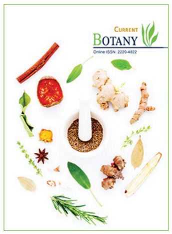
Current Botany 2025, 16: 286-291
doi: 10.25081/cb.2025.v16.9577
https://updatepublishing.com/journal/index.php/cb

Research Article



ISSN: 2220-4822

Seasonal variation and ecological significance of essential oil composition in *Gymnanthemum amygdalinum* (Delile) Sch. Bip. Ex Walp: A chemical and functional perspective

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Received: May 06, 2025
Revised: September 18, 2025
Accepted: October 08, 2025
Published: December 01, 2025

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ABSTRACT

The chemical makeup of essential oils extracted from Gymnanthemum amygdalinum stems varies seasonally, according to this study. Essential oils, noted for their ecological and economic applications, have dynamic chemical profiles that are impacted by ambient variables. Gas chromatography (GC) and mass spectrometry (GC-MS) were used to identify 33 chemicals, which included monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes. Winter has the highest overall essential oil content (92.56%), with spring having the lowest (76.41%). Monoterpenes and oxygenated monoterpenes peaked during winter, implying enhanced enzymatic activity at colder temperatures. Sesquiterpenes were most plentiful in winter and rainy seasons, whereas oxygenated sesquiterpenes peaked during the rainy season. Key chemicals including limonene, methyl eugenol, and linalool were consistently detected. These findings highlight the importance of environmental conditions including temperature, humidity, and light in the biosynthesis and accumulation of volatile oils. This seasonal variability has implications for optimal harvesting and commercial use.

KEYWORDS: *Gymnanthemum amygdalinum*, Essential oils, Terpenes, Monoterpenes, Oxygenated monoterpenes, Sesquiterpene and Oxygenated sesquiterpene

INTRODUCTION

The historical description of essential oil manufacturing in Europe demonstrates the enduring importance of plant-based aromatic compositions over time. Essential oil extraction dates back to the 13th century, particularly in Spain and France, where advances in distillation technique created the framework for a thriving aromatic industry. The early emphasis on herbs such as sage and rosemary demonstrates that medicinal and culinary plants were among the first to be prized for their volatile oils (Baser *et al.*, 2000). Over time, the industry grew in size and sophistication, with France becoming especially well-known for its spike lavender oil, which it began exporting widely. This shift marked the beginning of essential oils as global trade commodities. What is noteworthy is how the field increasingly attracted scientists, chemists, and medics who wanted to understand the chemical structures and physical properties of these aromatic molecules (Sacchettini & Poulter, 1997; Dewick, 2002). The complex composition of these oils has been extensively investigated using procedures like gas chromatography and mass spectrometry (Anitescu *et al.*, 1997), resulting in the identification of compounds responsible for specific medicinal or sensory effects. Today, the essential oil industry is a multibillion-dollar global market backed by numerous companies and marketplaces. Essential oils are widely used in pharmaceuticals, food flavourings, and wellness products (Newman & Cragg, 2020). Their ability to recombine specific chemical ingredients also enables customised fragrances, flavours, and therapeutic blends.

Ecological Functions of Essential Oils

Essential oils are complex blends of plant-derived chemicals that provide distinctive aromas, flavors, and therapeutic properties. Beyond human benefits, they play crucial ecological roles:

Pollinator Attraction

Aromatic compounds help guide pollinators to flowers, enhancing reproductive success (Harborne, 1991).

Allelopathy

Certain essential oils inhibit the germination of neighbouring seeds, reducing competition (Vokou & Liotiri, 1999).

Defense Mechanisms

Insects: Act as repellents or disruptors of feeding and reproduction (Gershenzon, 1994). *Microflora:* Exhibit fungicidal and bactericidal properties (Kalemba & Kunicka, 2003). *Browsing animals:* Deter grazing due to bitter taste or toxicity (Phillips & Croteau, 1999). The biochemical similarity of essential oils to human metabolic components (carbon, hydrogen, oxygen) makes them highly biocompatible (Anitescu *et al.*, 1997).

Chemistry of Essential Oils

Essential oils contain alcohols, aldehydes, esters, ethers, ketones, phenols, and terpenes, each contributing specific biological properties (Dewick, 2002). Lavender, hyssop, and patchouli oils, rich in ketones, promote cell regeneration, while oregano and thyme oils rich in phenols exhibit antimicrobial activity (Kalemba & Kunicka, 2003).

Terpenes

Terpenes form the largest group of plant secondary metabolites, with over 32,000 known members (Sacchettini & Poulter, 1997). They deter herbivores, inhibit pathogens, and seal plant wounds with resin (Phillips & Croteau, 1999). Terpenes arise from isoprene units (C5) and are essential components of plant defense.

Monoterpenoids (C10)

Monoterpenes are abundant in angiosperms and gymnosperms and function in herbivore deterrence and pollinator attraction (Harborne, 1991). They also influence forest atmospheric chemistry (Hakola *et al.*, 2000).

Sesquiterpenoids (C15)

Sesquiterpenes are the most diverse terpenoid class, comprising >1000 compounds. They are cytotoxic, antimicrobial, antioxidant, and strongly insect-repellent (Kalemba & Kunicka, 2003). Many form sesquiterpene lactones with high pharmacological activity.

MATERIALS AND METHODS

Plant Material Collection

Fresh stems of *Gymnanthemum amygdalinum* (Delile) Sch. Bip. exWalp. were collected from naturally growing populations in Landscape garden of Osmania University, Hyderabad, Telangana, India across four distinct seasons: summer (April-June), rainy (July-September), winter (October-December), and spring (January-March) during. Taxonomic identification was confirmed at the Herbarium Hyderabadensis is located at the Department of Botany, Osmania University, Hyderabad, Telangana, India and voucher specimens were deposited in the departmental herbarium for future reference.

Essential Oil Extraction

The stems were washed, air-dried under shade at room temperature for 48 hours and coarsely chopped. Essential oils were extracted by hydro-distillation using a Clevenger-type apparatus for 3 hours, following the standard procedure described by the European Pharmacopoeia (2004). The obtained oils were dried over anhydrous sodium sulfate to remove residual moisture, measured for yield (% w/w based on fresh weight) and stored in amber-sealed vials at 4 °C until analysis.

Gas Chromatography (GC) Analysis

Qualitative and quantitative analysis of the essential oils was performed using a Gas Chromatograph (GC) equipped with a flame ionization detector (FID) and a capillary column (DB-5MS, 30 m × 0.25 mm × 0.25 µm). The injector temperature was maintained at 250°C with a split ratio of 1:50. The oven temperature program was set as follows: initial temperature 60°C (2 min hold), increased at 3°C/min to 220°C and held for 10 min. Nitrogen was used as the carrier gas at a constant flow rate of 1.0 mL/min.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was performed using an Agilent 6890N Gas Chromatograph coupled to an Agilent 5973 Mass Selective Detector (MSD). The instrument was operated under the same chromatographic conditions described earlier.

Ionization of analyses was attained through Electron Impact (EI) ionization at 70 eV, a standard energy setting that ensures reproducible fragmentation of volatile compounds. The mass spectrometer was operated in scan mode with a mass-to-charge (m/z) range of 40-550, allowing for comprehensive detection and identification of low- to medium-molecular-weight compounds present in the sample.

The combination of GC separation and MS detection enabled precise identification of individual components based on their retention times and mass spectral fragmentation patterns, which were matched against reference libraries for compound confirmation.

Identification of Components

Chemical constituents were identified based on comparison of their retention times (RT) with authentic standards, retention indices (RI) calculated using a homologous series of n-alkanes (C8-C20) and mass spectral fragmentation patterns with those reported in NIST and Wiley libraries. Where available, co-injection with reference compounds was used to confirm identification.

Quantitative Analysis

Relative percentage composition of each component was calculated from GC peak areas without applying correction factors. Results were expressed as mean±standard error (SE) of triplicate analyses.

Statistical Analysis

Seasonal variation in oil yield and constituent composition was statistically evaluated using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Chemical Composition of Essential Oil from Stem of *G. amygdalinum* in Various Seasons

The hydro-distilled essential oil from *G. amygdalinum* stems was examined using GC and GC-MS at various seasons and the chemical composition was obtained and given in Tables 1 and 2. The GC chromatograms obtained are shown in Figure 1. In the current study, oil yields from the stem varied between 0.27 and 0.36%, however there was significant variance in the makeup of chemical compounds throughout all seasons. Sesquiterpene concentrations were found to be greater in all samples. A total of 33 compounds were discovered in stem essential oils, totalling 80.4, 88.42, 92.56, and 76.41% respectively in different seasons.

Monoterpene Hydrocarbons in Stem Essential Oil: Seasonal Variation

Seasonal variation observed in monoterpenes (such as limonene, β-pinene, sabinene) aligns with known temperature-regulated terpene emission patterns (Hakola *et al.*, 2000). Limonene, a well-known bioactive compound, is reported for anticancer and chemopreventive potential (Maltzman *et al.*, 1989).

The amount of monoterpene hydrocarbons fluctuated dramatically throughout time, with a low level during the summer and rainy seasons and a maximum in the winter and spring. Monoterpene hydrocarbons were detected throughout the winter (34.56%), wet (28.08%), spring (24.78%) and summer (24.75%) seasons.

Table 1 shows fourteen monoterpene hydrocarbons, with limonene, being the most prevalent in all seasons, reaching 3.78% in the winter.

The monoterpene hydrocarbons in stem essential oil varied as follows: camphene 0.87-1.32%, sabinene 1.01-2.43%, β-pinene 1.24-2.43%, myrcene 1.02-1.62%, α-phellendrene 0.41-1.78%, α-terpinene 0.97-1.47%, limonene 3.24-3.78%, Cis-β-ocimene 2.03-2.49 % and Trans-β-ocimene 2.02-2.66%.

Table 1: Chemical composition of Monoterpenes in essential oil from stems of *G. amygdalinum* in various seasons

S. No.	Compound	RI	% Composition in various seasons				MOI
			Summer	Rainy	Winter	Spring	
			Monoterpenes				
1	Camphene	945	0.87±0.12	1.16±0.12	1.32±0.17	1.02±0.13	a, b, c
2	Sabinene	977	1.01±0.11	1.54±0.13	2.43±0.36	1.27±0.16	a, b, c
3	β-pinene	981	1.43±0.23	1.79±0.31	2.43±0.22	1.24±0.18	a, b, c
4	Myrcene	984	1.02±0.13	1.19±0.14	1.62±0.16	1.09±0.11	a, b, c
5	α-phellendrene	1009	0.41±0.07	1.04±0.09	1.78±0.12	0.98±0.07	a, b, c
6	α-terpinene	1020	0.97±0.07	1.22±0.09	1.47±0.11	1.06±0.08	a, b, c
7	Limonene	1024	3.24±0.41	3.43±0.39	3.78±0.38	3.12±0.36	a, b, c
8	Cis-β-ocimene	1035	2.03±0.12	2.12±0.16	2.49±0.17	1.78±0.15	a, b, c
9	Trans-β-ocimene	1040	2.02±0.21	2.18±0.24	2.66±0.26	1.82±0.19	a, b, c
	Total		13	15.67	19.98	13.38	
			Oxygeneted monoteroenes				
10	Linalool	1085	2.53±0.23	2.68±0.21	2.98±0.32	2.34±0.27	a, b, c
11	Sabinene hydrate	1116	1.24±0.16	1.36±0.19	1.95±0.23	1.08±0.13	a, b, c
12	Borneol	1180	2.21±0.29	2.49±0.38	2.82±0.34	2.68±0.29	a, b, c
13	Thymol	1268	1.31±0.13	1.57±0.19	2.32±0.22	1.19±0.21	a, b, c
14	Methyl Eugenol	1403	4.46±0.31	4.24±0.33	4.51±0.39	4.11±0.31	a, b, c
	Total		11.75	12.34	14.58	11.40	
	Grand total		24.75	28.01	34.56	24.78	

MOI=Method of identification; a=Retention time; b=Retention indices; c=Mass spectra

Table 2: Chemical composition of Sesquiterpenes in essential oil from stems of *G. amygdalinum* in various seasons

S. No.	Compound	RI	% Composition in various seasons				MOI
			Summer	Rainy	Winter	Spring	
			Sesquiterpenes				
1	Delta elemene	1337	1.42±0.21	1.62±0.29	2.97±0.36	1.28±0.14	a, b, c
2	α-Cubebene	1360	1.98±0.27	2.34±0.31	2.89±0.38	1.61±0.17	a, b, c
3	α-copaene	1379	3.36±0.31	3.54±0.36	4.19±0.39	3.17±0.29	a, b, c
4	β-Bourbonene	1386	1.98±0.24	2.33±0.28	2.71±0.29	1.72±0.23	a, b, c
5	β-Elemene	1389	1.92±0.21	2.09±0.24	2.46±0.27	1.78±0.17	a, b, c
6	β-selinene	1419	3.11±0.19	3.45±0.21	3.98±0.28	3.03±0.23	a, b, c
7	β-caryo-phyllene	1421	3.12±0.23	3.34±0.27	3.68±0.28	2.86±0.22	a, b, c
8	α-Elemene	1440	1.39±0.17	1.47±0.16	1.79±0.19	1.28±0.13	a, b, c
9	α-humulene	1446	1.97±0.11	2.31±0.12	2.77±0.14	1.54±0.15	a, b, c
10	Germacrene D	1480	4.67±0.31	4.88±0.37	5.12±0.38	4.18±0.34	a, b, c
11	β-Calarene	1503	2.17±0.33	2.33±0.36	2.61±0.25	2.02±0.29	a, b, c
12	β-bisabolene	1507	3.11±0.21	3.29±0.22	3.36±0.26	3.06±0.24	a, b, c
13	δ-cadinene	1536	2.14±0.02	2.25±0.17	2.77±0.19	2.09±0.09	a, b, c
	Total		32.34	35.24	41.3	29.26	
			Oxygenated sesquiterepene				
14	Spathulenol	1564	6.11±0.22	6.61±0.29	4.87±0.13	5.98±0.37	a, b, c
15	Caryophyllene oxide	1574	5.16±0.33	5.42±0.28	5.94±0.34	4.96±0.26	a, b, c
16	τ-cadinol	1642	3.26±0.22	3.58±0.24	2.11±0.12	3.17±0.21	a, b, c
17	β-bisabolol	1672	2.43±0.31	2.77±0.23	1.18±0.11	2.32±0.19	a, b, c
18	Farnesol	1699	4.98±0.36	5.27±0.38	1.66±0.12	4.73±0.29	a, b, c
19	Farnesyl acetate	1790	1.37±0.11	1.52±0.12	0.94±0.14	1.21±0.12	a, b, c
	Total		23.31	25.17	16.7	22.37	
	Grand total		55.65	60.41	58.00	51.63	

MOI=Method of identification; a=Retention time; b=Retention indices; c=Mass spectra

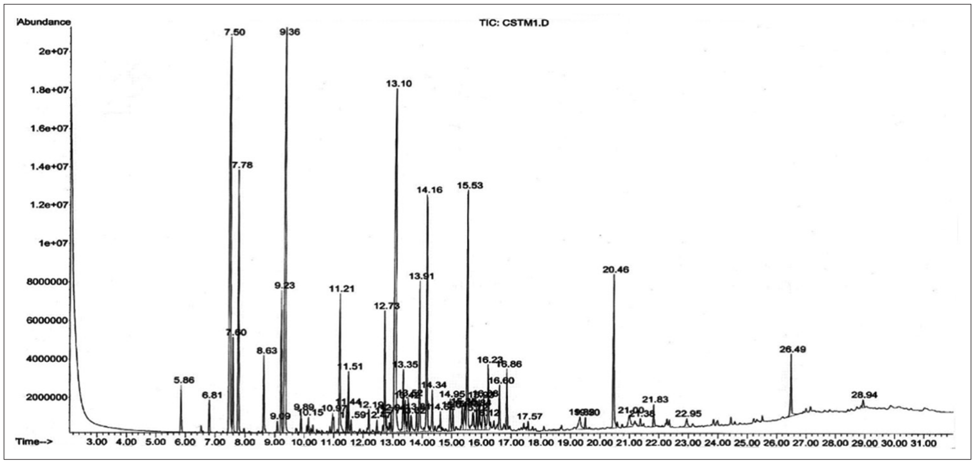

Figure 1: Typical GC chromatogram of essential oil from stems of *G. amygdalinum*

The primary oxygenated monoterpenes were linalool, sabinene hydrate, borneol, thymoland methyl eugenolall of which showed seasonal variations.

Surprisingly, stem oil contained limonene and methyl eugenol, which were missing in leaf oil.

Sesquiterpene Hydrocarbons in Stem Essential Oil: Seasonal Variation

Sesquiterpene dominance, including major compounds such as germacrene D and β-caryophyllene, agrees with known sesquiterpene defense roles in plants (Gershenzon, 1994; Phillips & Croteau, 1999).

Wethad the highest relative amounts of sesquiterpene hydrocarbons, at 60.41%, followed by thewinter (58.00%), summer (55.65%) and spring (51.63%) seasons.

The main sesquiterpenes found were germacrene D, β-caryophyllene, α-copaene, α-humulene, Delta elemene, and δ-cadinene, with considerable seasonal variations. α-Cubebene and β-Elemene were found in trace levels. Sesquiterpenes found in stem essential oil include δ-elemene, α-Cubebene, β-Bourbonene, β-Elemene, β-selinene, β-caryophyllene, α-Elemene, α-humulene, Germacrene D, β-Calarene, β-bisabolene, and δ-cadinene. Seasonal variations in composition were observed.

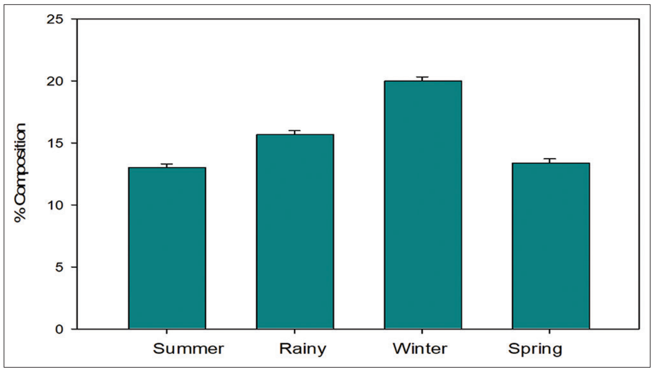

Figure 2: Variations in monoterpene hydrocarbons composition in stem essential oil during various seasons. Values presented as mean±SE ($p<0.05$)

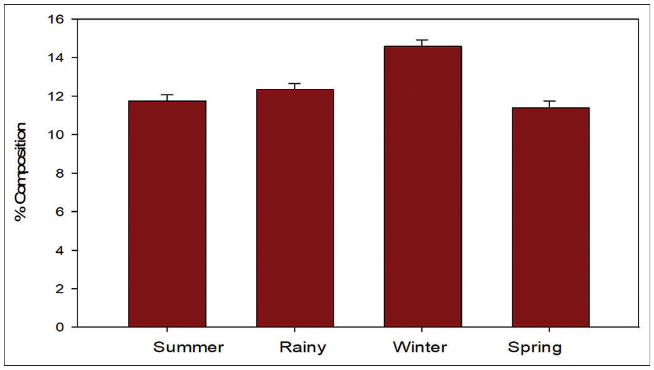

Figure 3: Variations in oxygenated monoterpene composition in stem essential oil during various seasons. Values presented as mean±SE ($p<0.05$)

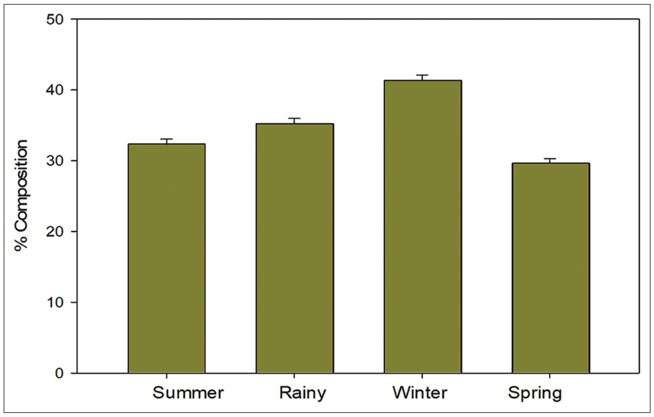

Figure 4: Variations in sesquiterpene hydrocarbons composition in stem essential oil during various seasons. Values presented as mean±SE ($p<0.05$)

These compounds (e.g., spathulenol, farnesol, β-cadinol) peaked in rainy season, possibly due to increased microbial pressure stimulating defense pathways (Vokou & Liotiri, 1999; Kalemba & Kunicka, 2003).

The stem oil contains six oxygenated sesquiterpenes with varying compositions: spathulenol (4.87-6.61%), caryophyllene oxide (4.96-5.94%), β-cadinol (2.11-3.58%), β-bisabolol (1.18-2.77%), farnesol (1.66-5.27%), and farnesyl acetate (0.94-1.52%).

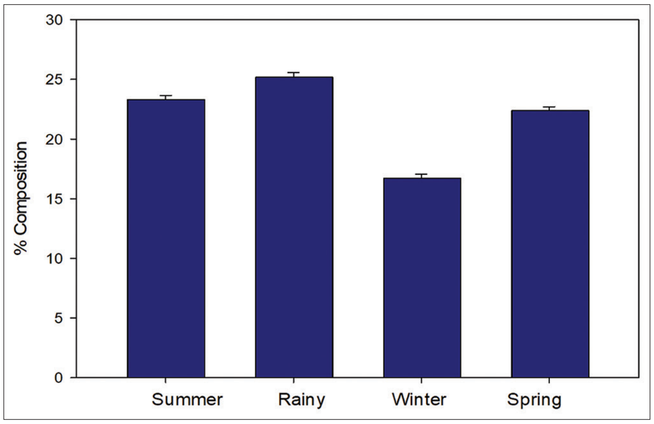

Figure 5: Variations in oxygenated sesquiterpene composition in stem essential oil during various seasons. Values presented as mean±SE ($p<0.05$)

Figures 2-5 showed the relative amounts of monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes in stem essential oils for each season.

Overall, seasonal shifts support the idea that terpenoid metabolism reflects environmental stress adaptation (Harborne, 1991; Gershenzon, 1994).

CONCLUSIONS

The chemical composition of stem essential oils of Gymnanthemum amygdalinum exhibited significant seasonal fluctuations across all major classes of compounds—monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes. These variations indicate a strong environmental influence on the biosynthesis and accumulation of volatile constituents. The overall essential oil profile demonstrated marked seasonal changes, with the maximum total concentration recorded in winter (34.56%), followed by the rainy season (28.01%), while the lowest levels occurred during summer (24.75%) and spring (24.78%). Limonene, methyl eugenol, and linalool were consistently detected throughout all seasons.

Monoterpene content was highest in winter (19.98%), suggesting enhanced production or improved stability of these compounds at lower temperatures. Oxygenated monoterpenes also peaked during winter (14.58%), likely due to increased enzymatic conversion of hydrocarbons under favourable climatic conditions. Sesquiterpenes dominated in winter (41.30%), followed by the rainy season (35.24%), and reached their minimum in spring (29.26%). Germacrene D, β-caryophyllene, and β-selinene remained the major sesquiterpene constituents across seasons. In contrast, oxygenated sesquiterpenes were most abundant during the rainy season (25.17%) and least abundant in winter (16.70%), indicating shifts in metabolic pathways in response to changing environmental factors.

Considering all compound classes, the total essential oil content was highest in winter (92.56%), followed by the rainy season (88.42%), and lowest in spring (76.41%). The statistically significant seasonal differences (MOI: a, b, c) confirm that environmental parameters such as temperature, humidity, and light exposure exert substantial influence on the production and accumulation of essential oil components. These findings are consistent with previous studies on atmospheric regulation of terpene emissions (Hakola *et al.*, 2000) and monoterpene biosynthesis (Lerdau *et al.*, 1997). Overall, winter favours the accumulation of monoterpenes and sesquiterpenes, whereas the rainy season promotes the formation of oxygenated derivatives, providing valuable insights for optimising harvest timing for commercial applications.

ACKNOWLEDGEMENT

The authors are thankful to Prof. Sabita Rani, Head department of Botany, University College of Science and also thanks to Prof. E. Sujatha, Director, Central Facilities for Research & Development (CFRD), Osmania University, Hyderabad, Telangana for the facilities and support.

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