibiscus rosa-sinensis* flowers has anticonvulsant properties (Kasture *et al*., 2000) [18]. Flowers are used in Diabetes, epilepsy and leprosy (Chatterjee and Prakashi 1992) [8].
With this view, the present study was aimed to Standardize and Authenticate the flower Drugs of *Hibiscus rosa-sinensis* L. by using Maceration, Palynology and GC-MS analysis.

Materials and Methods
Collection of the plant material
The fresh *Hibiscus* rosa-sinensis L. flowers were collected from Harsool Aurangabad, in February 2018. The plant material was identified and Authenticated by Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

Preparation of samples for maceration
The flowers of *Hibiscus rosa-sinensis* L. brought to the laboratory within 2 - 5 hours. Flower parts were preserved in 70% alcohol for their maceration work. The Xylem element were studied by using Jeffery's fluid method (Jeffrey, 1917) [14]. The Flower parts were macerated by using Jeffery's fluid Chromic acid 10% and Nitric acid 10% in equal proportion. The elements stained with aqueous, 1% safranin for 5 minutes. Excess stain was removed by washing with water and mounted in glycerine with DPX. The cellular illustrations of figures were taken by using canon digital camera. The dimensions of the element obtained during maceration were measured by ocular micrometry.

Preparation of samples for light and scanning electron microscopy
Pollen samples were obtained from fresh flowers that were collected during anthesis. These pollen grains were fixed in Glacial acetic acid and stored in bottle. After fixing, Material for SEM was prepared by mounting pollen on clean Aluminium stubs covered with double sided carbon tape. Sample is examined using FEI Quanta 200 Scanning electron microscope at 20 kV under low vacuum mode of pressure 65 Pascals in ICON analytical equipment pvt. Ltd, Lab, worli Mumbai.

Preparation of Flower powder and extract

The flowers of *Hibiscus rosa-sinensis* L. were washed thoroughly in running up tap water to remove soil particles and adhered debris and finally washed with sterile distilled water and shade dried for 14 days. Materials were ground with the help of mixer into a fine powder. Plant material (Flower 10 Gms) were extracted with 250 mL of Ethanol at 60°C for 8 hours in Soxhlet extractor. The ethanolic extracts were filtered through Whatmann No. 1 filter paper. The filtrate was evaporated to dryness at room temperature and stored until further analysis.

Preparation of stock solution

The extracts were reconstituted in Ethanol. Ethanolic extracts (1 µl) were injected for GC-MS analysis.

Gas Chromatography-Mass spectrometry analysis

The ethanolic extract of the Flower of *Hibiscus rosa-sinensis* L. were subjected to GC-MS analysis on a GC- MS system (GC-2010 plus) Shimadzu, (Agilent Technologies Inc.,) equipped with an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 mm, (Agilent Technologies Inc.,). The injection volume of sample was 1 µL. Helium (99.999%) was used as the carrier gas at a flow-rate of 1 mL/min. The temperature of the injection port was 250 ◦C, and the column temperature program was as follows: 50 ◦C for 2 min, followed by an increase to 180 ◦C at a rate of 5 ◦C/min, an increase to 270 ◦C at a rate of 20 ◦C/min, and maintenance at 270◦C for 5 min. The MS (QP-2020) conditions included an EI ion source temperature of 230 ◦C, an ionization energy of 70 eV, and a mass scan range of 40-500 amu. Mass spectra were taken at 70 eV; a 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 40 minutes.

Identification of compounds

Characterization of the mass spectrum the GC-MS were carried out with the use of the National Institute of Standards and Technology's database, which has over 62,000 patterns. The NIST library contains a spectrum of known components. The name, molecular weight, and structure of the components of the test materials were ascertained.

Results and Discussion

Maceration of flower shows single type of Tracheids Long, slender, Spiral, blunt ends with pits. Tracheids range 80-100× 4 µm. fig. 2a. Vessel elements are of three types Simple, elongated, without pits, perforated at end. Size range is 40- 55 × 4 - 6 µm. fig. 2b. Scalariform vessel, with pits, very long, perforated at end, size range is 25- 30 × 3 - 4 µm. fig. 2c. Reticulate vessel, with reticulate pits, very long, wide ends, size range is 25 - 30× 2-3 µm fig. 2d. Two types of parenchyma: Parenchyma Short, cells oblong, thick walled, size range is 6 - 10× 4 - 6 µm. fig. 2e. Parenchyma, long rectangular, thick walled, and size range is 10 - 16 × 2-6 µm. fig. 2f. Fibres are of two types: Simple fibre, slender, tapering and sharply pointed, outline entire, size range is 60-65× 2 µm. 2g. Labriform fibre, slender, tapering, sharply pointed, outline entire with thin lumen, size range is 50 - 55 × 2 µm. Fig 2h.

Hibiscus pollen grains was Pantoporate, spheroidal, Size: pollen polar diameter is 76.80 µm and equatorial diameter is 76.50 µm. A mature pollen grain's wall was made up of intine and exine that are overspread by echini of different height and width. Spines were dimorphic; longer spines with sharp and pointed apex and shorter ones with slightly obtuse apex, or the height of all these spines is inconsistent Fig. 3.

The nature of active compounds in edible medicinal flowers can be better understood using GC-MS analysis.

The compounds present in the ethanolic extract of *Hibiscus rosa-sinensis* flower, were identified by GC-MS analysis. These compounds were characterized using mass spectrometry in combination with gas chromatography.

The results of the present study were tabulated in Table 1.

The active principles along with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 1.

The National Institute of Standards and Technology Database is used to establish the compound prediction. Major Phytocomponents and their biological activities obtained through GC-MS study of *Hibiscus* flowers tabulated in Table 2. The results revealed that the presence of Methanethiol (42.83%), Propanedioic acid, dihydroxy- (2.45%), Glycerin (5.69%), n-Hexadecanoic acid (1.99%), Hexadecanoic acid, ethyl ester (0.70%), 4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester (0.22%), 9,12-Octadecadienoic acid (Z, Z)- (0.09%), Linoleic acid ethyl ester (0.57%), Ethyl Oleate (0.48%), Tetracontane (0.39%), 1-Methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-2-ol 100 (0.70%), 6a-.beta.-Aporphine, 1,2-dimethoxy- (3.29%), 1-Hexacosanol (0.38%), Dotriacontane (0.45%), 3,4-Dihydroisoquinoline, 1-[3-methoxybenzyl]-6-methoxy (2.29%), beta.-Sitosterol (2.34%), Spiro[2,5-cyclohexadiene-1,7'(1'H)-cyclopent[ij]isoquinolin]-4-one, 2',3',8',8'a-tetrahydro-5',6'-dimethoxy-1'-methyl-, (R)- (0.91%), dl-Laudanosine (0.82%), (-)-1,2,3,4 -6-ol-1-carboxylic acid, 7-methoxy-1-methyl-, methyl ester (0.79%), Tetracosane (1.53%), Tetrapentacontane (0.22%), Hexatriacontane (3.43%), Ethyl 3-(p-methoxyphenyl)-3-(3-methyl-2 benzofuranyl)propionate (0.89%), Triacontane (4.56%), Tetratriacontyl trifluoroacetate (18.28). The spectrum profile of GC-MS confirmed the presence of 23 major components with the retention time 1.390, 1.590, 2.435, 14.555, 14.990, 15.920, 17.075, 17.470, 17.560, 19.620, 19.620, 21.990, 22.475, 22.580, 22.695, 22.925, 23.085, 24.715, 24.845, 25.255, 25.685, 27.135, 28.695, 28.795, 28.795, 32.720, 32.890 respectively (Figure 4).

These phytochemicals are responsible for various pharmacological actions like antimicrobial, antioxidant, antifertility, antimutagenic, anti-inflammation, Anticancer, Hepato protective, Diuretic, Antiasthmatic activities etc. Thus this type of GC-MS analysis is the step to understanding the nature of active principles in this medicinal flowers and this type of study will be helpful for further studies. Among the 25 compounds identified, Methanethiol was found to be the major bioactive compound *Hibiscus rosa-sinensis* flower, which show 42.8380 %, peak area with retention time 1.390. Due to presence of such large number of useful metabolites in these *Hibiscus Rosa -sinensis* flower, hence as phytopharmaceutically important. Furthermore, GC-MS analysis of this type is the initial step toward determining the nature of active principles.

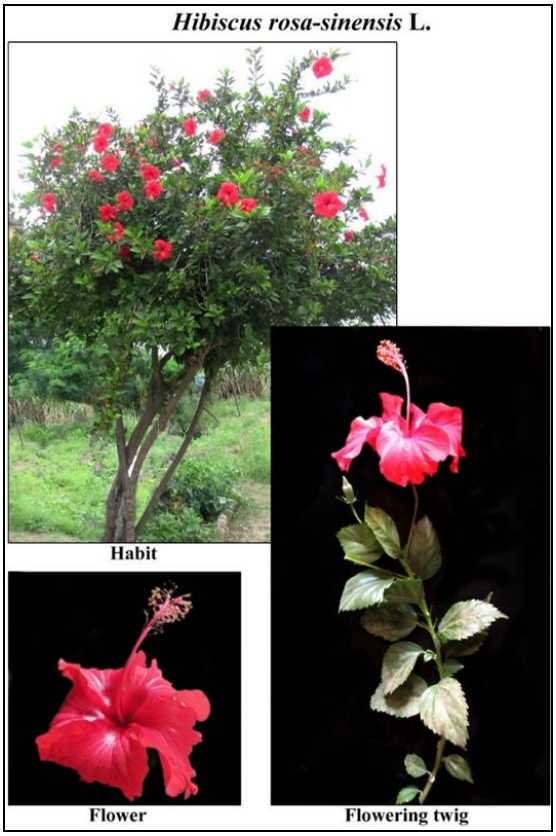


Fig 1: *Hibiscus rosa-sinensis* L

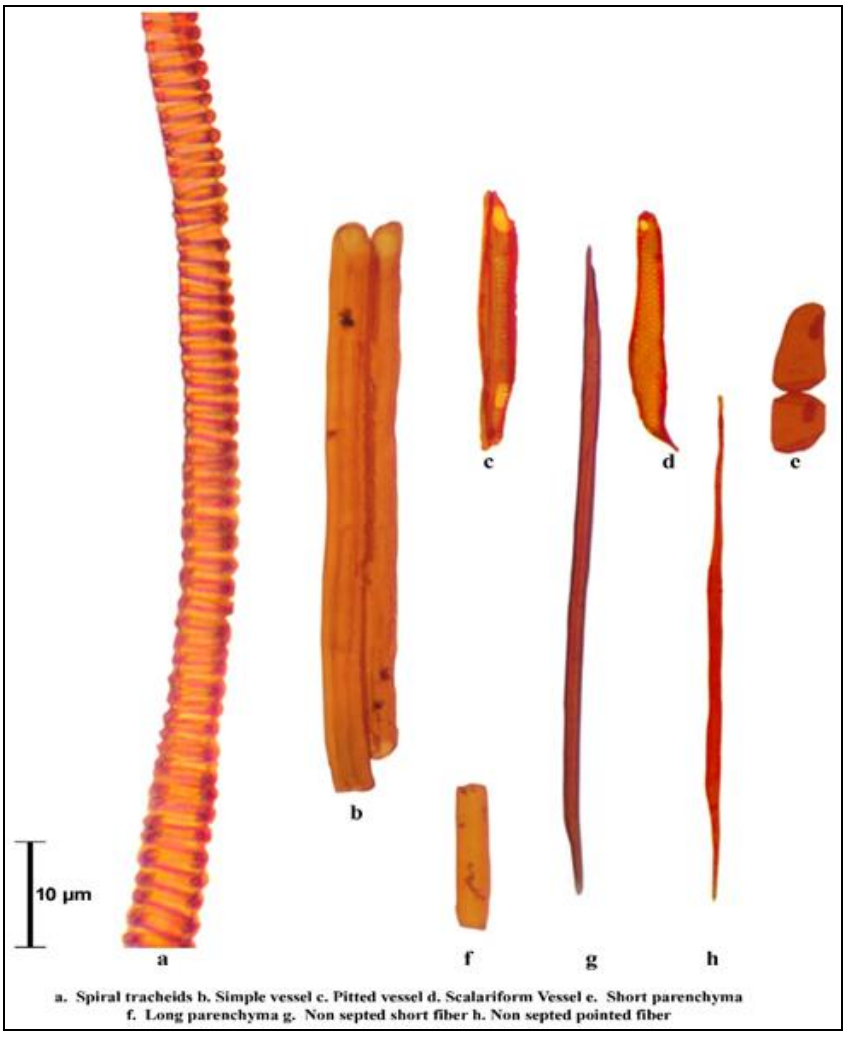


Fig 2: Maceration of *Hibiscus rosa-sinensis* L. Flower

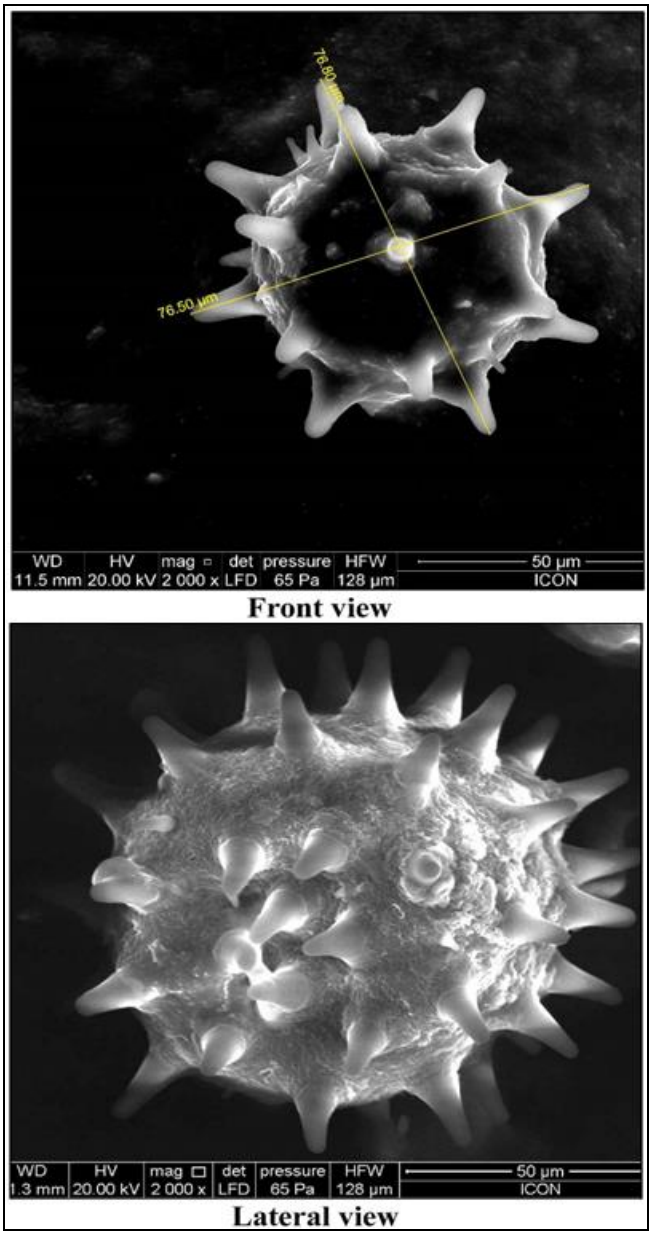


Fig 3: Pollen grain of *Hibiscus rosa-sinensis* L.

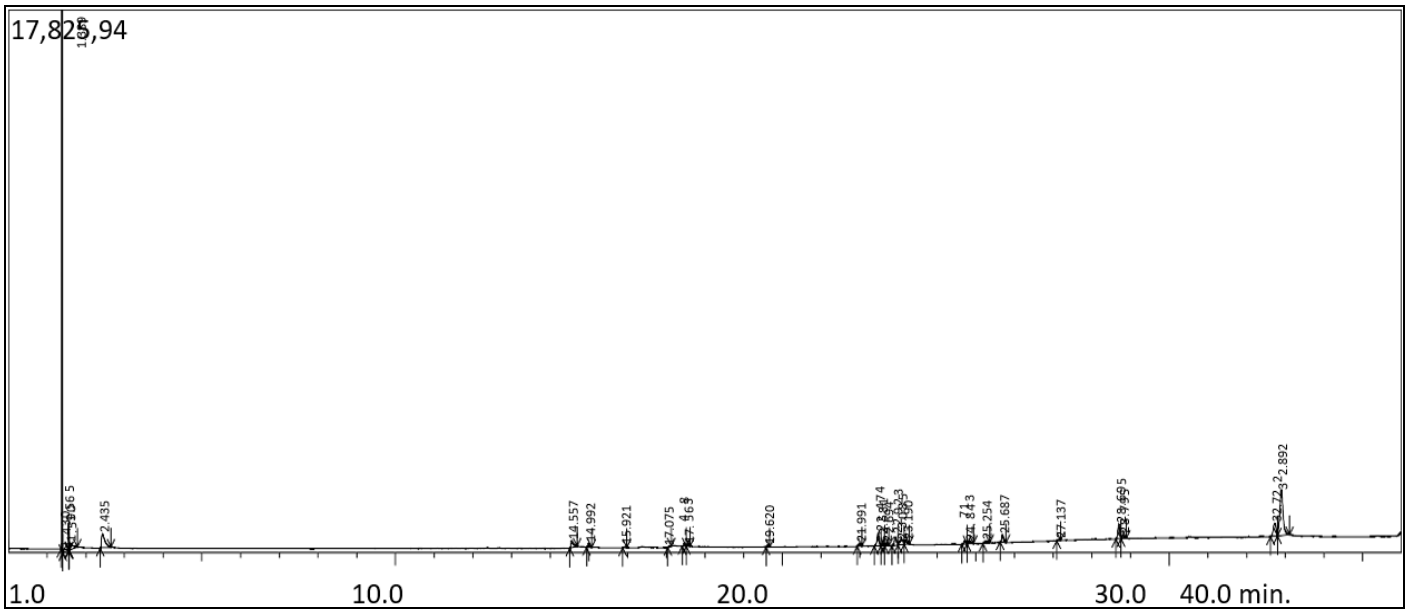
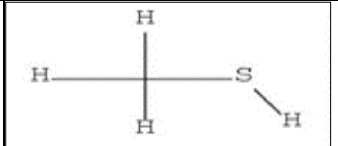
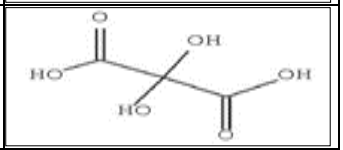
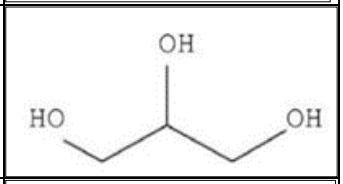
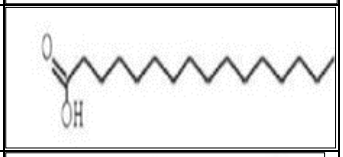
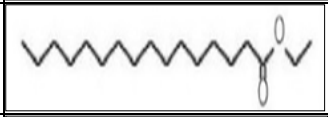
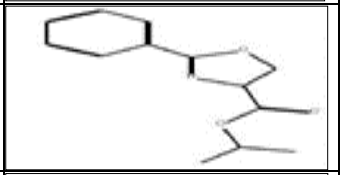
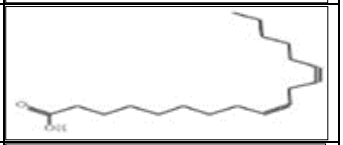
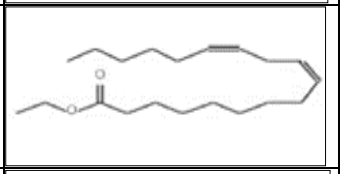
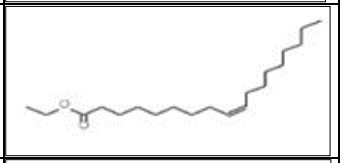
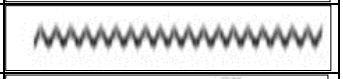
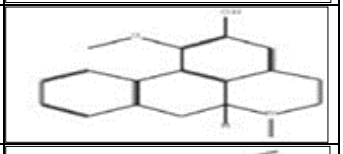
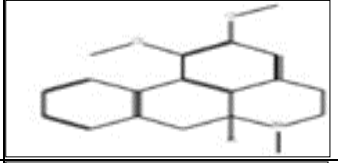
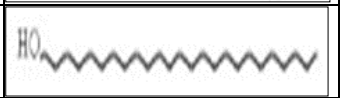
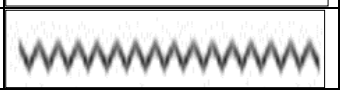


Fig 4: GC-MS Chromatogram of ethanolic extract of the *Hibiscus* flower.

Table 1: Phytocomponents Identification in Ethanolic Extract of *Hibiscus Rosa-Sinensis* L. Flower by GC-MS.

Peak no.	R.T.	Name of Compound	MW	MF	Peak area (%)	Compound Structure
1	1.390	Methanethiol	48	CH_4S	42.8380	
2	1.590	Propanedioic acid, dihydroxy-	136	$C_3H_4O_6$	2.4503	
3	2.435	Glycerin	92	$C_3H_8O_3$	5.6982	
4	14.555	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	1.9935	
5	14.990	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	0.7035	
6	15.920	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	233	$C_{13}H_{15}NO_3$	0.2247	
7	17.075	9,12-Octadecadienoic acid (Z, Z)-	280	$C_{18}H_{32}O_2$	0.0912	
8	17.470	Linoleic acid ethyl ester	308	$C_{20}H_{36}O_2$	0.5742	
9	17.560	Ethyl Oleate	310	$C_{20}H_{38}O_2$	0.4870	
10	19.620	Tetracontane	562	$C_{40}H_{82}$	0.3940	
11	21.990	1-Methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-2-ol 100	281	$C_{18}H_{19}NO_2$	0.7035	
12	22.475	6a-.beta.-Aporphine, 1,2-dimethox	295	$C_{19}H_{21}NO_2$	3.2914	
13	22.580	1-Hexacosanol	382	$C_{26}H_{54}O$	0.3822	
14	22.695	Dotriacontane	450	$C_{32}H_{66}$	0.4557	

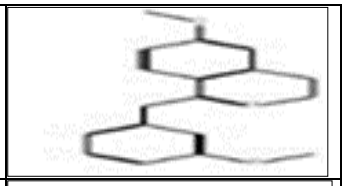
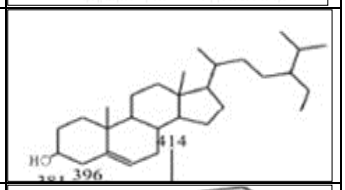
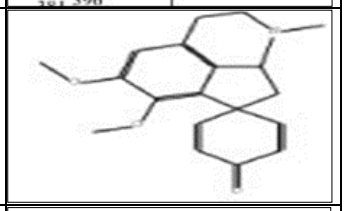
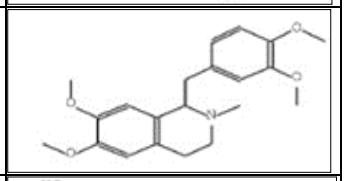
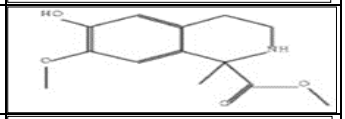
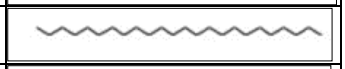
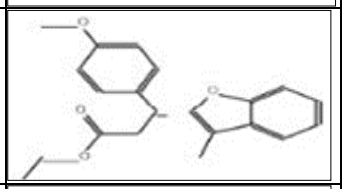
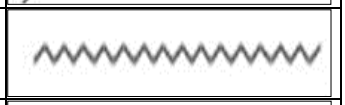
15	22.925	3,4-Dihydroisoquinoline, 1-[3-methoxybenzyl]-6-methoxy-	281	$C_{18}H_{19}NO_2$	2.2994	
16	23.085	beta.-Sitosterol	414	$C_{29}H_{50}O$	2.3488	
17	24.715	Spiro[2,5-cyclohexadiene-1,7'(1'H)-cyclopent[ij]isoquinolin]-4-one, 2',3',8',8'a-tetrahydro-5',6'-dimethoxy-1'-methyl-, (R)-	311	$C_{19}H_{21}NO_3$	0.9148	
18	24.845	dl-Laudanosine	357	$C_{21}H_{27}NO_4$	0.8207	
19	25.255	(-)-1,2,3,s4-Tetrahydroisoquinolin-6-ol-1-carboxylic acid, 7-methoxy-1-methyl-, methyl ester	251	$C_{13}H_{17}NO_4$	0.7976	
20	25.685	Tetracosane	338	$C_{24}H_{50}$	1.5334	
21	27.135	Tetrapentacontane	758	$C_{54}H_{110}$	0.2202	
22	28.695	Hexatriacontane	506	$C_{36}H_{74}$	3.4347	
23	28.795	Ethyl 3-(p-methoxyphenyl)-3-(3-methyl-2-benzofuranyl) propionate	338	$C_{21}H_{22}O_4$	0.8936	
24	32.720	Triacontane	442	$C_{30}H_{62}$	4.5671	
25	32.890	Tetratriacontyl trifluoroacetate	534	$C_{32}H_{61}F_{3O2}$	18.2867	

Table 2: Activity of Phyto-components identified in the ethanolic extracts of *Hibiscus rosa-sinensis* L. flower.

Sr. no.	Compound	Nature of Compound	Biological activity	Literature cited
1	Methanethiol	Organosulfur	Antifungal activity	El-Sayed *et al.*, 2011 [11]
2	Propanedioic acid, dihydroxy-	Dicarboxylic acids	Anti-Helicobacter pylori activity and Anti-Candida activity	Mohammed *et al*., 2016. [21]
3	Glycerin	Polyol compound	Preservative antimicrobial	Arockia, 2012 [3]
4	n-Hexadecanoic acid	Fatty acid	Anti-oxidant, Hypocholesterolemic, Nematicide, Anti-androgenic,	Tyagi, and Agarwal, 2017 [30]
5	Hexadecanoic acid, ethyl ester	Organic compound	Antioxidant, flavour, nematicide, pesticide,	Parthipan *et al*., 2015 [22]
5	9,12-Octadecadienoic acid (Z, Z)-	Polyunsatur-ated Fatty acid	Anti-inflammatory, hypocholesterolemic, cancer preventive,	Sudha *et al*., 2013 [27]
6	Linoleic acid ethyl ester	Polyunsatur-ated fatty acid	Cancer preventive Nematicide, Insectifuge, Antiarthritic,	Vadivel and Gopalakrishnan, 2011 [32]
7	Ethyl Oleate	Fatty acid ethyl ester	It is used for vehicle for intramuscular drug delivery,	Elaiyaraja, and Chandramohan, 2016 [10]

			Progesterone	
8	Tetracontane	Alkane	Antioxidant and antimicrobial activities	Swamy *et al*, 2017 [29]
9	1-Hexacosanol	Fatty alcohol	Lowering LDL-C	Jones and Jew 2016 [17]
10	Dotriacontane	Alkane	Antioxidant; antibacterial; antifungal	Asong *et al*., 2019 [4]
11	3,4-Dihydroisoquinoline, 1-[3-methoxybenzyl]-6-methoxy-	Alkaloid	Antimicrobial, Anti-inflammatory	Varadharajan *et al*., 2016 [33]
12	beta.-Sitosterol	Phytosterols	Antioxidant and antimicrobial	Hidayathulla *et al*., 2018 [13]
13	Spiro[2,5-cyclohexadiene-1,7'(1'H)-cyclopent[ij]isoquinolin]-4-one, 2',3',8',8'a-tetrahydro-5',6'-dimethoxy-1'-methyl-, (R)-	Alkaloid	Antimicrobial Anti-inflammatory	Varadharajan *et al*., 2016 [33]
14	dl-Laudanosine	Alkaloid	Neuromuscular-blocking drugs	Sayer *et al*., 2004 [25]
15	(-)-1,2,3,4-Tetrahydroisoquinolin-6-ol-1-carboxylic acid, 7-methoxy-1-methyl-, methyl ester	Alkaloid	Antimicrobial Anti-inflammatory	Varadharajan *et al*., 2016 [33]
16	Tetracosane	Alkane	Antioxiodant and Antibacterial	Huihua *et al*., 2013 [14]
17	Tetrapentacontane	Alkane	Antioxidant and Antimicrobial	Swamy, 2017 [29]
18	Hexatriacontane	Alkane	Radical scavenger	Bobbarala *et al*., 2011 [6]
19	Ethyl 3-(p-methoxyphenyl)-3-(3-methyl-2-benzofuranyl)propionate	Alkane	Insecticidal Effects	Al-Harbi *et al*., 2021 [1]
20	Triacontane	Alkane	Antibacterial, antidiabetic and antitumor activities	Amudha *et al*., 2018 [2]
21	Tetratriacontyl trifluoroacetate	Alkane	Antioxidant and Antimicrobial Properties	Sudrajat *et al*., 2016 [28]

Summary and Conclusion

Microscopy- The microscopical studies of Maceration and powder showed presence of abundant lignified, thick walled, Labriform type of fibres with pointed tips, Tracheids & vessel elements with oblique perforations plates having short, pointed tails, and parenchyma cells, which are distinguishing microscopic features.

Pollen grains of *Hibiscus rosa-sinensis* L. were found to be Pantoporate, spheroidal in shape. The Echinate, spines with bulbous or swollen apex, spaced, no basal cushion, central spines that do not differ too much making them monomorphic.

GC-MS analysis showed the various twenty-one type of bioactive compounds from the ethanol extract of *Hibiscus rosa-sinensis* L. flower were identified by Gas chromatography with Mass spectrometry (GC-MS) analysis. These studies have added scientific support to the plant's traditional medicinal uses.

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