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PRODUCTION AND CHARACTERIZATION OF PHYTOCOMPOUNDS AND PIGMENTS USING *PENICILLIUM PURPUROGENUM*

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ABSTRACT: In this study, the flower extract of various species was fermented using *Penicillium purpurogenum* strain, and characterized using analytical techniques like UV-Vis, FTIR, Proton-NMR spectroscopy and GC-MS. The phytochemical content of the soured extract of the flowers was investigated by GC-MS, and therefore the spectra of the compounds found within the extract were compared with the standard library. GC/MS analysis of the fermented extract of flowers unconcealed the existence of various compounds {1,3-Butanediol (66.89%), 2,3-Butanediol (6.64%), 1,2-Benzene dicarboxylic acid (6.36%), Butyric acid (2.46), and 1,2-Benzenediol (1.27%)} *Hibiscus rosa sinensis*, {1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid (51.63%), Sorbitol (19.06%), Acetic acid (7.51%)} *Ixora coccinea* L, {Silanediol (7.29%), Cyclohexylmethyl heptadecyl ester (3.41%), Hexadecanoic acid (2.85%), Cholesterol (2.54%), Di-n-octyl phthalate (2.22%)} *Gomphrena.globosa*, {Proponoic Acid (22.75%), 1,3,4,5-Tetra hydroxy-Cyclo hexane carboxylic Acid (13.55%), Mome Inositol (23.30%), Acetic acid (7.53%)} *Nerium.oleander* L. The phytochemicals obtained in this study offer a platform for studying bio-active compounds and further leads to the identification of microbial pigments as the alternative for the current synthetic dyes.

KEYWORDS: Ultraviolet spectroscopy, Antimicrobial agents, Synthetic dyes, NIST library.

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1.INTRODUCTION

Hibiscus, a prominent member of this Malvaceae family, it is a herbaceous plant. *Hibiscus* rosa-sinensis, perineal shrub, a tiny tree growing about 5 m height and 3 m wide, with shiny leaves and flowers in all seasons. This flower has been reportable to contain flavonoids, carbohydrates, proteins, and minerals[1]. The flowers have medicinal drug, hepatoprotective, anticancer, toxicity, bactericide, anti-inflammatory and inhibitory activities[2]. *Ixora.coccinea* Linn. (Rubiaceae) is additionally referred to as Jungle herb or Vetchi in writing. It's a natural shrub native to Asia. The plant was a dense, branched shrub, commonly grows four inches height. Leaves are sessile to short-petiolate, glossy, leathery, rectangular and are regarding ten cm long, with long margins and are organized in opposite pairs or whorled on the stem, stipules basally sheathing, Flowers small, sessile, tubular and are arranged in dense rounded clusters, calyx lobes short, triangular, persistent,corolla tube about 2 inches long. The plant is historically used as hepatoprotective, antimicrobial, as an antioxidant, and anti-incendiary activities[3]. This plant consists of phytochemicals such as ursolic acid, lupeol, anthocyanins, leucocyanidin, rutin, sitosterol, oleanolic acid, proanthocyanidins, and kaempferol and quercetin are the two types of glycosides[4]. *Gomphrena.globosa* Linn. is commonly called as globe amaranth, and vadamalli. It is a nutritious plant of the family Amaranthaceae. The globular flower has shades of magenta, purple, red, etc. It is a perennial grows all the year. It is not at all rustic and frost. The plant has an upright and bushy, well-branched with evergreen leaves, ovate, fifteen cm long, finely pubescent when young and less hairy as it ages. In history, the globe amaranth is used to relieve prostate and reproductive problems as a Caribbean folk medicine[5]. *Nerium. oleander*, a florid plant of Apocynaceae family, is generally appropriated in tropical and sub-tropical areas. The oleander also causes skin irritations, inflammation to eyes, and allergies to dermatitis[6]. Each the two (water and lipid) extract preparation obtained, was used as people medication within the treatment of assorted conditions like abscesses, corns, asthma, pain, allergy, epilepsy, epitheliomas, herpes, malaria, psoriasis, ringworm, scabies, sores, warts, and tumors[7]. Oleandrin, a cardiac glucoside obtained from this plant, used for the treatment of heart symptom in China for years[8]. A one of a kind, exclusive boiling water concentrate of oleander, Anvirzel TM has been utilized in Europe and the United States with an administrative endorsement on a constrained, caring premise to treat little quantities of patients with harmful infection. A Phase I preliminary of Anvirzel TM has additionally as of late been started in the US. Numerous ascomycetous organisms normally blend and discharge characteristic colorants with enhanced functionalities[9]. The decent variety of parasitic colorants isn't just found in their concoction structures, yet in addition in the shading scope of these compounds[10]. As the greater part of the examinations found inside the writing in regards to vegetation, colorants were performed with Monascus species and there's an outsized scope of elective parasites to be investigated, it's important to go searching for different colorant-delivering living beings. It has as of late been accounted for in the literature[11] that *Penicillium* strains are

potential makers of regular colorants, which have chromophore comparable to *Monascus* colorants. Among *Penicillium* species, *Penicillium.purpurogenum* can create colorants in a strong medium as well as in fluid media[12]. In studies performed by our exploration group[13], *Penicillium. purpurogenum* showed potential to create common colorants with critical antimicrobial exercises and aggregate nonattendance of poisonous quality against Artemia salina. The development and pigmentation of microorganism are extraordinarily influenced by two kinds of fermentation i.e. Solid state fermentation and submerged fermentation. The simple production and isolation of pigments have to lead to progressions in fermentation models. For the most part, submerged fermentation (SmF) is utilized for large scale pigment production. The solid-state fermentation (SSF) frameworks give off an impression of being promising because of the common potential and points of interest [14]. Microbial colors can be produced either by solid substrate fermentation or by submerged fermentation. In solid substrate fermentation (SSF), the microbial color biomass present on the surface of a solid substrate for the development of microbial coloring [15, 16]. This SSF method has numerous potential preferences incorporating funds in wastewater and higher yield of the metabolites. Then again, microorganisms are developed in fluid medium vigorously with legitimate unsettling to get homogenous development of cells and media segments in submerged fermentation system [17]. In addition, scientists examined the impact of different process parameters, for example, carbon source, nitrogen source, temperature, pH, air circulation rate for color production [18]. Because of the surprising expense of utilizing engineered medium, there is a need to grow new minimal effort process and extraction strategy for the production of shades. Endeavors are on to use the agro-modern waste for extensive scale production of microbial colors. A few examinations have concentrated on a generation of carotenoids from agro-modern waste, for example, whey, apple pomace, spent grain and squashed pasta and so forth [19]. Accordingly, such sort of agro-modern waste usage methodology drop down the generation cost as well as go about as viable waste administration device too. The generation of microbial colors depends upon the kind of microorganism and temperature is the important factor for microbial pigment production. Monascus desires 25 to 28°C temperature for the development and production of microbial shade though *Pseudomonas* sp. requires 35 to 36°C for its development and color generation [20]. The pH is likewise another critical factor for microbial color production. The pH of the medium is influenced by the development and kind of color produced in which the microorganism is developed. The marginal change in pH may change the shade of microbial color and it fluctuates starting with one microorganism then onto the next. The ideal pH for *Monascus* sp. is 5.5 to 6.5 and for *Rhodotorula* is 4.0 to 4.5 individually. The pH favors lycopene development from impartial to somewhat antacid while β-carotene arrangement shapes from unbiased to acidic [21]. The distinctive incubating time frames running from 24 to 96 hrs likewise impacts the development and pigmentation of the microorganism. While contemplating the parameters one

factor at that moment was considered while the other stayed steady for the yield of biomass and pigmentation. The incubating time frame 24-96 hrs for the improvement of Micrococcus which demonstrated the most astounding biomass production was chosen for every microorganism [22]. The color produced through mycelial development of microorganism is influenced by the sort of carbon sources like glucose, fructose, lactose, maltose, sucrose, galactose and so on. The carbon sources such as glucose and their oligosaccharides are the best for the development and pigment production. The volumetric color development by the Monascus is the best on starch and dextrin though moderate on glucose and maltose however poor on fructose. The cellobiose frames high pigmentation for Phaffia rohodozyma though glucose advanced both development and pigmentation. The shade of the color is additionally affected by the kind of sugar utilized for production of microbial shades [21].

2. MATERIALS AND METHODS

The *Penicillium purpurogenum* - was purchased from NCI Pune, and the stock culture was maintained on a Potato Dextrose Agar (PDA) slants. The flowers were collected in fresh polythene bags from Kakinada, East Godavari district, A.P.

Chemicals:- PDB (2%), $MgSO_4$ (1%), $MnSO_4$(1%), K_2HPO_4 (1%) and KH_2PO_4 (1%) and Urea(0.5%) with pH 5.5

Production of microbial pigments from various flowers

The leaves were at first washed in faucet water, at that point with distilled water to evacuate soil and different contaminants. They were weighed and ground into a paste and as the carbon source.

Fermentation

A loopful of well sporulated culture of the *Penicillium.purpurogenum* was inoculated into a relating 250 mL Erlenmeyer jar contains 100 mL of production medium composed of PDB (2%), $MgSO_4$ (1%), $MnSO_4$(1%), K_2HPO_4 (1%) and KH_2PO_4 (1%) and Urea(.5%) with pH 5.5. The inoculated jar was kept for incubation about 7-10 days in a rotating shaker (200 rpm) at 25ºC.

Pigment extraction

After incubation, the broth obtained was taken and heated on a heating mantle at 70 degrees Celsius for 2 hours. After heating, the broth was filtered, separating the biomass and the filtrate. The parameter pH of the colored filtrate was checked. The solution obtained was evaporated and concentrated at 70º Celsius. The water molecules were slowly removed on evaporation leaving the solid concentrate. The concentrate was cooled immediately. The crude extract acquired was allowed for crystallization to form crystals of the pigment. The pigment obtained was purified and weighed.

UV-Vis Spectroscopy (UV-Vis)

The maximum absorbance of extracted and dried red pigment powder was determined by spectrophotometer (SPECORD 210-222K333 UV-Vis) at 500 nm wavelength[23].

FTIR spectroscopy

The FTIR spectrum was documented on a Bruker FT-IR spectrophotometer and the unearthly range was 4000 – 500 cm-1[24]. The dried powder of the red shade was filtered by a Shimadzu spectrophotometer FT-IR 8000 in the range level 4000–400 cm-1 utilizing the KBr method at 27 °C.

NMR Spectroscopy

The purified pigment was dissolved with dimethylsulfoxide(DMSO d6) and the sample was injected into a nuclear magnetic resonance spectrometer (Bruker 400 MHz)[25].

GC-Mass Spectrometry

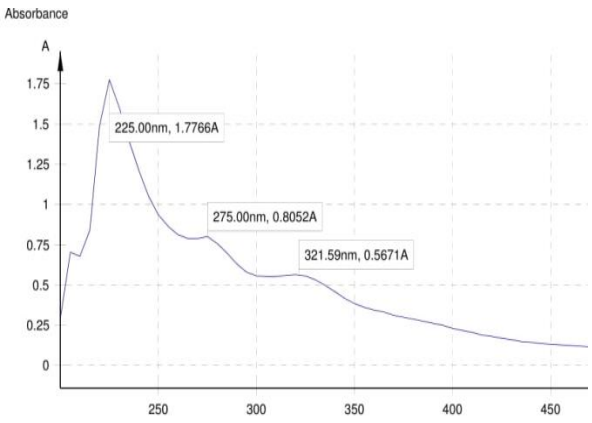
In the current study, the microbial extract of flowers was screened for the existence of phytochemical compounds by qualitative test procedures followed by GC-MS assay of novel compounds. This examination was encouraged by utilizing GCMS Spectrometry. The mass spectra of compounds in the microbial filtrate were coordinated with NIST and Wiley library.

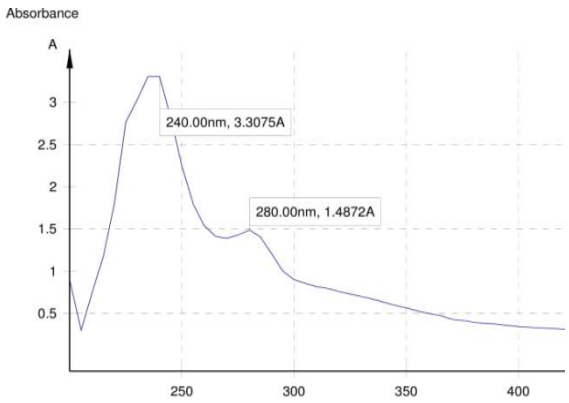
RESULTS AND DISCUSSION

The ideal parameters for extreme production of the colored pigment were observed to be pH 5.5, 37ºC and 8 days respectively. The comparative study of pigments was characterized using different techniques like UV Visible Spectroscopy, FTIR and 1H NMR and GC-MS techniques.

Table No 1: UV-Visible Absorbance of microbial pigments

S.no	Scientific Name of the Flower	UV-Vis Absorbance (nm)	The concentration at Maximum (A)	Yield gms/L	Yield %
1	*Hibiscus.rosa sinensis*	225 nm	1.77	24	2.5
2	*Ixora.coccinea*	240 nm	3.30	11.8	1.05
3	*Gomphrena.globosa*	225 nm	1.07	16.8	4.8
4	*Nerium.oleander*	229 nm	2.68	12.8	1.28





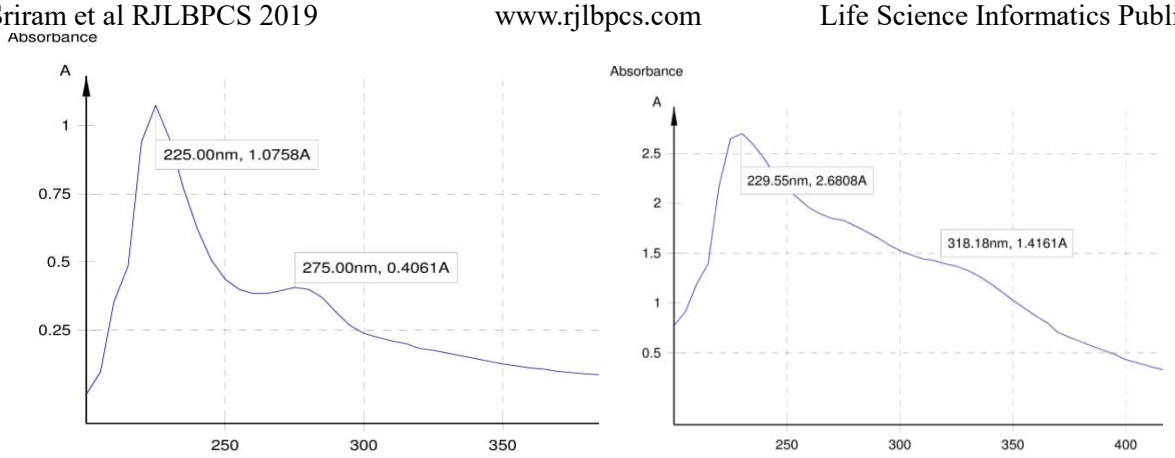


Figure No 1:- UV-Visible Spectrum of pigments

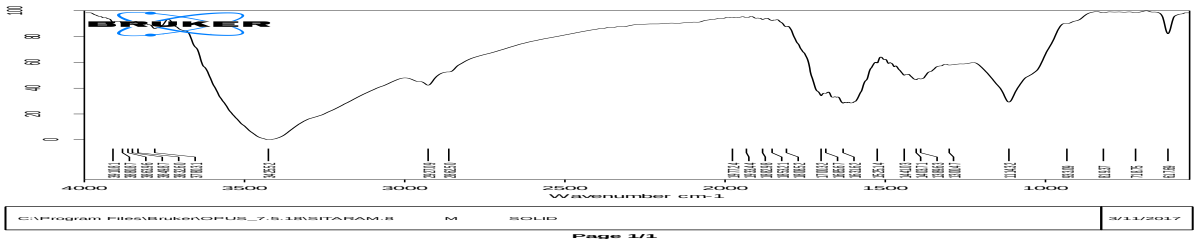


Figure No 2: The FTIR data show the presence of following vibration and functional groups for *Hibiscus rosa-sinensis* :

FTIR peak value and specific type of bond of pigment extracted from *Hibiscus rosa-sinensis*

Absorption Peak value	**Absorption range**	**Specific type of bond**
3425.52	3500-3300	1^{O} amines (doublet), 2^{O} amines N-H stretch
2927.09	3000-2830	Alkanes C─H stretch
2862.50	3000-2830	Alkanes C─H stretch
1631.62	1640-1550	Amides, 1O and 2O amines N-H stretch
1403.71	1450-1375	Alkane CH3 bend
1114.32	1300-1000	Alcohols, esters, ethers, -COOH, Anhydrides C-O stretch
617.69	800-600	Chloride C-Cl stretch

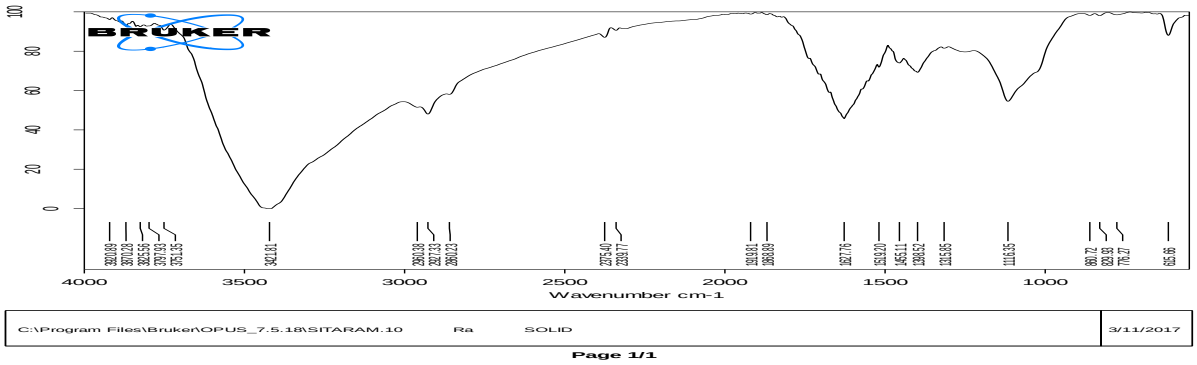


Figure No 3: The FTIR data show the presence of following vibration and functional groups for *Ixora coccinea* :

FTIR peak value and specific type of bond of pigment extracted from *Ixora coccinea*

Absorption Peak value	**Absorption range**	**Specific type of bond**
3421.81	3500-3300	1^{O} amines (doublet), 2^{O} amines N-H stretch
2960.38	3000-2830	Alkanes C─H stretch
2927.33	3000-2830	Alkanes C─H stretch
2860.23	2900–2700	Aldehydes C─H stretch
1627.76	1640-1550	Amides, 1O and 2O amines N-H stretch
1398.52	1400–1000	Fluoride C- F stretch
1116.35	1300-1000	Alcohols, esters, ethers, -COOH, Anhydrides C-O stretch
615.66	800-600	Chloride C-Cl stretch

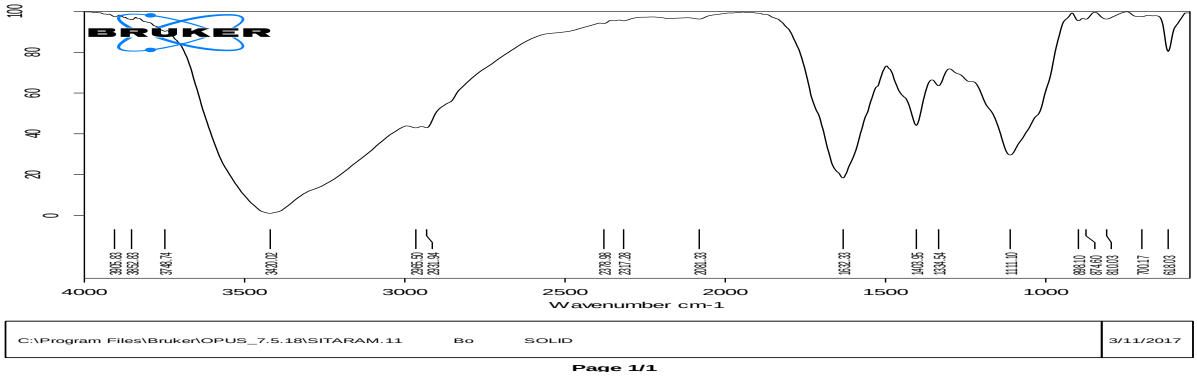


Figure No 4: The FTIR data show the presence of following vibration and functional groups for *Gomphrena.globosa* :

FTIR peak value and specific type of bond of pigment extracted from *Gomphrena.globosa*

Absorption Peak value	**Absorption range**	**Specific type of bond**
3420.02	3500-3300	1^{O} amines (doublet), 2^{O} amines N-H stretch
2965.50	3000-2830	Alkanes C─H stretch
2931.94	3000-2830	Alkanes C─H stretch
1632.33	1640-1550	Amides, 1O and 2O amines N-H stretch
1403.95	1450-1375	Alkane CH3 bend
1334.54	1375-1300	Sulfones, sulfonyl chlorides, sulfates, sulfonamides S=O stretch
1111.10	1300-1000	Alcohols, esters, ethers, -COOH, Anhydrides C-O stretch
618.03	800-600	Chloride C-Cl stretch

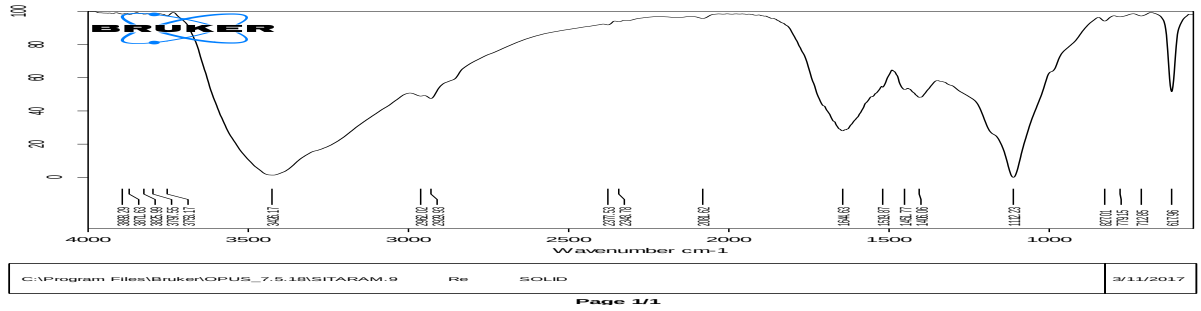
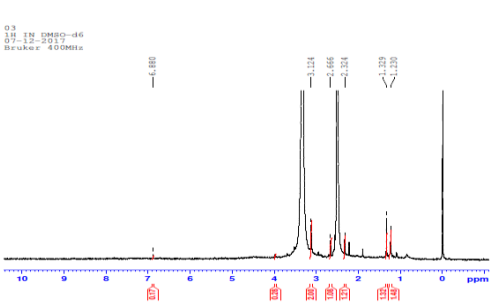


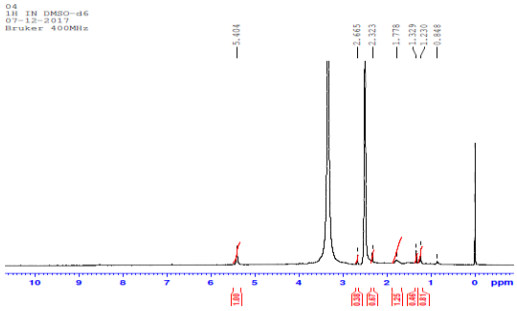
Figure No 5: The FTIR data show the presence of following vibration and functional groups for *Nerium.odoratum*

FTIR peak value and specific type of bond of pigment extracted from *Nerium.odorantum*

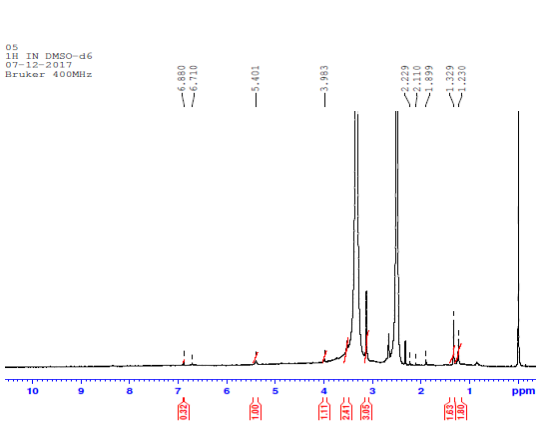
Absorption Peak value	**Absorption range**	**Specific type of bond**
3426.17	3500-3300	1^{O} amines (doublet), 2^{O} amines N-H stretch
2962.02	3000-2830	Alkanes C─H stretch
2929.93	3000-2830	Alkanes C─H stretch
1644.63	1670-1640	AmidesC=O stretch (Amide II band)
1406.06	1450-1375	Alkane CH3 bend
1112.23	1300-1000	Alcohols, esters, ethers, -COOH, Anhydrides C-O stretch
617.96	800-600	Chloride C-Cl stretch



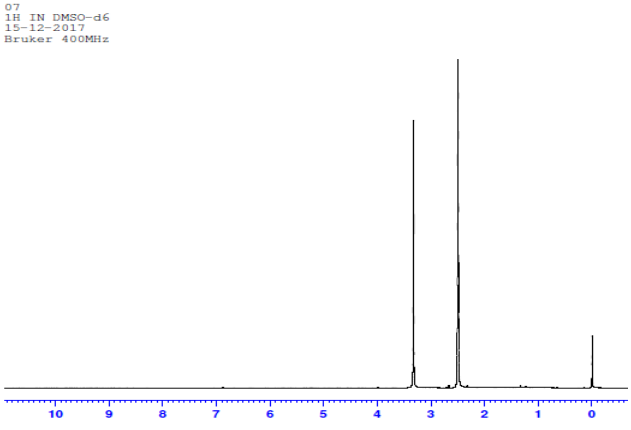
Hibiscus



Ixora



Gomphrena



Nerium

Figure No 6: – H1-NMR Spectrum of pigments

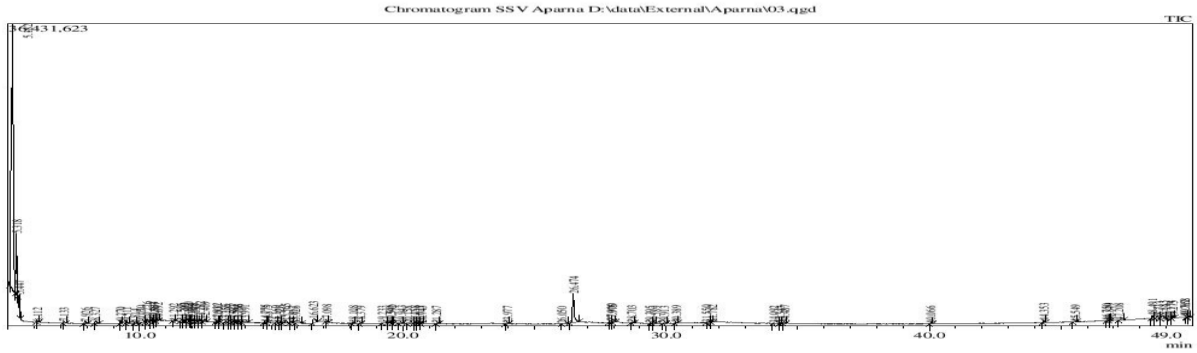
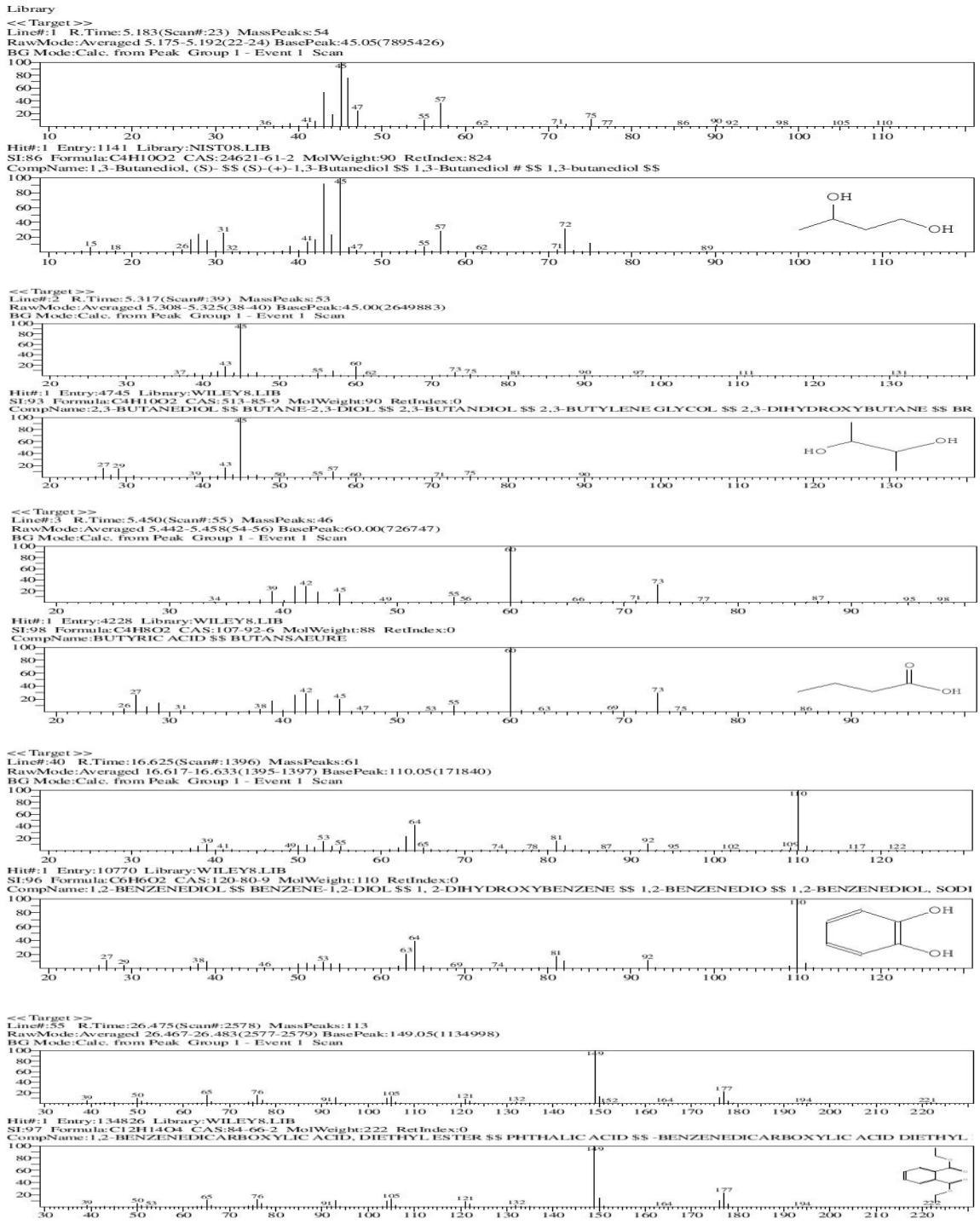


Figure 7: Chromatogram of *Hibiscus*

Phytochemicals structures matched with NIST library of *Hibiscus rosa sinensis* are



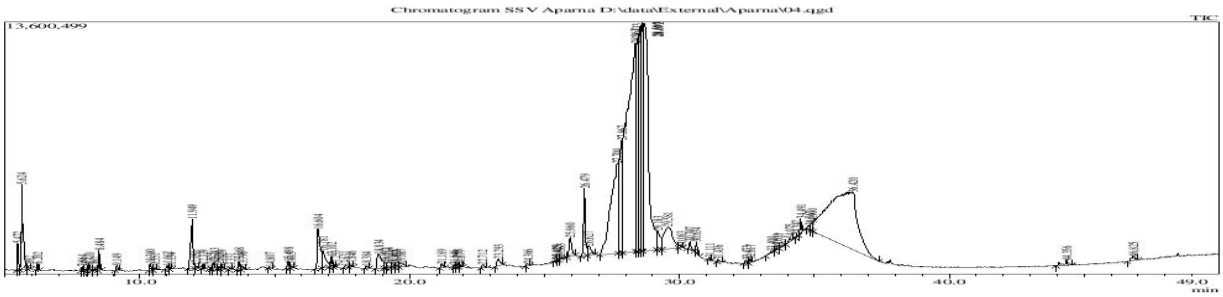
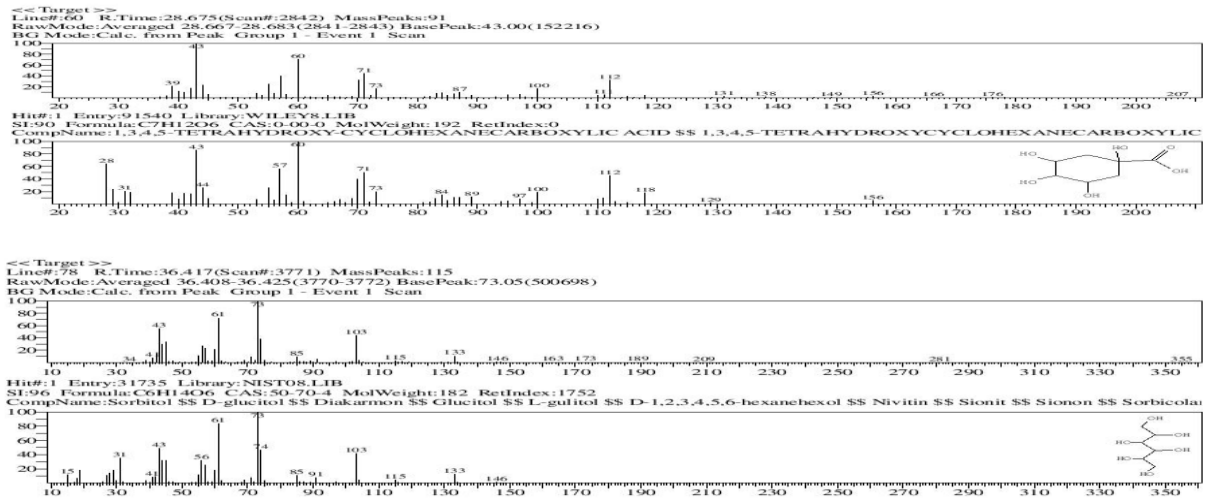
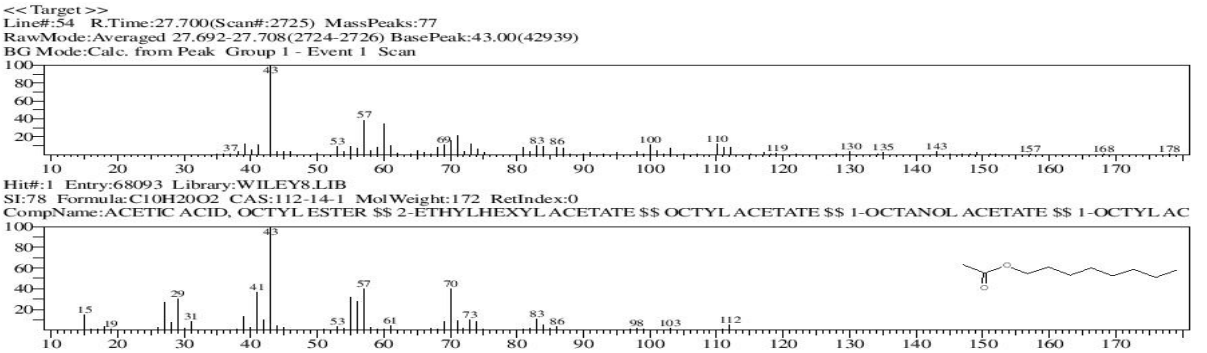


Figure 8: Chromatogram of *Ixora*

Phytochemicals structures matched with NIST library of *Ixora.coccinea* are





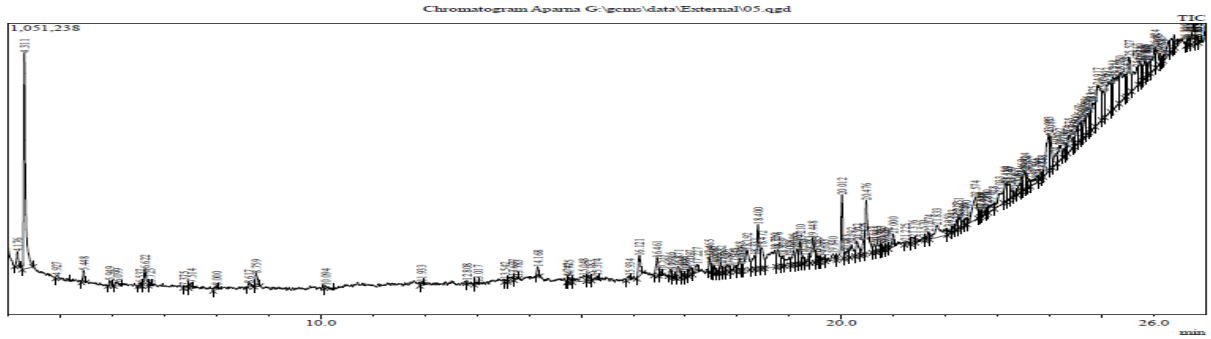
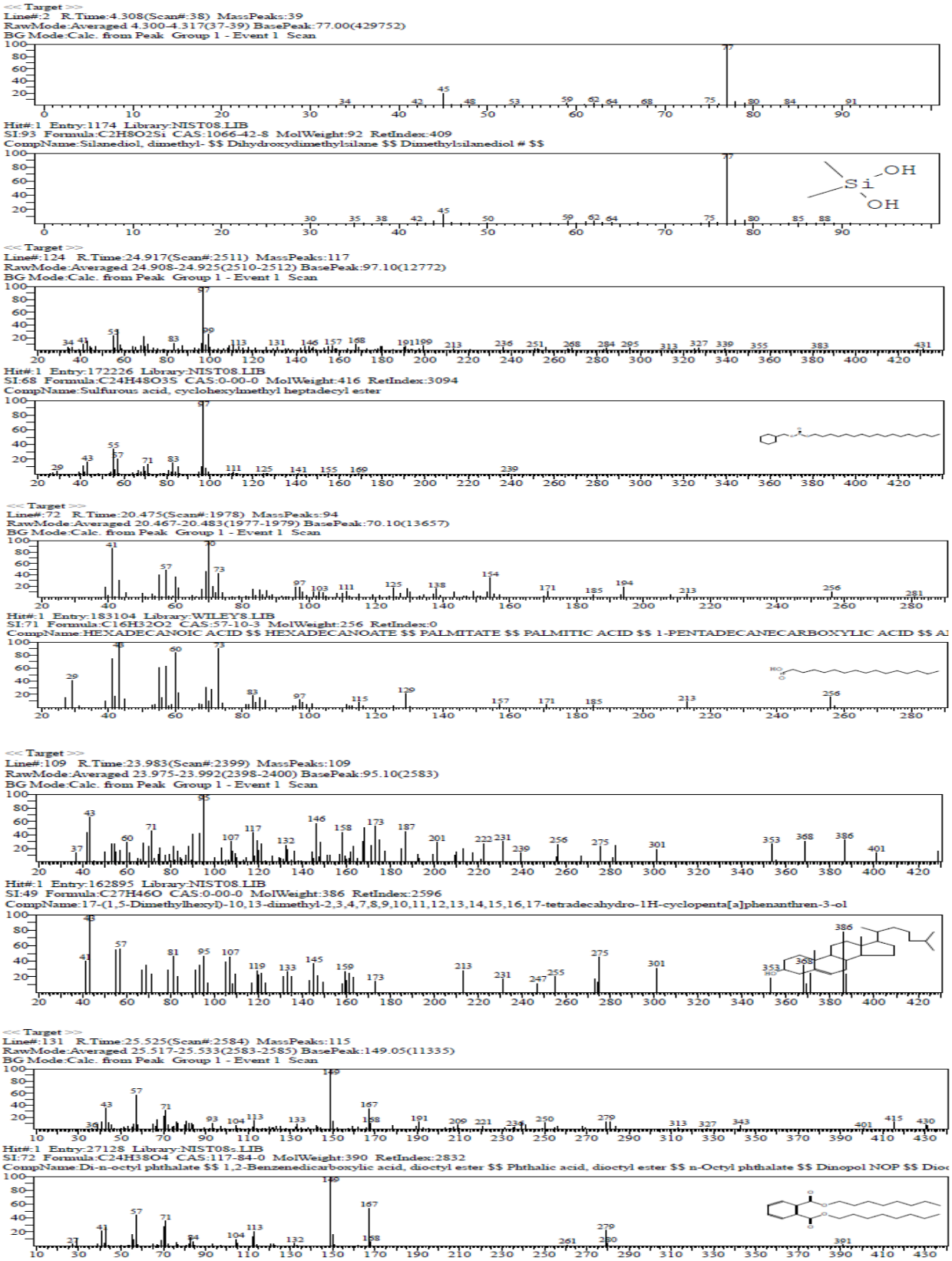


Figure 9: Chromatogram of *Gomphrena*

Phytochemicals structures matched with NIST library of *Gomphrena.globosa* are



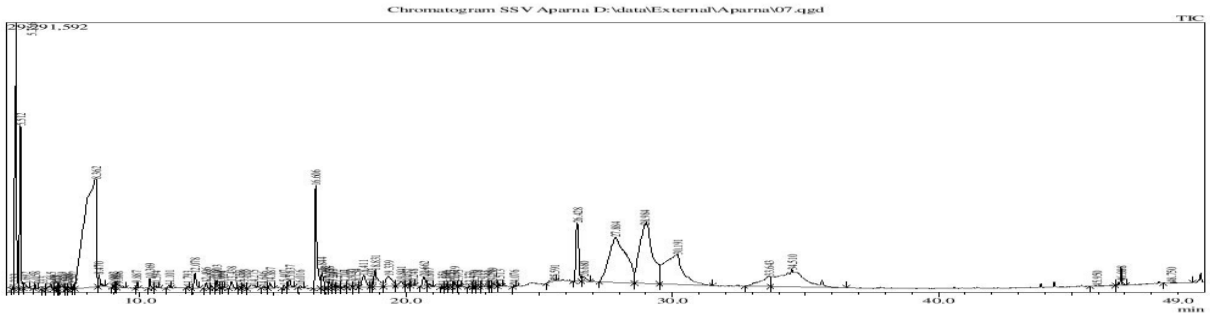


Figure 10: Chromatogram of *Nerium*

Phytochemicals structures matched with NIST library of *Nerium.oleander* is

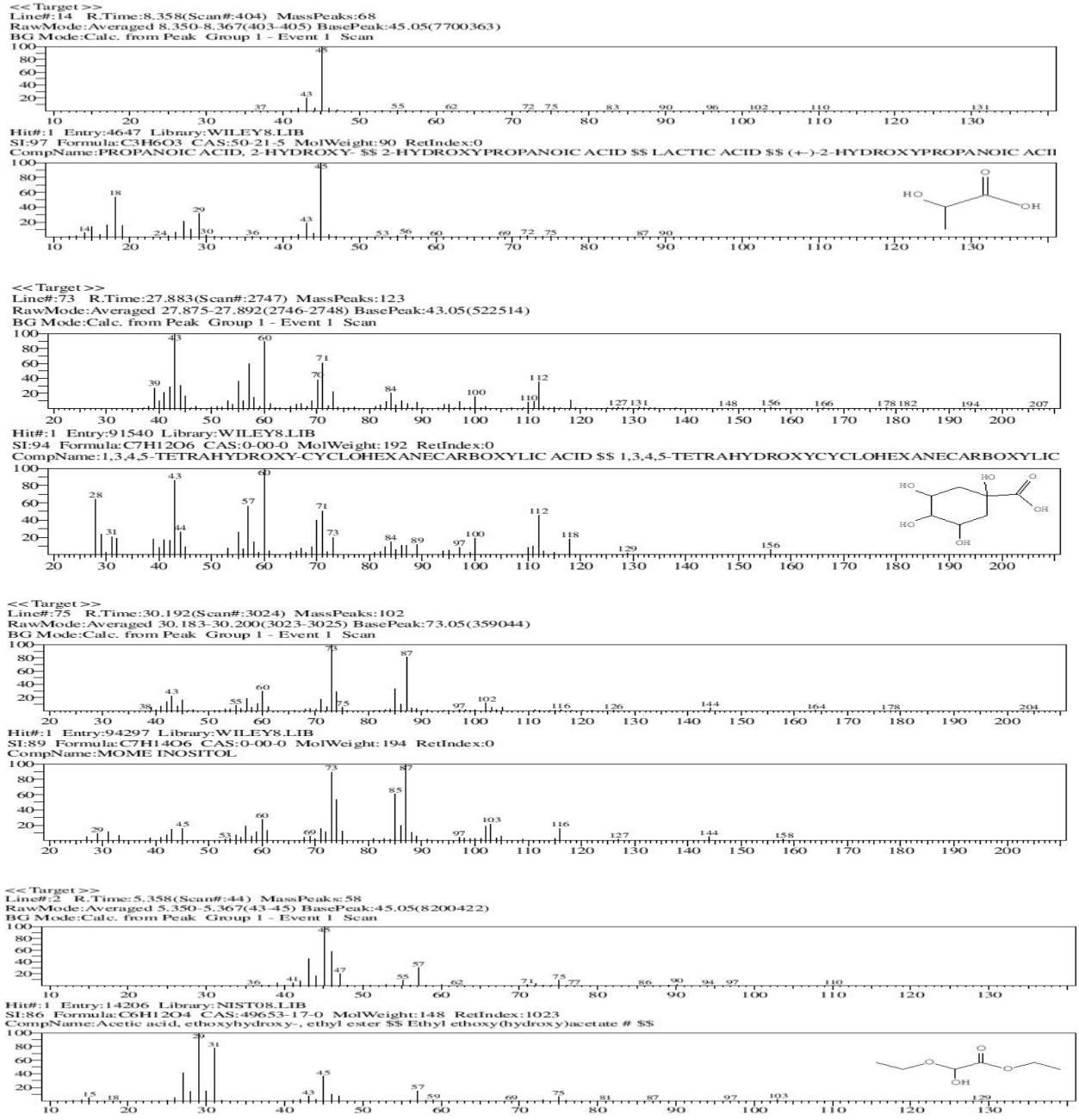


Table No 2: Nature of the compound and pharmacological purpose of compounds

S.no	RT(min)	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %	Pharmacological purpose
1	5.182	1,3-Butanediol	C4H10O2	90	66.89	Flavoring agent
2	5.318	2,3-Butanediol	C4H10O2	90	6.64	A Glycol And A Secondary Alcohol.
3	5.447	Butyric Acid	C4H8O2	88	2.46	Flavoring agent, Fragrances
4	16.623	1,2-Benzenediol	C6H6O2	110	1.27	Enhancing agent, Catechol, photographic developer, antioxidants in rubber and lubricating oils,
5	26.474	1,2-Benzenedicarboxylic Acid	C12H14O4	222	6.36	Flavoring agent, Metabolites
6	27.867 28.367 28.560 28.617 28.672	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid	C7H12O6	192	51.63	Quinic acid
7	33.717 34.900 36.420	Sorbitol	C6H14O6	182	19.06	Glucitol, a sugar alcohol with a sweet taste
8	27.700	Acetic Acid	C10H20O2	172	7.51	Flavoring agents, Indicators
9	**4.311**	**Silanediol**	**C2H8O2Si**	**92**	**7.29**	Hydrogen Bond Donor
10	**24.917**	**Cyclohexylmethyl heptadecyl ester**	**C24H48O3S**	**416**	**3.41**	**No activity reported**
11	**20.476**	**Hexadecanoic acid**	**C16H32O2**	**256**	**2.85**	**Flavoring agents, Enzyme Indicators**
12	**23.983**	**Cholesterol**	**C27H46O**	**386**	**2.54**	**Lipid Molecule**
13	**25.527**	**Di-n-octyl phthalate**	**C24H38O4**	**390**	**2.22**	**Flavoring agents, Plasticizer**

14	8.362	Propanoic Acid	C3H6O3	90	22.75	Flavoring agents, Fungicide, Bactericide
15	27.884	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid	C7H12O6	196	13.55	Quinic Acid
16	28.984 30.191	Mome Inositol	C7H14O6	194	23.30	Food Additive, natural sugar
17	5.356	Acetic acid	C6H12O4	148	7.53	Flavoring agents, Indicators

The present investigation of the bio-active compounds from plants and their activity has expanded. Gas Chromatography-Mass Spectrometry (GC-MS) is a valuable tool for reliable identification of bioactive compounds[26]. In the present study, 150 compounds have been identified from the microbial extract of each type of flowers by GC - MS analysis. The most abundant 17 components found in all the flowers were {1,3-Butanediol (66.89%), 2,3-Butanediol (6.64%), 1,2-Benzene dicarboxylic acid (6.36%), Butyric acid (2.46), and 1,2-Benzenediol (1.27%)} ***Hibiscus rosa sinensis***, {1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid (51.63%), Sorbitol (19.06%), Acetic acid (7.51%)} ***Ixora coccinea*** **L**, {Silanediol (7.29%), Cyclohexylmethyl heptadecyl ester (3.41%), Hexadecanoic acid (2.85%), Cholesterol (2.54%), Di-n-octyl phthalate (2.22%)} ***Gomphrena.globosa***, {Proponoic Acid (22.75%), 1,3,4,5-Tetra hydroxy-Cyclo hexane carboxylic Acid (13.55%), Mome Inositol (23.30%), Acetic acid (7.53%)} ***Nerium.oleander*** **L.** Although, many colored pigments are also identified.

4.CONCLUSION

The most significant outcome of this study was the production of microbial pigment from *P.purpurogenum* under various nutritional conditions. It could be seen that *P.purpurogenum* responded by producing high concentrations of pigment from flowers. The results of the optimization, spectroscopic characterization indicates that the isolated pigments having different phytochemicals, which can also be used as food additives or flavoring agents and indicators. To the best of our knowledge, this is the first study to report bioactive compounds produced using *P.purpurogenum* from various flowers.

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