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RESEARCH ARTICLE

Phytochemical profiling and molecular characterization of different *Adenium* genotypes

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Abstract

Adenium obesum, or desert rose, is a member of the Apocynaceae family and is highly valued in horticulture for its ornamental appeal and resilience in container cultivation. The current study employed Inter Simple Sequence Repeat (ISSR) markers to explore the genetic diversity of 20 distinct *Adenium* genotypes. ISSR markers help to identify genetic differences by targeting repeating DNA regions across the genome and they are easy to use, cost-effective and do not require prior genome information, making them useful for studying diversity in many types of organisms. In this study, ISSR analysis revealed a high level of polymorphism (81.51 %), demonstrating significant genetic variability. A total of 206 loci were identified using 25 primers, showcasing the genetic richness of the *Adenium* species. The genetic relationship between the genotypes was further analyzed through the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), a clustering technique that grouped the genotypes based on their genetic similarities, revealing clear genetic differentiation and confirming the robustness of ISSR markers in genetic diversity studies. In addition to the genetic analysis, Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of the ethanolic leaf extracts of *Adenium obesum* revealed the presence of 30 bioactive metabolites, including commonly identified compounds such as vitamin E, phenol, phytol and n-hexadecanoic acid. These results identified several bioactive compounds with potential medicinal properties, underscoring the pharmacological significance of the plant. Overall, this study demonstrates the effectiveness of ISSR molecular markers in both genetic evaluation and the identification of bioactive compounds in *Adenium* species.

Keywords: *Adenium*; docking; ISSR markers; phytochemicals; secondary metabolites

Introduction

The desert rose, or *Adenium obesum* (Forssk.) Roem and Schult is a member of the Apocynaceae family. This genus consists of striking succulents with a highly heterozygous genetic makeup and Adenium species are primarily diploid (2n=24). Due to their slow growth, ability to withstand salinity and excellent response to pruning, *Adenium* plants are well-suited for container cultivation. These plants are heterozygous and cross-pollinated and their ease of vegetative propagation allows for developing newer genotypes. As a result, primary RAPD (Random Amplified Polymorphic DNA) markers were considered essential for evaluating the genetic diversity of *Adenium* genotypes. Molecular markers, including RAPD, have become crucial tools in cultivar development and breeding, aiding in the correct identification of cultivars, precise evaluation of genetic relationships, diversity and the effective tagging and mapping of desirable genes, as well as early selection of superior genotypes (1). In addition to RAPD markers, other systems like minisatellites, microsatellites and restriction fragment length polymorphism (RFLP) have also proven beneficial for analyzing genetic diversity (2). Apart from its ornamental value, *Adenium* plants produce numerous secondary metabolites such as flavonoids, phenolic acids, lignans, quinones, coumarins and alkaloids. All the metabolites exhibit notable antioxidant and other bioactive properties (3). In fact, approximately 80 % of people in developing countries rely on herbal or natural remedies for treating various illnesses (4, 5). Indigenous people and traditional healers have long utilized the therapeutic properties of plants to address a wide range of medical conditions. India is the birthplace of the three widely recognized traditional healthcare systems: Ayurveda, Siddha and Unani (6). The pharmacological properties of plants are largely attributed to the presence of secondary metabolites like flavonoids, phenolic compounds, alkaloids, terpenoids, glycosides and steroids (7). Chemical analysis of Adenium foliage has revealed the presence of compounds such as pregnanes, cardenolides, triterpenes, flavonoids and carbohydrates. Recognizing the significance of traditional medicine, the World Health Organization (WHO) advocates for its integration into healthcare systems to prevent and treat diseases (8).

Material and Methods

Plant material

The present investigation was conducted at Department of Floriculture and Landscaping Architecture, TNAU Coimbatore from 2023 to 2024. Twenty genotypes viz Pink Beauty, Golden Crown, Adenium Soft, Sudharsan, Mung Siam, Picotee, Harry Potter, Home Run, Buttons, Mor Lok Dork, Deang Siam, Miss India, My Country, Noble Queen, White Lucky, Nilakaan, Arrogant, Red Giant, Artic Snow and Triple Star were collected from different geographical locations (Table 1).

Molecular markers

ISSR (Inter Simple Sequence Repeat) markers are widely used to identify molecular diversity because they are highly polymorphic, allowing genetic variation detection across individuals or populations. They do not require prior sequence information, making them suitable for a broad range of species. ISSRs are also reproducible, cost-effective and relatively simple to use in the lab. These features make them valuable tools for assessing genetic diversity, population structure and evolutionary relationships in both plant and animal studies.

Extraction

Samples of Adenium foliage were first rinsed under running water and then allowed to air-dry (50 °C) for about 6 to 7 days. The leaves were then carefully divided into sections and ground into small pieces with a grinder. The resulting powder was gathered with care and kept dry in containers. Encased in a Soxhlet apparatus, the powdered specimens were extracted using a solution consisting of 150 mL of 70 % ethanol and 30 % water. This solution was selected due to its superior extraction efficiency. The resulting mixture was then filtered using an evaporator that was set to 40 °C. The compound was dried and then stored at -20 °C to facilitate easier analysis. Semi-solid ethanolic extract (AOE) from *A. obesum* was prepared and stored at 4 °C. The yield of obtaining (%) was calculated using the methodology outlined in Eqn. 1 (9).

$$\text{Extract yield } \% = \frac{\text{(Weight of the extract after evaporating solvent and freeze drying)}}{\text{(Dry weight of the sample)} \times 100} \quad \text{(Eqn. 1)}$$

Gas-chromatography coupled with mass spectroscopy analysis

Using previously published methods, GC-MS was used to characterize secondary metabolites in the ethanol-based extract leaves of *A. obesum* (10). Agilent GC 7890A/ MS5975C was used for the experiment and a capillary column was used after the extract was dissolved in 100 % ethanol prior to analysis. The sample was placed into the Agilent DB5MS apparatus with a column length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 microns, with an injector running in split mode and helium present. The retention time and fragmentation pattern were evaluated using the NIST spectral library to determine the extract's bioactive components (Table 2). The GC -MS analysis was repeated twice for each sample and the mean values were taken for ascertaining the availability of phytochemicals.

Data analysis

The experimental data was analyzed statistically using the XLSTAT plugin v. 2009.3.02 for Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). The raw data obtained by GC-MS was processed using Mass Hunter Qualitative Analysis software (Version B 07.00, Agilent Technologies). The correlation between the volatile profiles of various germplasms has been analyzed using principal component analysis (PCA). Agglomerative Hierarchical Clustering (AHC) was used to identify the variables contributing to group classification.

DNA isolation

DNA isolation was performed using a modified CTAB (Cetyl Trimethyl Ammonium Bromide) method as described by (11). A 1 g leaf sample was placed in a mortar and 1 mL of 2 % CTAB was added. The sample was finely ground, then transferred to a 2 mL Eppendorf tube. 5 µL of mercaptoethanol and a small pinch of polyvinylpyrrolidone (PVP) were added, followed by thorough mixing. The mixture was incubated in a water bath at 65 °C for 45 min. After incubation, 600 µL of chloroform: isoamyl alcohol (24:1) was added, mixed well and centrifuged at 10000 rpm for 15 min. The supernatant was carefully transferred to a fresh 1.5 mL tube and an equal volume of 600 µL ice-cold isopropanol was added. The sample was then incubated at 4 °C overnight. Following the overnight incubation, the sample was centrifuged again at 10000 rpm for 15 min. The supernatant

Table 1. List of *Adenium* genotypes collected from different locations

S. No.	Name of the genotype	Source	Soil type	Climatic zone
G1	Pink Beauty	Walayar	Slightly acidic laterite soil	Hot and Humid
G2	Golden Crown	Walayar	Slightly acidic laterite soil	Hot and Humid
G3	Soft	Walayar	Slightly acidic laterite soil	Hot and Humid
G4	Sudharsan	Walayar	Slightly acidic laterite soil	Hot and Humid
G5	Mung Siam	Walayar	Slightly acidic laterite soil	Hot and Humid
G6	Picotee	Trichy	Slightly Alkaline and red soil	Tropical and dry
G7	Harry Potter	Trichy	Slightly Alkaline and red soil	Tropical and dry
G8	Home Run	Trichy	Slightly Alkaline and red soil	Tropical and dry
G9	Buttons	Trichy	Slightly Alkaline and red soil	Tropical and dry
G10	Mor Lok Dork	Trichy	Slightly Alkaline and red soil	Tropical and dry
G11	Deang Siam	Trichy	Slightly Alkaline and red soil	Tropical and dry
G12	Miss India	Pondicherry	Sandy loam and red soil	Coastal humid
G13	My Country	Pondicherry	Sandy loam and red soil	Coastal humid
G14	Noble Queen	Pondicherry	Sandy loam and red soil	Coastal humid
G15	White Lucky	Thrissur	Slightly acidic laterite soil	Hot and Humid
G16	Nilakaan	Thrissur	Slightly acidic laterite soil	Hot and Humid
G17	Arrogant	Thrissur	Slightly acidic laterite soil	Hot and Humid
G18	Red Giant	Kanyakumari	Slightly acidic loamy soil	Coastal humid
G19	Artic Snow	Kanyakumari	Slightly acidic loamy soil	Coastal humid
G20	Triple Star	Kanyakumari	Slightly acidic loamy soil	Coastal humid

Table 2. Parameters that are employed in GC-MS analysis

Parameter	Value
Instrumentation	Agilent GC 7890A / MS5975C
Column	Agilent DB5MS
Column length	30 m
Internal diameter	0.25 mm
Film thickness	0.25 µm
Sample injection mode	Split mode
Carrier gas	Helium
Sample preparation	Extract dissolved in 100 % ethanol
Compound identification	Retention time and fragmentation pattern matched with the NIST spectral library
Reference method	(10)

was discarded, leaving a white pellet. To wash the pellet, 200 µL of 70 % ethanol was added, followed by centrifugation at 10000 rpm for 5 min. The ethanol was discarded and the pellet was air-dried for 2 hr. Finally, the pellet was resuspended in 50 µL of 1X TE buffer by gentle tapping and stored at -20 °C.

Data scoring and analysis

Manual scoring was applied to well-resolved fragments ranging in size from 100 bp to 2.5 kb. Every band was used as a reference point. Bands were scored according to whether they were present in the gel (1) or not (0). By computing the Jaccard's similarity coefficient for pairwise comparisons based on the pro portion of shared bands generated by the primers, the genetic associations were assessed. The cluster analysis of the unweighted pair group method with arithmetic averages (UPGMA) was applied to the similarity matrix and NTSYS-pc version 2.1 software was utilized to generate a dendrogram (12).

Molecular docking

Selected ligands with PubChem ID

⇒ Tocopherol - 483926424

Protein structure preparation

A type of protein was selected for docking studies. Protein structure preparation ensures that the structure's physio-chemical characteristics, such as bond distance and torsion angle, are optimized for computational analyses. Using BIOVIA Discovery Studio software (DS4.5, Accelrys, Inc., San Diego, CA, USA), the target receptors were prepped for docking analysis by adding hydrogen atoms and other heteroatoms and removing the monomeric chain and unnecessary water molecules. The other parameters, such as protonation and building loops, were set to TRUE by default. Proteins are cleaned, missing residues are inserted, loops are refined and minimized and then the proteins are protonated using the CHARMm force field.

Molecular Modelling and Docking

Molecular docking was carried out using AutoDock4.2 (13) and the Lamarckian Genetic Algorithm while considering the docking parameters from our previously published method (14). For every ligand, a total of 50 separate docking runs were conducted. Considering a difference of less than 2.0 Å of root mean square deviation (RMSD), the conformations were categorized under clusters. The lowest inhibition constant (Ki) and lowest free energy of binding (DG) were considered to determine the most advantageous binding pose. LigPlot + v 1.4.5 was used to investigate the molecular interactions between the compounds and receptors (15).

PCA and UPGMA

Principal compound analysis (PCA) identifies a plant's most abundant or biologically active compounds, linking its chemical composition to potential therapeutic or functional properties. This aids in understanding medicinal value and supports species differentiation and genetic variation analysis. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) complements this by grouping samples based on genetic or chemical similarities. It generates a dendrogram visually representing genotype relationships, revealing genetic diversity and chemotypic clusters. Together, these methods enable effective classification, comparison and selection of elite plant varieties for research and breeding.

Results

GCMS

Overall, 30 peaks of each sample were recorded in the GC–MS graph of the extracts of *A. obesum,* which corresponded to the bioactive substances identified by assessing the duration of retention and dissection pattern with the help of the NIST spectrum archive and GC-MS Real-Time Evaluation. (Table 3, Fig. 1a & 1b) contain the recognized phytoconstituents. The extract's chromatogram demonstrated the presence of several phytoconstituents that were linked to anticancer activity in various extracts. The ethanolic extract of AOE contained multiple compounds, including Gamma-sitosterol, neoisolongifolene, campesterol, indeno(1,2,3-ij) isoquinoline, cholesta-5,20,24-trien -3-ol and dl-alpha-tocopherol, which are all significant metabolites. At the same time, vitamin E is the only common metabolite consistently found across the genotypes.

Principal component analysis (PCA)

A total of 30 variables (volatile compounds) of 15 *Adenium* Genotypes. The biplot diagram illustrates the relationship between principal components and samples based on their concentrations and variances. The first two principal components (PC1 and PC2) explain 33.26 % and 19.70 % of the variance, respectively. Key compounds such as Thiophene, Lupeol, dl-alpha-Tocopherol, Hexadecenoic Acid and Gamma-Sitosterol were represented by arrows, indicating their influence on the distribution of samples. For instance, Sample A1 strongly associates with Thiophene, while Sample A9 aligns with Alpha-Amyrin and Phytol. The proximity of samples (A1 to A15) to the arrows reflects the relative concentration of these compounds in each sample, helping to explain the variation in chemical profiles across the dataset. This analysis highlights which compounds were predominant in different samples, providing insights into the chemical composition (Fig. 2).

Table 3. List of various phytochemicals identified by GC-MS analysis that are contained in the ethanolic leaf extracts of *A. obesum*

Si No	RT time	Compounds	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
			Area %														
1	22.562	gamma-Sitosterol	5.50	-	-	-	-	4.67	-	4.00	4.53	7.13	3.70	7.02	5.27	4.39	1.08
2	22.440	Neoisolongifolene	2.93	-	-	-	-	-	-	-	-	-	-	3.69	-	-	-
3	21.507	Campesterol	1.41	-	-	-	-	-	-	-	-	-	1.19	1.79	1.98	-	-
4	21.129	Indeno(1,2,3-ij) isoquinoline	1.13	-	-	-	-	-	-	-	5.69	-	-	-	-	-	3.89
5	20.563	Cholesta-5,20,24-trien-3-ol	1.27	-	-	-	-	-	-	-	-	6.46	-	-	-	-	-
6	20.329	dl-alpha-Tocopherol	11.34	-	-	-	-	-	-	1.27	1.31	4.78	1.55	1.67	1.96	-	1.20
7	19.863	Phenol	1.08	0.89	1.32	1.42	-	-	1.09	1.96	-	0.85	-	3.99	-	0.53	0.85
8	19.118	3-Formyl-6-(4-methoxyphenyl)-2H-thiopyran	1.97	-	-	-	-	-	-	-	-		-	2.96	-	-	-
9	18.818	2H-1-Benzopyran-6-ol	0.77	2.83	1.11	0.53	4.85	-	4.11	-	1.31	4.78	-	5.85	5.08	-	-
10	17.485	Benzo[h]quinoline	0.69	1.07	1.03	0.35	1.85	4.73	-	0.93	5.69	-	-	1.79	0.83	3.94	1.06
11	17.396	Tridecanedioic acid	2.05	-	-	-	-	-	-	1.61	0.95	-	-	-	-	1.61	-
12	17.308	Lupeol	2.92	2.70	-	-	-	-	-	4.13	-	-	2.72	-	-	-	-
13	17.185	9,9'-Biphenanthrene	2.40	-	-	-	-	-	-	-	-	-	-	-	3.69	-	-
14	16.330	Hexadecanoic acid	1.46	0.92	0.64	11.32	-	-	-	-	0.75	-	-	4.88	-	1.61	1.80
15	16.152	Vitamin E	1.44	3.86	4.40	5.91	4.85	4.85	1.59	1.27	3.53	4.78	4.01	1.67	1.96	7.99	5.45
16	15.919	Acetamide	1.45	1.96	0.99	5.77	2.45	0.44	-	-	0.31	1.56	1.59	1.47	3.38	1.11	-
17	15.641	Tricosanoic acid	1.35	-	-	-	-	-	-	0.83	-	-	-	-	-	-	-
18	15.419	alpha-Amyrin	12.61	-	-	-	-	-	-	4.13	18.76	-	3.00	4.60	4.22	8.20	-
19	14.475	1,4-Dimethyl-8-isopropylidenetricyclo [5.3.0.0(4,10)]	1.50	-	-	-	-	-	-	-	-	-	1.89	-	-	-	-
20	14.197	9,12,15-Octadecatrienoic acid	1.91	-		-	-	-	-	-	-	0.67	-	2.97	-	-	-
21	14.030	Phytol	3.60	1.36	1.77	2.87	4.33	-	3.00	4.19	5.29	-	3.07	3.52	2.85	2.96	-
22	13.053	n-Hexadecanoic acid	5.02	0.92	0.64	2.58	1.53	0.58	2.64	-	-	-	0.86	1.31	1.38	0.50	0.99
23	12.986	Diphenyl sulfone	0.78	2.09	2.63	3.21	2.27	-	-	1.56	0.61	-	1.27	-	1.12	2.20	1.98
24	12.875	beta-Amyrin	2.13	-	-	-	-	-	-	8.37	3.07	5.61	3.40	-	-	-	3.74
25	11.642	Myo-Inositol	5.75	-	-	-	-	-	-	-	6.19	-	5.62	-	0.73	-	-
26	11.064	Thiophene	4.57	8.56	-	-	-	-	-	-	-	-	-	3.99	0.42	-	-
27	10.675	Butanoic acid	2.44	0.58	1.09	2.87	1.34	7.66	-	-	-	5.22	5.62	1.62	5.55	6.51	-
28	9.897	Benzoic acid	1.46	5.07	0.63	3.49	5.14	0.50	-	4.55	2.68	-	3.34	1.34	0.47	-	1.63
29	7.098	Benzofuran	2.56	-	0.70	-	-	-	-	0.46	-	0.85	-	0.76	0.57	-	-
30	5.398	1,3-Dioxane	1.17	-	-	-	-	-	-	-	-	-	2.87	4.91	-	-	-

"-" not present

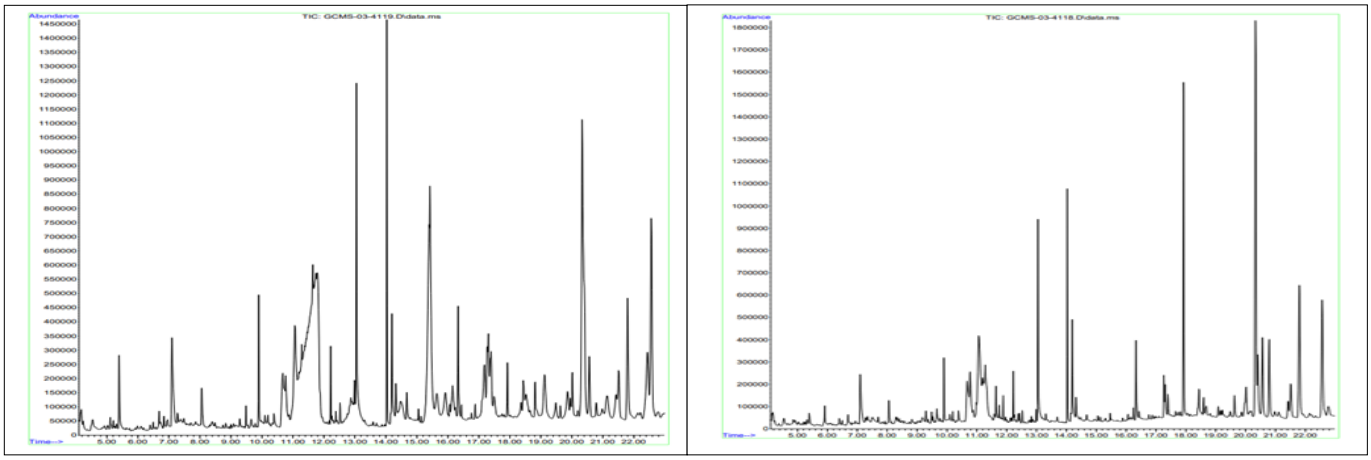

Fig. 1. A. Chromatogram of the genotype G4; B. Chromatogram of the genotype G16.

Molecular markers

DNA amplification of inter inter-SSR was carried out using a set of primers. Among the 25 ISSR primers screened, primers produced clear polymorphic loci in all 20 genotypes tested. A total of 206 loci were detected from 20 genotypes, among which 165 (81.51 %) loci were polymorphic, revealing the higher degree of polymorphism among the genotypes (Table 4). The range of polymorphic loci in *Adenium* genotypes ranged from 4 to 12. The average number of polymorphic loci per primer was 6.6. The highest number of polymorphic loci i.e., 12 were generated by the primer TriCAC3 RC followed by TriCAC3 YC (9), TriAAG3 RC (8) and DICA3 RG (8). Cluster analysis was performed using in NTSYS software (UPGMA method) using the ISSR data from 25 primers. The PIC (Polymorphic Information Centre) value considers the overall number of alleles at a locus and their relative frequencies. In the present study, the PIC values ranged between 0.10 - 0.32. The mean PIC score for all loci was 0.21. The linkage and divergence properties of the ecotypes were further examined by constructing a dendrogram for 20 *Adenium* genotypes using UPGMA cluster analysis in NTSYS software. The dendrogram based on 25 ISSR markers depicted the *Adenium* genotypes' variability using the Hierarchical clustering method. It separated 20 genotypes into two major clusters I and II (Table 5 & Fig. 3). The similarity coefficient value ranged from 0.35 to 0.72, revealing significant variation at the genetic level among 20 genotypes. Cluster I was divided into two sub clusters as I A and I B. Sub cluster I A comprised of the genotypes Golden Crown, Sudharsan, Mung Siam, Harry Potter, Picotee, Home Run, Buttons, Mor Lok Dork and Soft where sub cluster I B comprised of Deang Siam, Miss India, My Country, Noble Queen, Nilakaan, Red Giant, White Lucky and Arrogant. Likewise, cluster II comprised of Artic Snow and Triple Star and the Genotype Pink Beauty (Table 6 & 7).

Similarity matrix for 20 *Adenium* genotypes was determined using Jaccard's Coefficient based Distance method. From this matrix, similarity indices were calculated among *Adenium* genotypes (Table 8). The genotype Miss India and My Country were found to have the highest similarity (0.7165), followed by the genotype Home Run and Buttons (0.6960). The genotype Artic Snow exhibited the least similar index (0.2517).

Table 4. Band statistics of the analyzed primers for *Adenium* genotypes

S. No.	Primer name	Primer sequence	Total no. of bands	Polymorphic bands	% Polymorphism	PIC
1	TriGGA3 RC	GGA GGA GGA GGA GGA RC	10	7	70.00	0.24
2	DIGA3 T	GAG AGA GAG AGA GAG AT	7	6	85.71	0.14
3	TriCAG3 RC	CAC CAC CAC CAC CAC RC	9	5	55.55	0.17
4	TriAAG3 RC	AAG AAG AAG AAG AAG RC	10	8	80.00	0.29
5	DICA3 G	CAC ACA CAC ACA CAC AG	7	6	85.71	0.20
6	DICA3 RG	CAC ACA CAC ACA CAC ARG	9	8	88.88	0.29
7	DiCA3 YG	CAC ACA CAC ACA CAC AYG	4	4	100.00	0.10
8	DIGA3 C	GAG AGA GAG AGA GAG AC	4	4	100.00	0.13
9	DIGA3 RC	GAG AGA GAG AGA GAG ARC	7	6	85.71	0.22
10	TriCAC3 RC	CAC CAC CAC CAC CAC RC	13	12	92.30	0.27
11	TriCAC3YC	CAC CAC CAC CAC CAC YC	11	9	81.81	0.32
12	TriCAC5 CY	CAC CAC CAC CAC CAC CY	8	8	100.00	0.23
13	TriGTG3 YC	GTG GTG GTG GTG GTG YC	8	7	87.50	0.28
14	TriTGT3 YC	TGT TGT TGT TGT TGT YC	6	5	83.33	0.19
15	TriAAC3 RC	AAC AAC AAC AAC AAC RC	8	7	87.50	0.26
16	TriACG3 RC	ACG ACG ACG ACG ACG RC	11	8	72.72	0.31
17	TriAGA3 RC	AGA AGA AGA AGA AGA RC	8	5	62.50	0.18
18	TriTGG3 RC	TGG TGG TGG TGGTGG RC	10	6	60.00	0.16
19	TriCGA3 RC	CGA CGA CGA CGA CGA RC	8	5	62.50	0.17
20	TriCGC3 RC	CGC CGC CGC CGC CGC RC	8	7	87.50	0.20
21	TriGAC3 RC	GAC GAC GAC GAC GAC RC	9	7	77.77	0.21
22	TriGCA3 RC	GCA GCA GCA GCA GCA RC	7	7	100.00	0.23
23	TriGCC3 RC	GCC GCC GCC GCC GCC RC	10	6	60.00	0.16
24	834	AGA GAG AGA GAG AGA GYT	8	7	87.54	0.25
25	837	ACA CAC ACA CAC ACA GC	6	5	83.33	0.12
		Total	206	165	81.51	0.21

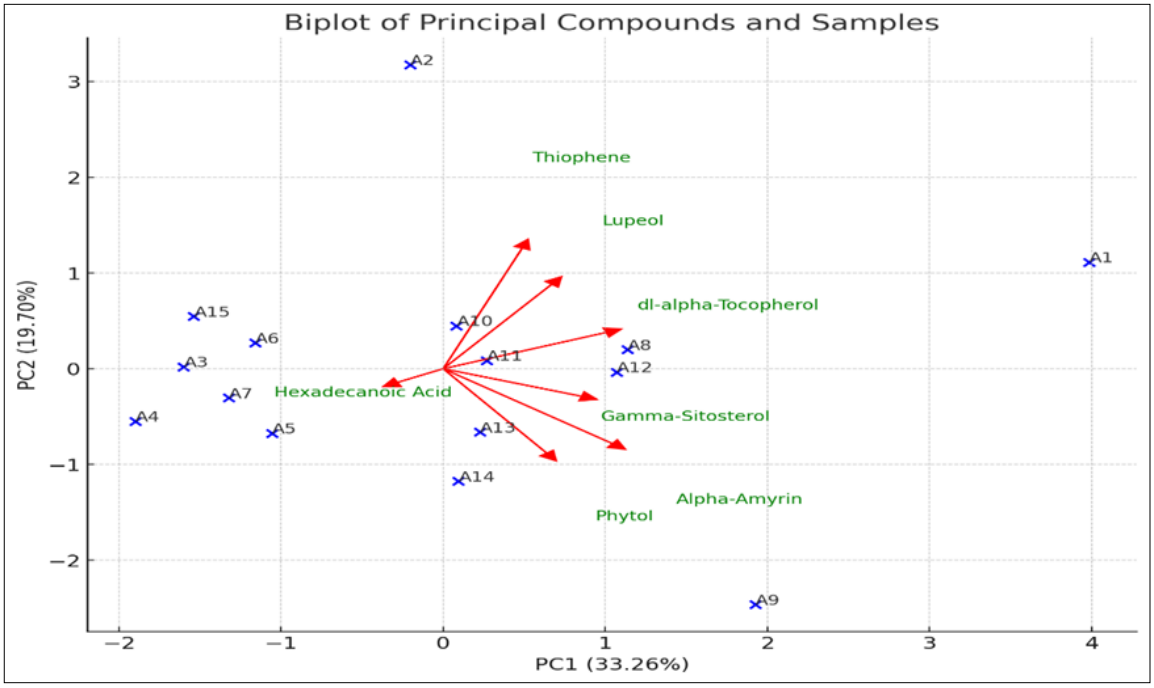

Fig. 2. PCA of *Adenium* genotypes.

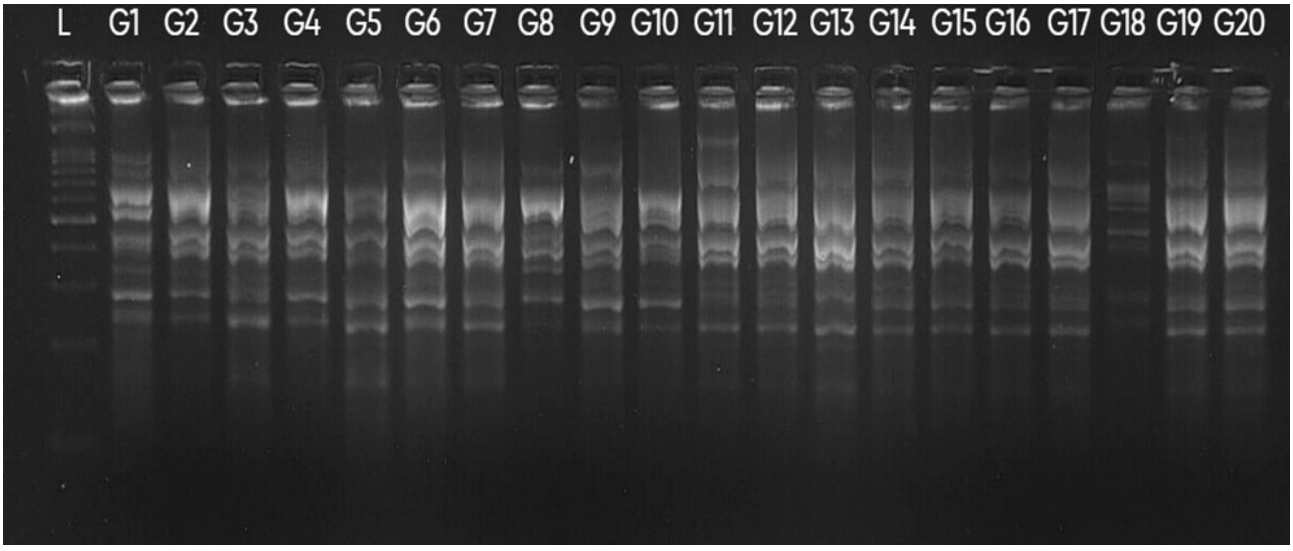

Fig. 3. DICA3 RG primer.

Table 5. Distribution of 20 *Adenium* genotypes into different clusters based on ISSR data

Major clusters	Sub clusters	Genotypes
I	I A	Golden Crown Sudharsan Mung Siam Harry Potter Picotee Home run Buttons Mor Lok Dork Soft
	I B	Deang Siam Miss India My Country Noble Queen Nilakaan Red Giant White Lucky Arrogant
II		Artic Snow Triple Star

Docking

The three-dimensional (3D) structure of vitamin E, the primary compound identified from *Adenium obesum*, was computationally modelled and energetically optimized to reflect its most stable conformation. This optimized structure was then used in molecular docking studies to investigate its potential interaction with *Escherichia coli (E. coli)* receptor proteins. Before conducting these docking simulations, the docking protocol and algorithm were validated through a redocking experiment, which involved re-docking a known ligand into its receptor to ensure the accuracy and reliability of the docking method used. This validation step is critical to confirm that the docking software can accurately predict binding poses and interaction energies.

The molecular docking results revealed that vitamin E did not form any hydrogen bonds with the *E. coli* receptor, suggesting a lack of strong, specific polar interactions in the binding site. However, the analysis of the docking diagrams (Fig. 4 & 5) indicated a likely non-bonding interaction between vitamin E and the tryptophan residue at position 77 (Trp77) of the receptor. This interaction is likely a hydrophobic or π-π

Table 6. Cluster I mean performance for the morphological traits of *Adenium* genotypes

Parameters	Genotypes															
	G1	G3	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G18	G19	Mean
PH	40.66	29.06	39.00	39.28	36.32	23.33	30.82	37.80	37.43	36.32	34.90	40.81	40.03	40.61	37.62	36.27
PS	20.58	22.40	21.36	22.61	22.61	20.78	23.30	25.63	21.54	23.24	22.78	22.27	21.92	20.68	21.90	22.24
NB	1.97	1.47	2.30	1.63	1.97	1.67	1.70	2.53	2.07	2.13	2.00	1.97	1.60	1.53	1.90	1.90
NL	33.93	30.67	34.83	44.17	49.97	58.90	44.60	48.07	51.90	48.73	43.70	42.70	62.73	60.90	67.37	48.21
CC	17.04	17.00	16.98	16.96	15.92	18.16	18.33	18.30	18.16	19.16	18.77	18.01	17.75	16.98	16.84	17.62
LW	4.16	4.68	5.55	4.43	4.53	4.94	3.84	4.29	4.14	4.31	3.88	5.08	4.18	4.91	4.41	4.49
LL	11.77	10.83	8.90	10.13	9.05	9.71	9.48	7.50	8.58	11.27	10.90	10.85	11.80	10.11	10.02	10.06
LA	25.23	32.00	32.03	27.11	21.44	24.91	21.82	24.79	23.23	33.31	22.88	25.59	28.95	25.18	28.21	26.45
LT	0.41	0.24	0.33	0.28	0.30	0.36	0.30	0.33	0.40	0.36	0.32	0.31	0.33	0.39	0.38	0.34
FD	9.00	6.85	8.14	8.25	7.34	6.89	7.95	9.02	7.26	7.85	8.58	6.79	7.29	8.99	8.31	7.90
LCT	3.65	3.14	4.02	3.88	3.12	2.97	3.06	3.30	3.15	3.20	3.08	3.25	3.08	3.54	3.49	3.33
DCT	1.85	1.30	1.86	1.90	1.54	1.96	1.64	1.92	2.06	1.81	1.80	1.46	1.39	1.41	1.62	1.70
PT	0.17	0.15	0.16	0.17	0.14	0.19	0.20	0.14	0.15	0.13	0.16	0.12	0.13	0.12	0.12	0.15
WF	1.66	1.39	2.33	1.30	1.16	1.32	1.27	1.49	2.14	1.04	1.29	1.59	1.24	1.18	1.14	1.44
LAP	3.06	2.38	3.07	3.37	3.09	2.50	2.96	3.42	3.13	2.66	3.57	3.44	3.04	3.13	3.60	3.09

PH: Plant height (cm), PS: Plant spread (cm), NB: Number of branches per plan, NL: Number of leaves per plant, CC: Caudex circumference (cm), LW: Leaf width (cm), LL: Leaf length (cm), LA: leaf area (cm2), LT: Leaf thickness (mm), FD: Flower diameter (cm), LCT: Length of corolla tube (cm), DCT: Diameter of corolla tube (cm), PT: Petal thickness (mm), WF: Weight of flower (g), LAP: Length of anther appendages (cm)

Table 7. Cluster II mean performance for morphological traits of *Adenium* genotypes

Parameters	Genotypes					
	G2	G4	G16	G17	G20	Mean
PH	39.21	44.02	42.04	39.31	38.04	40.52
PS	22.33	26.55	23.20	25.63	22.20	29.98
NB	2.53	5.07	3.70	2.31	2.30	3.18
NL	35.20	74.70	80.01	66.73	64.53	64.23
CC	17.26	17.10	17.45	16.97	16.10	16.98
LW	2.83	4.79	3.74	4.88	3.23	3.89
LL	13.56	11.91	10.04	10.77	9.61	11.18
LA	24.79	39.45	24.42	24.47	20.96	26.82
LT	0.35	0.32	0.38	0.34	0.30	0.34
FD	8.07	8.87	8.13	7.87	9.69	8.53
LCT	3.54	3.23	3.86	3.30	4.01	3.59
DCT	2.06	1.51	1.65	1.40	2.29	1.78
PT	0.14	0.13	0.13	0.11	0.24	0.15
WF	2.68	1.99	1.65	1.14	2.83	2.06
LAP	3.23	3.95	4.47	4.03	4.91	4.12

PH: Plant height (cm), PS: Plant spread (cm), NB: Number of branches per plan, NL: Number of leaves per plant, CC: Caudex circumference (cm), LW: Leaf width (cm), LL: Leaf length (cm), LA: leaf area (cm2), LT: Leaf thickness (mm), FD: Flower diameter (cm), LCT: Length of corolla tube (cm), DCT: Diameter of corolla tube (cm), PT: Petal thickness (mm), WF: Weight of flower (g), LAP: Length of anther appendages (cm)

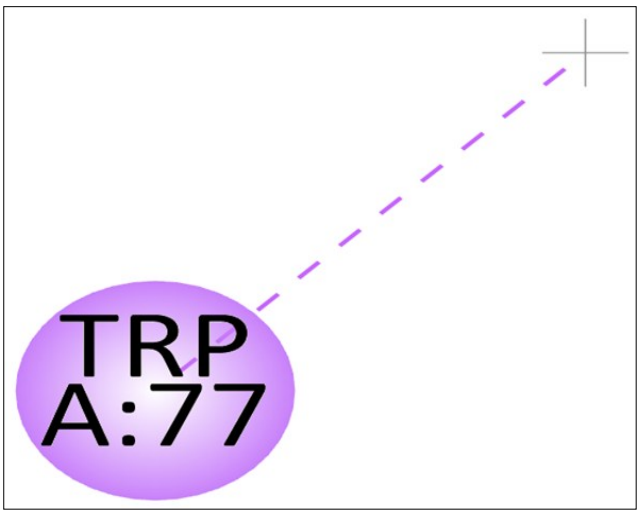

Fig. 4. Tryptophan structure.

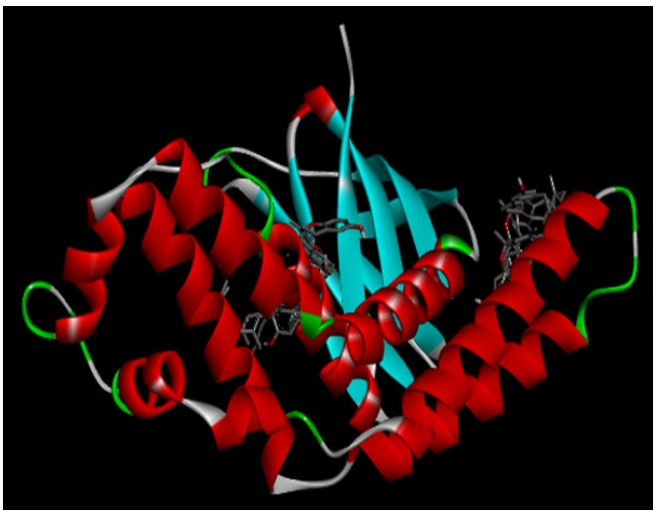

Fig. 5. Docking structure of *Adenium*.

Table 8. Similarity matrix for 20 *Adenium* genotypes

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20
G1	1.0000																			
G2	0.4521	1.0000																		
G3	0.3510	0.4789	1.0000																	
G4	0.4026	0.5310	0.5970	1.0000																
G5	0.3974	0.5203	0.5175	0.6594	1.0000															
G6	0.3649	0.5109	0.4741	0.5474	0.6090	1.0000														
G7	0.3457	0.6000	0.5319	0.6056	0.6739	0.6641	1.0000													
G8	0.3439	0.5175	0.5036	0.5034	0.6159	0.5969	0.6767	1.0000												
G9	0.3618	0.5435	0.4964	0.5500	0.5532	0.5859	0.6466	0.6960	1.0000											
G10	0.3537	0.5606	0.4887	0.4823	0.5362	0.5520	0.6107	0.5938	0.6475	1.0000										
G11	0.3660	0.4658	0.5259	0.5245	0.5385	0.5338	0.5493	0.6015	0.5597	0.5781	1.0000									
G12	0.3789	0.4805	0.4966	0.5570	0.5743	0.5461	0.6027	0.5548	0.5486	0.5926	0.6842	1.0000								
G13	0.3289	0.4752	0.5152	0.5143	0.5735	0.5000	0.5852	0.5333	0.5263	0.5600	0.6063	0.7165	1.0000							
G14	0.3191	0.4191	0.5164	0.4924	0.5308	0.4640	0.5076	0.4545	0.4803	0.4836	0.5667	0.6290	0.6697	1.0000						
G15	0.3309	0.3955	0.3876	0.4806	0.4370	0.4508	0.4621	0.4646	0.4677	0.4380	0.4409	0.4380	0.5000	0.4862	1.0000					
G16	0.3194	0.4627	0.4574	0.5267	0.5075	0.4531	0.5659	0.5000	0.4806	0.4839	0.5565	0.5373	0.5583	0.5221	0.4435	1.0000				
G17	0.3022	0.3852	0.4553	0.4692	0.4511	0.4390	0.4511	0.4419	0.4331	0.3810	0.4959	0.5154	0.5128	0.5000	0.4444	0.5865	1.0000			
G18	0.3087	0.4672	0.4733	0.5414	0.5109	0.4922	0.4892	0.4599	0.5077	0.4651	0.5469	0.5515	0.5159	0.5304	0.4286	0.6126	0.6346	1.0000		
G19	0.2517	0.3971	0.3788	0.4697	0.4737	0.4286	0.4519	0.3597	0.4122	0.4240	0.4308	0.5267	0.5126	0.4602	0.4071	0.4956	0.4722	0.6321	1.0000	
G20	0.2867	0.4179	0.4109	0.3857	0.4275	0.3740	0.4101	0.3897	0.3603	0.3937	0.4198	0.4706	0.4754	0.4103	0.3947	0.4322	0.4455	0.5175	0.5577	1.0000

stacking interaction, given the aromatic nature of vitamin E and the tryptophan side chain. Although weaker than hydrogen bonds, such non-covalent interactions can still play a role in molecular recognition and contribute to the overall binding affinity, albeit with potentially lower specificity. These findings provide insight into vitamin E's interaction with bacterial targets and could inform future structural modifications to enhance its biological activity.

Discussion

GCMS

Chemicals found in medicinal plants have therapeutic properties and serve as building blocks for chemical and pharmaceutical semi-synthesis (16). Many different medical conditions were treated with plants and the variety of products they produced. Medicinal plants were used extensively in most countries' traditional medical systems to treat basic health needs. Thus, since ancient times, developed nations have utilized plant-based herbal remedies to treat various medical ailments. Herbal medicine, derived from different plants, was a good source of medicinal compounds. The medications were either pharmaceutical or plant-based products sold worldwide; in essence, they were plant-based active ingredients (17, 18). The drug-producing industries used these medically significant plants to create new medications. Scientists worldwide have already conducted numerous biological and pharmacological studies on a wide range of constituents. Thus, the current focus was on screening the chosen plant species and portions to verify their application in complementary and substitute medical care additionally the mainstream healthcare framework (19).

The ethanolic extracts from *A. obesum* leaves analyzed in this study indicated the existence of several plant-based constituents, including terpenoids, prenylated flavonoids, carbohydrates, glycosides and cardiac. This work has examined the anti-inflammatory, anticancer and antioxidant characteristics of the extracts from *A. obesum* leaves. These plant-based biologically active constituents may give the ethanolic extracts of *A. obesum* their therapeutic qualities. The extract(ethanol) of *A. obesum* foliage contained heptadecane, an unstable ingredient of Spirulina platensis that has been demonstrated to possess anti-proliferative effectiveness towards HepG2 cells, which are cancerous cells found in the liver of humans (20). It has been shown that phytol, currently in the ethanolic extract of *A. obesum*, possesses antimicrobial, anti-inflammatory, anticancer and diuretic attributes (21). Adenium Obesum Extract was found to be cytotoxic against A549 lung cancer cells and inhibited the viability of A549 lung cancer cells by inducing nuclear condensation and fragmentation. Furthermore, the anti-inflammatory potential of AOE in murine alveolar macrophages (J774A.1) showed its potential in reducing the levels of inflammatory mediators, including the proinflammatory cytokines and TNF-α (22).

Vitamin E was the only compound commonly found in all 15 genotypes we examined. Vitamin E, found in *Adenium obesum* leaves, is a fat-soluble antioxidant that plays various roles in plant and human health. In plants, Vitamin E helps protect cell membranes from oxidative stress, particularly during environmental challenges like drought, high light and temperature extremes, common in the habitats where Adenium thrives. Vitamin E's primary function in human health is as an antioxidant, protecting cells from damage caused by free radicals. This property is linked to several potential health benefits, such as enhancing immune function, reducing oxidative stress and lowering the risk of chronic diseases like heart disease and cancer. It is also known for promoting skin health by reducing UV damage and improving wound healing. Similar results were reported by (23) in *Cassia alata.*

Principal component analysis (PCA)

In this study, the biplot analysis reveals key insights into the chemical composition of the samples based on their principal components (PC1 and PC2), which together explain 52.96 % of the variance (33.26 % from PC1 and 19.70 % from PC2). The biplot visually illustrates the relationship between samples and principal components, allowing us to observe compound concentration and variance patterns. Similar studies were found in the crop Murraya.

Molecular markers

In addition to morphological classification, molecular markers can be used to investigate genetic diversity both within and between species (24). The genetic relationships between genotypes in a variety of flower crops, including jasmine (25), chrysanthemum (26), gerbera(27), rose (28), hibiscus (29) and others, have been studied using RAPD markers. Research indicates the inter-simple sequence repeat (ISSR) analysis (30). It uses the SSR motif alone as the single marker in PCR amplifications and has a number of benefits, including the need for only smaller amounts of template DNA, the lack of sequence data needed to construct the marker, random distribution across the genome, etc. The detection of polymorphisms in flower crops, including tuberose (31), jasmine (32), chrysanthemum (33), lilium (34), etc., has been extensively done through the use of inter-simple sequence repeat (ISSR) analysis. The current study attempted to analyze genetic diversity among genotypes of Adenium using ISSR markers. Molecular markers and the identification of polymorphic nucleotide sequences scattered throughout the genome have made it feasible to assess genetic diversity and identify intra and intraspecific genetic connections (35).

Twenty-five (ISSR) primers were used in the current study for molecular characterization of 20 genotypes. PIC was determined by the total number of detectable alleles and gene diversity was equal to the distribution of their frequency. For dominant markers viz RAPD, the maximum PIC limit was 0.5 and effective multiple ratio (EMR) was found to be 9.09–17:00, while for ISSR markers EMR was 6.40–56.00 (36-38).The proven duplicability of ISSR banding patterns was a crucial pre-requisite for detecting variation and estimating crop variability. The accuracy and efficacy of these markers have been verified by numerous other researchers (39). They found that if an experimental technique was carefully tested and care was taken to avoid changing any of the experimental parameters, repeatable results can be produced when a specific template DNA and primer combination was used.

All of the 25 ISSR primers that were screened produced distinct polymorphic loci in the 20 genotypes that were examined (Fig. 6). Out of 206 loci, 165 were polymorphic and the

average number of polymorphic loci per primer was 6.60, meaning that the average polymorphism in the current study was 81.51 %. The percentage of polymorphism (81.51 %) in the DNA analyses of the 20 genotypes indicated the degree of genetic variation among the genotypes. The high level of variability among the adenium genotypes may be due to the natural cross-pollination during evolution. The selection procedure usually employed in breeding studies of different genotypes with desired traits relied heavily on these polymorphic loci.

Primers TriCAC3 RC displayed the highest percentage of polymorphic loci (12 out of 13 loci exhibiting polymorphism; 92.30 %), TriCAC3 YC with 9 polymorphic loci out of 11 loci resulting in (81.81 %) polymorphism, TriAAG3 RC with 8 polymorphic loci out of 10 loci resulting in (80.00 %) polymorphism and DICA3 RC with 8 polymorphic loci out of 9 loci exhibiting (88.88 %) polymorphism. The variety in the various Adenium genotypes used may be the cause of the highest polymorphism. The same has been reported earlier in Gladiolus species using RAPD markers in saffron (40-43).

To determine the marker's discriminating power, the PIC value considers the total number of alleles at a locus and the relative frequencies of the alleles. The PIC values in this investigation varied from 0.10 to 0.32. For every locus, the average PIC score was 0.21. The highest PIC value of 0.32 % was recorded in the primer TriCAC3YC, followed by TriACG3 RC (0.31) and DICA3 RG (0.29) and the lowest was DiCA3 YG (0.10). Similar results were obtained in ginger, where the average PIC value was 0.7532 and the range of PIC values was 0.6560 to 0.8496 (44). Similar reports were obtained in gladiolus and saffron (45, 46).

A dendrogram was constructed using cluster analysis and the Jaccard's similarity coefficient matrices were generated from ISSR markers. Two main clusters were formed due to the variation in amplification pattern. Cluster I formed two subclasses viz IA and IB. Sub cluster I A comprised of the genotypes Golden Crown, Sudharsan, Mung Siam, Harry Potter, Picotee, Home Run, Buttons, Mor Lok Dork and Soft where sub cluster I B comprised of Deang Siam, Miss India, My Country, Noble Queen, Nilakaan, Red Giant, White Lucky and Arrogant. Likewise, cluster II comprised of Artic Snow and Triple Star and the Genotype Pink Beauty stands apart from all clusters, existing independently from them. Similar findings were reported in Ginger with RAPD markers, where 12 genotypes of ginger were separated into two major clusters (46).

Jaccard's Coefficient generated a similarity matrix for 20 Adenium genotypes. The genotypes Miss India and My Country (0.7165) and Home Run and Buttons (0.6960) were found to have the highest similarity. Artic Snow genotype, however, showed the lowest Similar Index (0.2517). A similar pattern was observed in the Gladiolus accessions, where in Punjab Dawn and Wine & Roses had the highest genetic similarity (0.824), while Her Majesty and Eurovision had the lowest genetic similarity (0.172) (47). The UPGMA method was used to examine genetic variation in plants and was predicated on the notion that the mutation rate between distinct ecotypes was constant. It was frequently used to assess ISSR polymorphism. Twenty genotypes and 25 primers were used in this study to produce 206 distinct loci. The findings given here demonstrate the value of ISSR in the analysis of the genetic variability distribution within this significant ornamental species. The ISSR seems to have many benefits and a high-resolution capacity when it comes to determining genetic distances. In addition to portion to conserve the Adenium genome, this marker shows promise in identifying the genetic variation among genotypes of the Adenium. The data it yields will also be useful in precisely identifying the variations.

Docking

The molecular docking study was performed to explore the interaction between vitamin E, a primary compound from *Adenium obesum* and *Escherichia coli* (*E. coli*) proteins. Vitamin E's antioxidant properties contribute to the observed protective effects by reducing oxidative stress and supporting immune function. The three-dimensional structure of vitamin E was modelled and optimized to ensure its stability for docking. A redocking experiment was conducted to validate the docking protocol and ensure the algorithm's accuracy. The results indicated that vitamin E did not form any hydrogen bonds with E. coli proteins. This can be explained by the predominantly hydrophobic nature of vitamin E. The molecule consists mainly of a chromanol ring with a single hydroxyl group (-OH) and a long hydrophobic tail, which limits its potential for hydrogen bonding interactions. Instead, vitamin E likely interacts with the *E. coli* protein through hydrophobic forces, as evidenced by the absence of hydrogen bonds in the docking results. Hydrophobic interactions, such as van der Waals forces, are likely to dominate the interaction between vitamin E and the bacterial proteins, as is typical for nonpolar compounds in a hydrophobic environment. The diagram accompanying the docking results further supports this conclusion, showing a non-bonding interaction between vitamin E and the tryptophan (TRP) residue at position 77 of the *E. coli* receptor. Tryptophan is an aromatic amino acid that is commonly involved in hydrophobic interactions, which aligns with the observed lack of hydrogen bonding. This suggests that hydrophobic contacts primarily drive vitamin E's interaction with the bacterial protein.

Conclusion

The study highlights the effectiveness of ISSR markers in revealing genetic polymorphism and variability among *Adenium obesum* genotypes. GC-MS analysis of ethanolic leaf extracts identified bioactive compounds, indicating potential medicinal properties. The current investigation focuses on the analysis of volatile compounds alone through GC-MS. Further studies must be carried out for anthocyanin characterization and other pigments through HPLC / LCMS. This research emphasizes Adenium's importance in genetic studies, conservation and pharmacological exploration and it is also possible to develop improved varieties with greater benefits.

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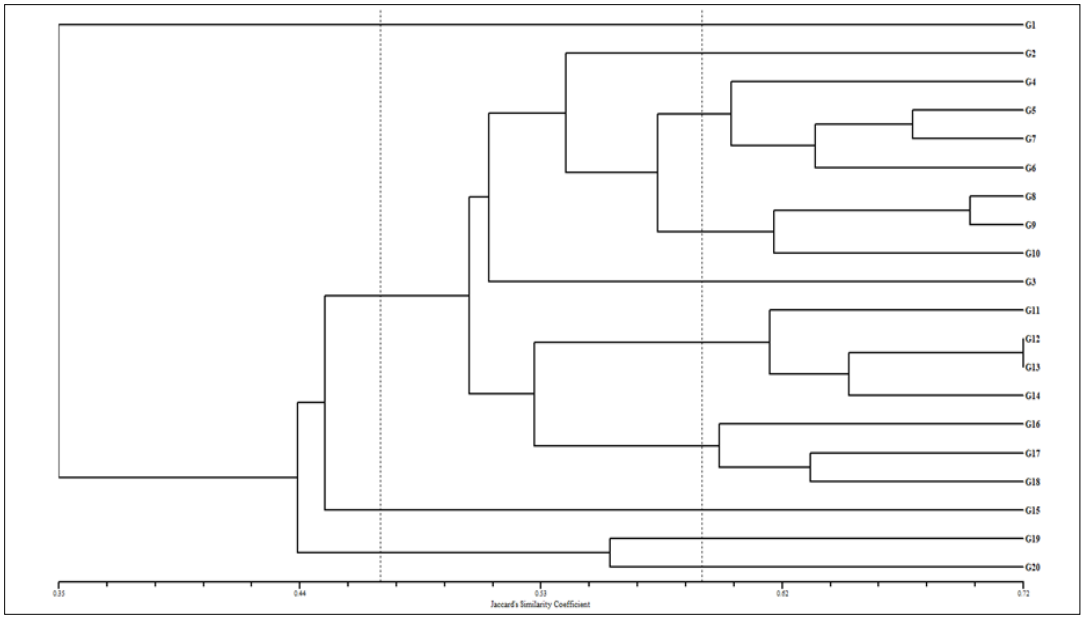

Fig. 6. Cluster diagram based on ISSR data for *Adenium* genotypes.

Authors' contributions

SP guided the research by formulating the research concept, securing funds and approving the final manuscript. SD assisted in experimenting with and analysing the data. VAS contributed by developing the ideas, reviewing the manuscript and helping procure research grants. RR contributed by imposing the experiment and helped edit, summarise and revise the manuscript. VPS helped in summarizing and revising the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

Ethical issues: None

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