Stem epidermal properties of four *Caralluma* (*Apocynaceae*) species in Yemen and their taxonomic significance

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Abstract. About 19 epidermal succulent stem characters (five qualitative and 14 quantitative) of four medicinal and often confused *Caralluma* species in Yemen (*C. penicillata*, *C. quadrangula*, *C. subulata* and *C. tuberculata*) were investigated using compound light microscopy. The results indicated that the studied epidermal characters showed high significance in distinguishing between the investigated taxa. Furthermore, based on the studied epidermal properties, statistical analysis, Two-Way Cluster Analysis (TWCA), and Principal Component Analysis (PCA) illustrate the taxonomic relationship between the studied species, which agrees with the earlier taxonomic studies on the genus *Caralluma*.

Key words: *Caralluma penicillata, C. quadrangula, C. subulata, C. tuberculata,* epidermal cells, stomata properties

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Introduction

Caralluma R. Br. is an essential medicinal genus that belongs to the family *Apocynaceae*, subfamily *Asclepiadoideae*, and is a member of a stem succulents group named stapeliads (Bruyns & al. 2010; Sharawy & al. 2015). *Caralluma* comprises *ca*. 120 species, mainly distributed in dry to semidry tropical regions in Africa, Southwest Europe (Spain), Asia in Arabia, Pakistan, and India (Mabberley 1987; Bruyns & al. 2010; Sharawy & al. 2015).

The taxonomic status of the genus *Caralluma* is still unclear, which may be due to the fact that it has gradually become crowded with species that cannot be placed in other genera and frequently bear little resemblance to one another (White & Sloane 1937; Plowes 1995), or because many plants of the genus *Caralluma* exhibit intermediate features in their habitat because of their potency to inter-hybridize (Mahmood & al. 2010). Brown (1810) described the genus *Caralluma*, which included an Indian species characterized by very distinctive elongated flowering stems.

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Schumann (1895) distinguished three sections, namely *Eucaralluma* Schumann, *Lalacruma* Schumann, and *Boucerosia* Schumann.

Moreover, White & Sloane (1937) grouped the species of the genus *Caralluma* into nine categories (Eucaralluma, Umbellata-europaea, Pauciflora-quadrangula, Corrugata, Ango, Mammillaris, Carnosa-incarnata, Marlothii, and Maculata-lutea), which included 50 species from southern parts of Africa in four of their nine informal groups.

Furthermore, taxonomists who studied the southern African *Caralluma* species considered that the nine groups suggested by White and Sloane (1937) were artificial, and thus transferred three groups of *Caralluma* species into the distinct genera *Orbeopsis* L.C. Leach, *Pachycymbium* L.C. Leach, and *Quaqua* N.E. Br. (Bruyns 1983).

Another decrease in the number of *Caralluma* species was also brought about by the transfer of six species to the genera *Orbea* Haw., *Tridentea* Haw., and *Pectinaria* Haw., leaving only a few species from the southern parts of Africa listed within the genus *Caralluma* (Leach 1978, 1980; Bruyns 1981; Gilbert 1990; Plowes 1995).

Based on the stem and flower morphology, Gilbert (1990) identified 56 species of *Caralluma*, classifying them into four subgenera: *Boucerosia* (Wight & Arn.) M. Gilbert (27 species), *Caralluma* (R. Br.) M. Gilbert (21 species), *Desmidorchis* (Ehrenb.) M. Gilbert (five species), and *Urmalcala* M. Gilbert (three species).

Moreover, according to Plowes (1995), 70 species of the genus *Caralluma*, including the species reported by Gilbert in 1990, were reclassified into 17 genera listed in two groups on the basis of their morphological concept. Group 1 included eight genera characterized by slender stems and thin racemose or tapering flowering tips (*Caralluma* R. Br., *Saurolluma* Plowes, *Spiralluma* Plowes, *Australluma* Plowes, *Spathulopetalum* Chiov., *Somalluma* Plowes, *Cryptolluma* Plowes & *Caudanthera* Plowes); Group 2 comprised nine genera with flowers in terminal umbels or sub-terminal pseudoumbels; stem apices not slenderly tapered nor deciduous (*Desmidorchis*, *Boucerosia*, *Apteranthes* J.C. Mikan, *Sulcolluma* Plowes, *Cylindrilluma* Plowes, *Borealluma* Plowes, *Monolluma* Plowes, *Sanguilluma* Plowes & *Crenulluma* Plowes).

However, none of the latest regional studies or reports on the genus *Caralluma* (Gilbert 2003; Albers & Meve 2004; Lavranos 2006; Bruyns & al. 2010; Shrawy & al. 2015) followed the Plowes classification.

Furthermore, Plowes (2010) has reviewed the genus *Caralluma* R. Br. and reported 24 genera split from the genus *Caralluma* and listed by him into three groups based on their morphological aspects: Group A (species with slender stems and thin racemose or tapering flowering tips) includes nine genera (*Caralluma*, *Saurolluma*, *Spiralluma*, *Australluma*, *Pleuralluma* Plowes, *Spathulopetalum, Somalluma, Cryptolluma,* and *Caudanthera*), Group B (species with flowers in terminal umbels or sub-terminal pseudoumbels; stem apices not slenderly tapered nor deciduous) also includes nine genera (*Desmidorchis*, *Boucerosia*, *Apteranthes*, *Sulcolluma*, *Cylindrilluma*, *Borealluma*, *Monolluma*, *Sanguilluma,* and *Crenulluma*) and Group C (other species listed earlier in the genus *Caralluma* which differ from the groups A and B) comprises six genera (*Angolluma* R. Munster, *Anomalluma* Plowes, *Orbea*, *Orbeopsis*, *Orbeanthus* L.C. Leach, and *Pachycymbium* L.C. Leach).

Additionally, in the same year, Bruyns & al. (2010) have studied the evolutionary relationships of *Caralluma* R. Br. taxa (including some genera proposed by Plowes 1995), the largest genus in stapeliad by analyzing the data from four molecular markers: two plastid regions, psbA, trnH, and trnT-F, and two nuclear regions, ITS and ncpGS. They have concluded that the genus *Caralluma* includes only two entities that should be recognized taxonomically.

On the other hand, using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Random Amplified Polymorphic DNA (RAPD-PCR) techniques, Sharawy & al. (2015) revised systematically the thirteen species belonging to the genus *Caralluma* (comprising some genera generated by Plowes 1995) grown in Saudi Arabia. Based on that study, it was concluded that the genus *Caralluma* was variable, and taxonomists have proposed diverse suggestions about which species should be listed under it. Classification of this genus is still under discussion.

Furthermore, Bruyns & al. (2017) transferred 357

species of highly succulent stapeliads, including 45 *Caralluma* taxa, and keyed them out among the seven sections within the genus *Ceropegia* L. This has not been generally followed, and websites with species lists, such as POWO (2024) and WFO (2024) did not even list the *Ceropegia* names as synonyms.

Earlier research (Gilbert 1990; Plowes 1995, 2010) has indicated that some stem morphological characteristics (stem apices shape, stem shape, and stipular rudiments shape and arrangement on the stem) and certain floral properties (pollinar shape, perianth, and flower arrangement on the raceme inflorescence) were used to classify the genus *Caralluma*.

However, Bruyns & Jonkers (1993) noted that in dried sterile specimens the stem properties of certain *Caralluma* did not enable identification. Furthermore, because flowers were only seen during specific seasons, it could be challenging to differentiate between different *Caralluma* species when the flowers were absent. Moreover, Bruyns & Jonkers (1993) mentioned that field experience is necessary to distinguish between *C. penicillata* (Deflers) N.E. Br. and *C. quadrangula* (Forssk.) N.E. Br.

In Yemen, the genus *Caralluma* is represented by 27 species (including 11 genera created by Plowes 1995), including *C. penicillata*, *C. quadrangula*, *C. subulata* (Forssk.) Decne., and *C. tuberculata* N.E. Br. (Wood 1997; Al Khulaidi 2013), which have a great curative potential in folk medicine in Yemen (Abdo 2009; Esmail 2010; Masdoos 2012; Saleem 2013; Albaser & al. 2014; Al-Mulaiki 2018) and some other Asian countries such as Saudi Arabia, Oman and Pakistan (Adnan & al. 2014; Albaser & al. 2014; Waheed & al. 2011; Al-Massarani & al. 2019). *Caralluma penicillata* is utilized for antihyperglycemic and antitrypanosomal treatment of gastric ulcers, and in some communities is used to cure snake and scorpion bites (Adnan & al. 2014; Albaser & al. 2014).

Nonetheless, *C. quadrangula* is traditionally used for wound healing, diabetes, and general tonic vegetable activity (Adnan & al. 2014; Al-Massarani & al. 2019). Also, *C. subulata* has traditionally been used to treat diabetes (Alamier & al. 2023).

Caralluma tuberculata is widely used for its anti-inflammatory and anti-tumor properties. It is also applied for various conditions, including blood purification, diabetes, liver disease, rheumatism, dysentery, ulcers, constipation, hepatitis B and C, freckles, and pimples. (Waheed & al. 2011; Adnan & al. 2014).

Caralluma penicillata [*Desmidorchis penicillata* (Deflers) Plowes] is an erect, branched succulent herb, with a quadrangular slightly winged grey-green stem and flowers with a pale brown corona, growing in the low and medium altitude mountains of Yemen, within the altitudinal range of 300-1600 m. a.s.l. (Wood 1997; Al Khulaidi 2013).

Caralluma quadrangula [*Monolluma quadrangula* (Forssk.) Plowes] is a strongly branched erect succulent herb, with a grey quadrangular stem with blunt teeth and flowers with pale yellow to rich yellow corona, growing in the low, medium, and high-altitude mountains in Yemen, within the altitudinal range of 500-2500 m a.s.l. (Wood 1997; Al Khulaidi 2013).

Caralluma subulata is a strongly branched, erect succulent herb, with a grey-green quadrangular stem with blunt teeth and flowers with a white outer corona and a red-brown inner corona, growing in the low and medium altitude mountains in Yemen, within the altitudinal range of 300-1300 m a.s.l. (Wood 1997; Al Khulaidi 2013).

Caralluma tuberculata [*Borealluma tuberculata* (N.E. Br.) Plowes] is a strongly branched erect succulent herb, with a grey-green or glaucous quadrangular stem with blunt teeth, flowers with a pale purple corona, growing in high mountain areas of Yemen, within the altitudinal range of 2000-2500 m a.s.l. (Wood 1997; Al Khulaidi 2013).

According to Goldberg (1988), the anatomical structure of the plant body is believed to be simple and composed of just a few cell types that can differ morphologically. In the succulent stem of *Caralluma* species, the epidermis layer is the outermost cell layer. It is made of several differentiated cell types that vary in their structure and function, including epidermal cells, guard cells and subsidiary cells, as well as stomata pores (Mauseth 2004; Masrahi & al. 2012).

It has been mentioned in many studies that the epidermal layer's qualitative and quantitative characteristics are sufficient to distinguish between the different genera, and occasionally, these characteristics can

be used to differentiate between the species. (Grace & al. 2009; Coopoosamy & Naidoo 2011; Ibrahim & al. 2016, 2018, 2021).

However, no attempts appear to have been made to study the stem epidermal properties of *Caralluma* species. Thus, the present study aims to investigate the epidermal features of four *Caralluma* species, namely, *C. penicillata*, *C. quadrangula*, *C. subulata*, and *C. tuberculata,* and to assess their taxonomic significance in studying the relationships between the four species.

Material and methods

1. Plant material

Four *Caralluma* species, *C. penicillata*, *C. quadrangula*, *C. subulata*, and *C. tuberculata*, were collected from medium and high-altitude mountains in Yemen in July 2022 (Table 1, Fig. 1). Plant materials were identified by using the available taxonomical and floristic literature (Wood 1997; Collenette 1999; Al Khulaidi 2013).

A specimen of each species (*C. penicillata, C. quadrangula*, *C. subulata*, and *C. tuberculata*) has been assigned a herbarium number (BHSS743, BHSS730, BHSS740, and BHSS733, respectively) and deposited at the Faculty of Science Herbarium for future reference.

Succulent branches of each collected and identified species were planted in the experimental farm at the Faculty of Science, Sana'a University, in August 2022 under uniform nursery conditions. Eight subpopulations (two subpopulations for each species, and each subpopulation including 15 individuals) were produced (Ibrahim & al. 2020). The stem epidermal properties of the four species were investigated in speci-

Table 1. Locality and sample collection of the investigated *Caralluma* species.

No	*Caralluma* species	Location	Date	Coordinates		Altitude (m a.s.l.)	Herbarium No
				longitude	latitude		
1.	*C. penicillata*	Al khofage village, Toor Al-Baha District, Lahaj Governorate, Yemen	27 July, 2022	44°29'34.46"E	13°17'73.14"N	720 m.a.s.l.	BHSS 743
2.	*C. quadrangula*	Jabal An-Nabi Shu'ayb, Bani Matar, District, Sana'a Governorate, Yemen	22 July, 2022	43°58'52.02"E	15°14'10.08"N	2850 m.a.s.l.	BHSS 730
3.	*C. subulata*	Al khofage village, Toor Al-Baha District, Lahaj Governorate, Yemen	26 July, 2022	44°30'26.68"E	13°17'66.88"N	600 m.a.s.l.	BHSS 740
4.	*C. tuberculata*	Jabal An-Nabi Shu'ayb, Bani Matar, District, Sana'a Governorate, Yemen	23 July, 2022	44° 0'24.48"E	15°14'32.34"N	2787m.a.s.l.	BHSS 733

Fig. 1. *Caralluma taxa* (flowering branches): **A,** *C. penicillata*; **B,** *C. quadrangular*; **C,** *C. subulata*; **D,** *C. tuberculata.*

mens of the obtained subpopulations (under uniform nursery conditions) in the experimental farm at the Faculty of Science, Sana'a University.

2. Epidermal investigation

Longitudinal sections were made in 30 succulent branches from each species (15 succulent branches from each subpopulation) by a razor blade, each section measuring about 10 cm^2 (modified after Ibrahim & al. 2021).

To get a clear, one-cell-thick epidermal layer, all 30 sections of each species were cleaned with water and a camel hair brush (Ibrahim & Ayodele 2013; Ibrahim & al. 2021). The epidermal layers were stained with safranin, and then each stained layer was mounted in glycerol on clear slides under a cover slide (Sreelakshmi & al. 2014).

A light microscope (Leica ATC 2000) with magnification ×400 was used for observing the slides; photographs of the epidermal view at ×400 magnification were taken by a Canon (IXUS255 HS) digital camera to determine the qualitative and quantitative characteristics of the epidermal layer in each species under investigation.

Furthermore, the quantitative characteristics were measured with an ocular micrometer calibrated by a stage micrometer (magnification ×400, one ocular small division = 2.5 μm) and Image J software (Ibrahim & al. 2021).

About 19 epidermal characteristics (five qualitative and 14 quantitative characters) were studied (Tables 2 & 3) by using the epidermal layer nomenclature mentioned by Dilcher (1974).

Table 2. List of the examined epidermal stem qualitative characters of the four *Caralluma* species under investigation.

No.	Characters		
1	Shape of epidermal cells	Isodiametric [1]	Not so [0]
2	Arrangement of epidermal cells	Random [1]	Non-random [0]
3	Epidermal cell outline	Straight [1]	Not so [0]
4	Epidermal cell surface ornamentation	Absent [1]	Present [0]
5	Stomata type based on subsidiary cells	Polycystic, Anisocytic, Paracytic, Tetracytic [1]	Anisocytic, Paracytic, Tetracytic [2]

Table 3. List of the examined epidermal stem quantitative characters of the four *Caralluma* species under investigation.

No.	Characters
1	No. of epidermal cells
2	Cell wall thickness (µm)
3	Length of epidermal cells (µm)
4	Width of epidermal cells (µm)
5	Area of epidermal cells (µm^2)
6	No. of stomata
7	Length of guard cells (µm)
8	Width of guard cells (µm)
9	Area of guard cells (µm^2)
10	Area of guard cells × Franc'os constant 0.79 (µm^2)
11	Length of stomata (µm)
12	Width of stomata (µm)
13	Area of stomata (µm^2)
14	Stomata index %

3. Statistical analysis

The taxonomic value of quantitative epidermal properties was confirmed by a one-way ANOVA test using Graph Pad Prism software version 8.0.2 (263); when the P-value was <0.05, the quantitative properties were deemed to be substantially different. The relationship between the four investigated *Caralluma* species has been demonstrated in a dendrogram based on the obtained stem epidermal data (Tables 2 & 3), by creating a data matrix for numerical analysis with a PC-ORD Windows version 7.09 (TWCA) (Alhadi & al. 2023).

Furthermore, PCA was utilized to emphasize the results obtained from cluster analyses using a Minitab software version 14.1.

Results

About 19 (five qualitative and 14 quantitative) stem epidermal characters of four *Caralluma* species (*C. penicillata, C. quadrangula, C. subulata*, and *C. tuberculata*) were investigated to evaluate their taxonomic significance. The qualitative stem epidermal properties in Table 4 and Figs 2-5 indicate that the epidermal cells of the studied species were found to be isodiametric in shape, randomly arranged (pentagonal, hexagonal, and polygonal) and with a smooth surface.

Based on the shape and arrangement of the subsidiary cells, the stem epidermis of *C. quadrangula*, *C. subulata*, and *C. tuberculata* was characterized by the presence of four types of stomata; polycytic, an-

Table 4. Data matrix of the stem epidermal qualitative characters of the four *Caralluma* species under investigation.

Character	*C. penicillata*	*C. quadrangula*	*C. subulata*	*C. tuberculata*
Shape of epidermal cells	1	1	1	1
Arrangement	1	1	1	1
Cell outline	1	1	1	1
Surface ornamentation	1	1	1	1
Stomata type based on Subsidiary cells	2	1	1	1

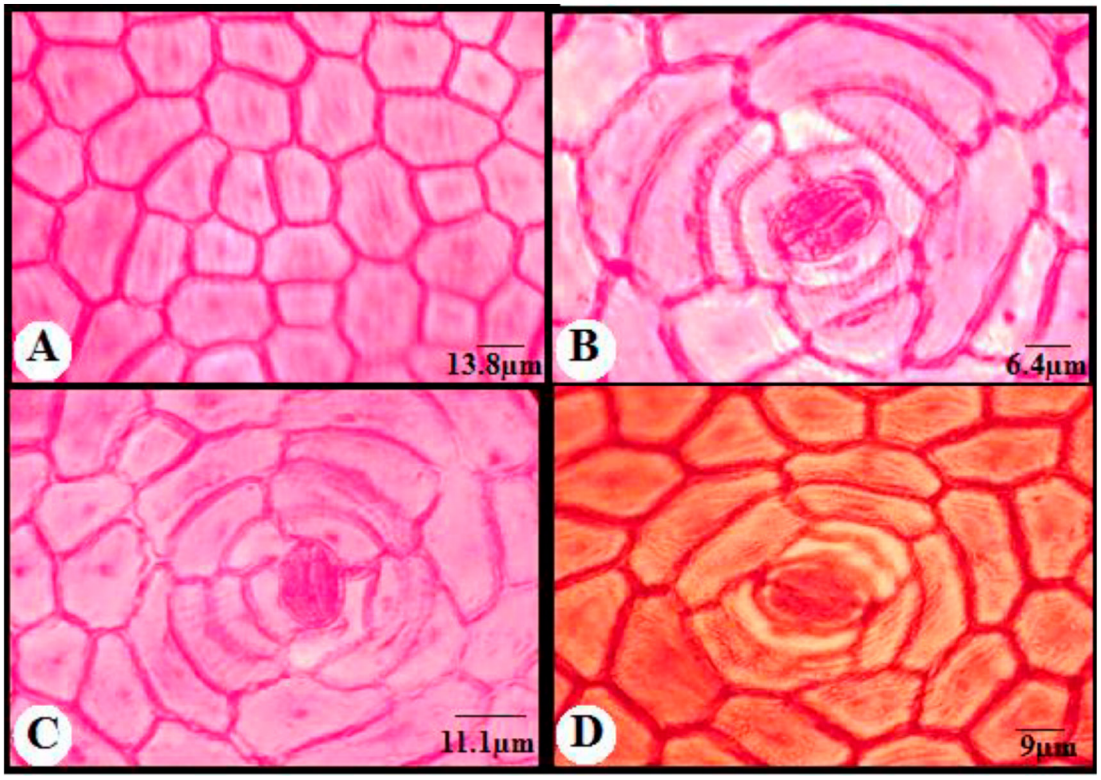

Fig. 2. Stem epidermal characters of *C. penicillata*: **A,** epidermal cells; **B–D,** types of stomata; **B,** anisocytic stomata; **C,** tetracytic stomata; **D,** paracytic stomata.

isocytic, tetracytic, and paracytic. *C. penicillata* was

differentiated from the other four studied species by having only three types of stomata: anisocytic, tetracytic and paracytic.

On the other hand, the quantitative characters (Table 5) of the studied species have revealed that *C. subulata* had the highest mean epidermal cell density (211 epidermal cells) in the ×400 field of view, followed by *C. quadrangula* (208 epidermal cells), *C. penicillata* (179 epidermal cells), and *C. tuberculata* (120 epidermal cells).

Moreover, *C. quadrangula* possessed the highest mean epidermal cell-wall thickness (3.7µm), followed by *C. subulata*, *C. penicillata,* and *C. tuberculata*, with a mean epidermal cell-wall thickness of 2.8, 2.7, and 2.3 µm, respectively (Table 5).

The largest epidermal cells with a mean area of 1492.4 µm^2 were noted in the stem epidermis of *C. tuberculata* (Table 5), followed by *C. penicillata* and *C. quadrangula* (mean area of 719.8 and 699 µm^2, respectively), while *C. subulata* had the smallest epidermal cells in the area (mean area of 643.4 µm^2).

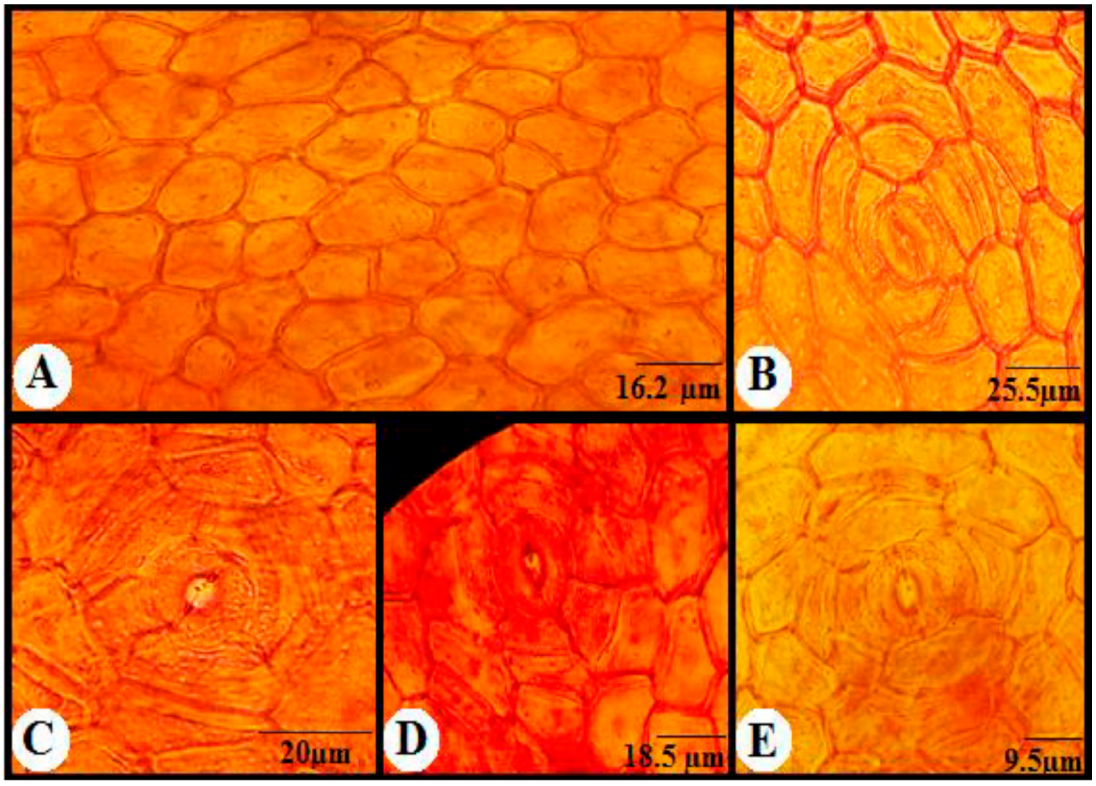

Fig. 3. Stem epidermal characters of *C. quadrangular*: **A,** epidermal cells; **B–E,** types of stomata; **B,** anisocytic stomata; **C,** tetracytic stomata; **D,** polycytic stomata; **E,** paracytic stomata.

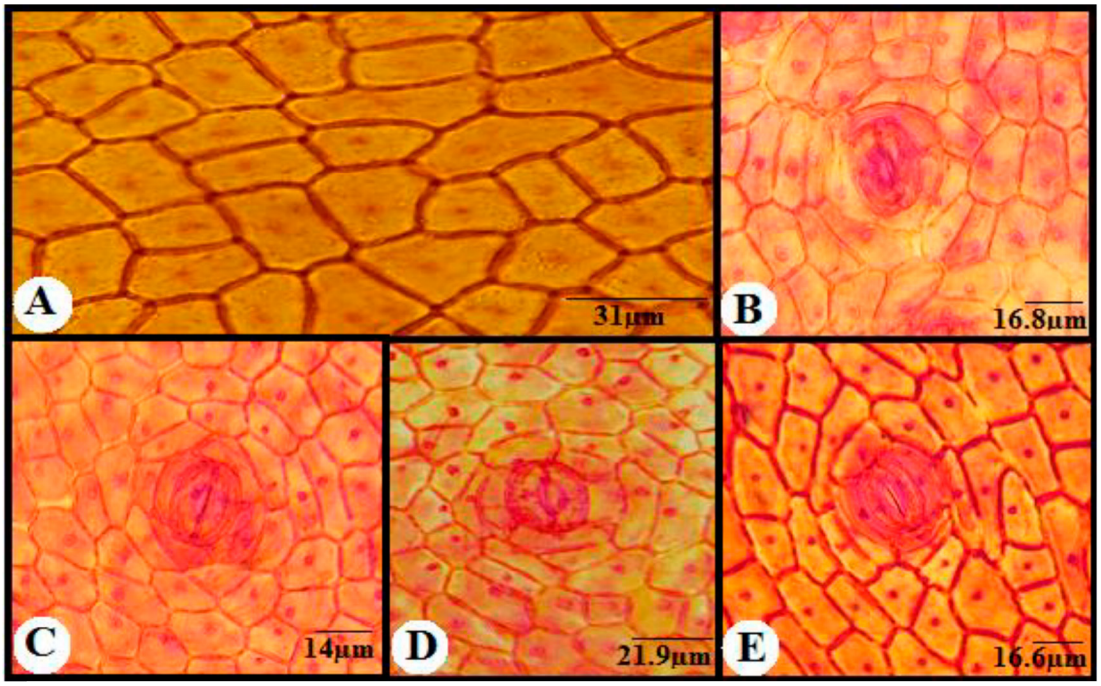

Fig. 4. Stem epidermal characters of *C. subulata*: **A,** epidermal cells; **B–E,** types of stomata; **B,** anisocytic stomata; **C,** tetracytic stomata; **D,** polycytic stomata; **E,** paracytic stomata.

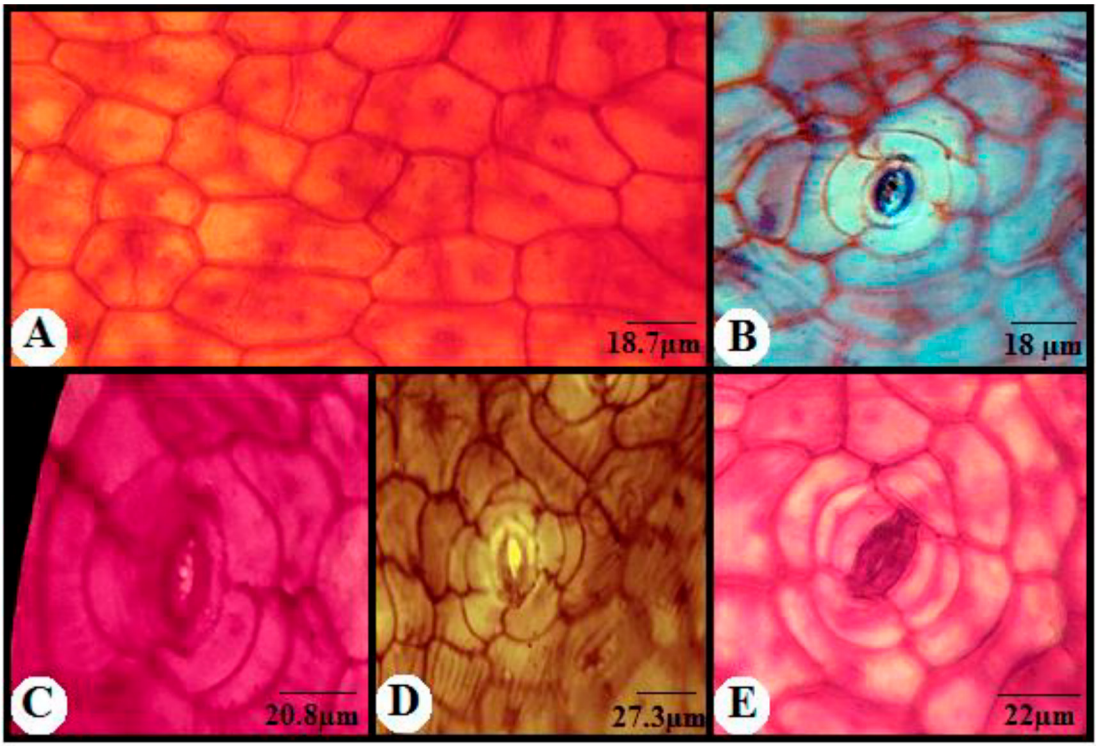

Fig. 5. Stem epidermal characters of *C. tuberculata*: **A,** epidermal cells; **B–E,** types of stomata; **B,** anisocytic stomata; **C,** tetracytic stomata; **D,** polycytic stomata; **E,** paracytic stomata.

On the other hand, *C. tuberculata*, *C. penicillata,* and *C. quadrangula* had the same mean frequency of stomata appearance (three stomata / ×400 field of view), while *C. subulata* showed lower mean stomata frequency (two stomata per ×400 field of view) (Table 5). However, *C. subulata* possessed the largest guard cells, with a mean area × Francos' constant of about 278.6µm^2 (Table 5), followed by *C. quadrangula*, *C. tuberculata,* and *C. penicillata,* with a mean guard cells area × Francos constant of 261.6, 260.5, and 223.7µm^2, in sequence.

Moreover, the stem epidermis of *C. tuberculata* had the largest stomata area (with a mean stomata area of 1039.1 µm^2), followed by *C. quadrangula*, *C. subulata*, and *C. penicillata* with a mean stomata area of 919.1, 864.7, and 582 µm^2 successively (Table 5).

The highest stomata index was found to be 2.1 in *C. tuberculata* stem epidermis, followed by 1.8 and 1.5 in *C. penicillata* and *C. quadrangula* stem epidermis, respectively, while the smallest stomata index (0.8) was recorded in the stem epidermis of *C. subulata* (Table 5).

Table 5 illustrates that six epidermal quantitative characteristics (number of epidermal cells in ×400 view, cell wall thickness, length of guard cells, length, width, and area of stomata) show an extremely high taxonomic significance in distinguishing between *C. penicillata* and *C. quadrangula*.; while the number of epidermal cells and the number of stomata in ×400 view, length of guard cells, length, width, area of stomata, and stomata index display an extremely high taxonomic value in differentiating between *C. penicillata* and *C. subulata* (Table 5).

The number of epidermal cells in ×400 view, length, width, and area of epidermal cells, length of guard cells, length, width and area of stomata exhibit an extremely high taxonomic relevance in separating *C. penicillata* from *C. tuberculata* (Table 5).

Table 5 indicates that three epidermal quantitative characteristics - epidermal cell wall thickness, number of stomata in ×400 view, and stomata index show an extremely high taxonomic significance in differentiating between *C. quadrangula* and *C. subulata*, while the number of epidermal cells in ×400 view,

Table 5. Quantitative stem epidermal characters of the four *Caralluma* species under investigation.

	Characters/Species	*C. penicillata*	*C. quadrangula*	*C. subulata*	*C. tuberculata*
1.	No. of Epidermal cells Min (Mean ±SD) Max	151(179 ± 22.8) 222	186(208 ± 13.8) 228 a^{****}	177(211 ± 14.9) 234 a^{****}	103 (120 ± 13.6) 146 a^{****}, b^{****}, c^{****}
2.	Epidermal Cell wall thickness(µm) Min (Mean ±SD) Max	2.1(2.7± 0.3)3.1	2.7(3.7 ± 0.4)4.3 a^{****}	2.3(2.8 ± 0.3) 3.2 b^{****}	1.2(2.3 ± 0.5)3.3 a^{**}, b^{****}, c^{****}
3.	Length of Epidermal cells (µm) Min (Mean ±SD) Max	17.3(31.1 ± 8.4) 46.6	22(30.5 ± 4.8) 40.4	16(27 ± 5.1) 36.3	28.4(45.9 ± 11.5) 74.9 a^{****}, b^{****}, c^{****}
4.	Width of Epidermal cells (µm) Min (Mean ±SD) Max	13.9(24.8 ±7.3) 48.4	15.9(22.7 ±4.1)33.2	17.6(24.3 ±5.3) 36.1	19.6(32.4 ±7.4) 57.1 a^{****}, b^{****}, c^{****}
5.	Area of Epidermal cells (µm^2) Min (Mean ±SD) Max	581(719.8 ±95.3) 935.4	408.7(699 ±203.6) 1323.5	381.6(643.4 ±138.6) 885.9	747.5(1492.4 ±512.1) 2445.5 a^{****}, b^{****}, c^{****}
6.	No. of Stomata Min (Mean ±SD) Max	2(3 ±1.1) 5	2(3 ±0.9) 4	1(2±0.7) 3 a^{****}, b^{****}	1(3 ±0.9) 4 c^{****}
7.	Length of Guard cells (µm) Min (Mean ±SD) Max	24.3(28.3 ±2.4) 32.6	28.3(34.3 ±3.1) 38.8 a^{****}	28.6(34.8 ±4.3) 43.9 a^{****}	27.3(38.7 ±3.8) 44.3 a^{****}, b^{****}, c^{***}
8.	Width of Guard cells (µm) Min (Mean ±SD) Max	6.7(9.9 ±1.6) 13.8	7.2(10.1 ±1.3) 11.9	6.9 (9.9 ±1.4) 12.1	6.4 (8.6 ± 1.1) 10.5 a^{***}, b^{***}, c^{***}
9.	Area of Guard cells (µm^2) Min (Mean ±SD) Max	201.7(284.8 ±63.8) 472.6	196.6(333.1 ±63.6) 437.5 a^{*}	230.5 (354.8 ±61.8) 438.6 a^{***}	214.8(331.7 ±57.2) 436.3 a^{*}
10.	Area of Guard cells × Franc'os constant (µm^2) Min (Mean ±SD) Max	158.4(223.7 ± 50.1) 371.1	154.4(261.6 ±49.9) 343.6 a^{*}	181(278.6 ±48.5) 344.4 a^{***}	168.7(260.5 ±44.9) 342.6 a^{*}
11.	Length of Stomata (µm) Min (Mean ±SD) Max	24.3(28.3 ±2.4) 32.6	28.3(34.3 ±3.1) 38.8 a^{****}	28.6(34.8 ±4.3) 43.9 a^{****}	27.3(38.7 ±3.8) 44.3 a^{****}, b^{****}, c^{***}
12.	Width of Stomata (µm) Min (Mean ±SD) Max	16.6(20.5 ±2) 24.6	21.2(26.7 ±2.6) 29.8 a^{****}	18.7(24.6 ±3.3) 32.6 a^{****}, b^{*}	22(26.8 ±2.4) 31.2 a^{****}, c^{**}
13.	Area of Stomata (µm^2) Min (Mean ±SD) Max	426.6(582 ±94.1) 789.4	617.7(919.1 ±152.9) 1112 a^{****}	609.8(864.7 ±204.4) 1430.4 a^{****}	654.9(1039.1 ± 159.2) 1356.4 a^{****}, b^{*}, c^{***}
14.	Stomata index % Min (Mean ±SD) Max	1.2(1.8 ±0.5) 2.4	0.9(1.5 ±0.4) 1.9 a^{*}	0.4(0.8 ±0.3) 1.4 a^{****}, b^{****}	0.9(2.1 ±0.6) 2.8 b^{****}, c^{****}

a. Significant differences in epidermal characters compared to *C. penicillata* epidermal, b. compared to *C. quadrangula* epidermal, c. compared to *C. subulata* epidermal,

*: P< 0.05, **: P< 0.01, ***: P< 0.001 and ****: < 0.0001.

epidermal cell wall thickness, length, width, and area of epidermal cells, length of guard cells, length of stomata, and stomata index exhibit an extremely high taxonomic value in distinguishing between *C. quadrangula* and *C. tuberculata* (Table 5).

The number of epidermal cells in the ×400 view, epidermal cell wall thickness, length, width, and area of epidermal cells, number of stomata in the ×400 view, and stomata index - display an extremely high taxonomic relevance in separating *C. subulata* from *C. tuberculata* (Table 5).

Table 5 shows that five epidermal quantitative characteristics - number of epidermal cells in ×400 view, length of guard cells, length, width, and area of stomata - exhibit an extremely high taxonomic value in distinguishing between *C. penicillata* and the other three studied species, *C. quadrangula, C. subulata,* and *C. tuberculata*.

Moreover, one epidermal quantitative property (epidermal cell wall thickness) displays an extremely high taxonomic value in differentiating between *C. quadrangula* and the other investigated species, *C. penicillata, C. subulata,* and *C. tuberculata* (Table 5), while the number of stomata in ×400 view and stomata index show an extremely high taxonomic significance in distinguishing between *C. subulata* and the other studied species, *C. penicillata, C. quadrangula,* and *C. tuberculata* (Table 5).

Furthermore, four epidermal quantitative properties - number of epidermal cells in ×400 view, length, width, and area of epidermal cells - display an extremely high taxonomic relevance in separating *C. tuberculata* from the other species (*C. penicillata, C. quadrangula,* and *C. subulata*) under investigation (Table 5).

Taxonomic key to the four often confused species, based on the qualitative and quantitative characteristics of epidermal stem

1. Presence of three types of stomata (anisocytic, tetracytic and paracytic) in the epidermal stem peels……***C. penicillata***
– Presence of four types of stomata (anisocytic, tetracytic, polycytic, and paracytic) in the epidermal stem peels……2
2. The number of epidermal cells in ×400 view of epidermal stem peels is below 150 cells……
……***C. tuberculata***
– The number of epidermal cells in ×400 view of epidermal stem peels is above 150 cells……3
3. Stomata index less than 1.5% ……***C. subulata***
– Stomata index higher than 1.5% …***C. quadrangula***

Numerical study

Based on Fig. 6, TWCA shows that the studied species were divided into two main groups (I and II) based on the 19 investigated epidermal characters, with a similarity distance level of 91.4%, where Group I included *C. tuberculata*; while Group II comprised the three remaining species (*C. penicillata, C. quadrangula,* and *C. subulata*).

Furthermore, Group II was subdivided into two subgroups (A & B): Subgroup A included *C. quadrangula* and *C. subulata*, while Subgroup B comprised *C. penicillata* at a similarity distance level of 95% (Fig. 6).

The species in Subgroup A were separated into two clusters. Cluster 1 included *C. quadrangula*, and Cluster 2 included *C. subulata* at a similarity distance level of 98.23% (Fig. 6).

On the other hand, based on the first and second components (Fig. 7), PCA separated *C. tuberculata* from the other studied species. In contrast, the second component separated *C. penicillata* from *C. quadrangula* and *C. subulata* (Fig. 7).

Therefore, the PCA results in Fig.7 confirmed the finding of TWCA in Fig. 6.

Discussion

The current findings show that there is a relationship between the two major groups (Group I and II), indicated by the similarity level (91.4%) between Group I (which includes *C. tuberculata*) and Group II (which includes *C. subulata*, *C. penicillata*, and *C. quadrangula*) in TWCA and the presence of few *C. tuberculata* mem-

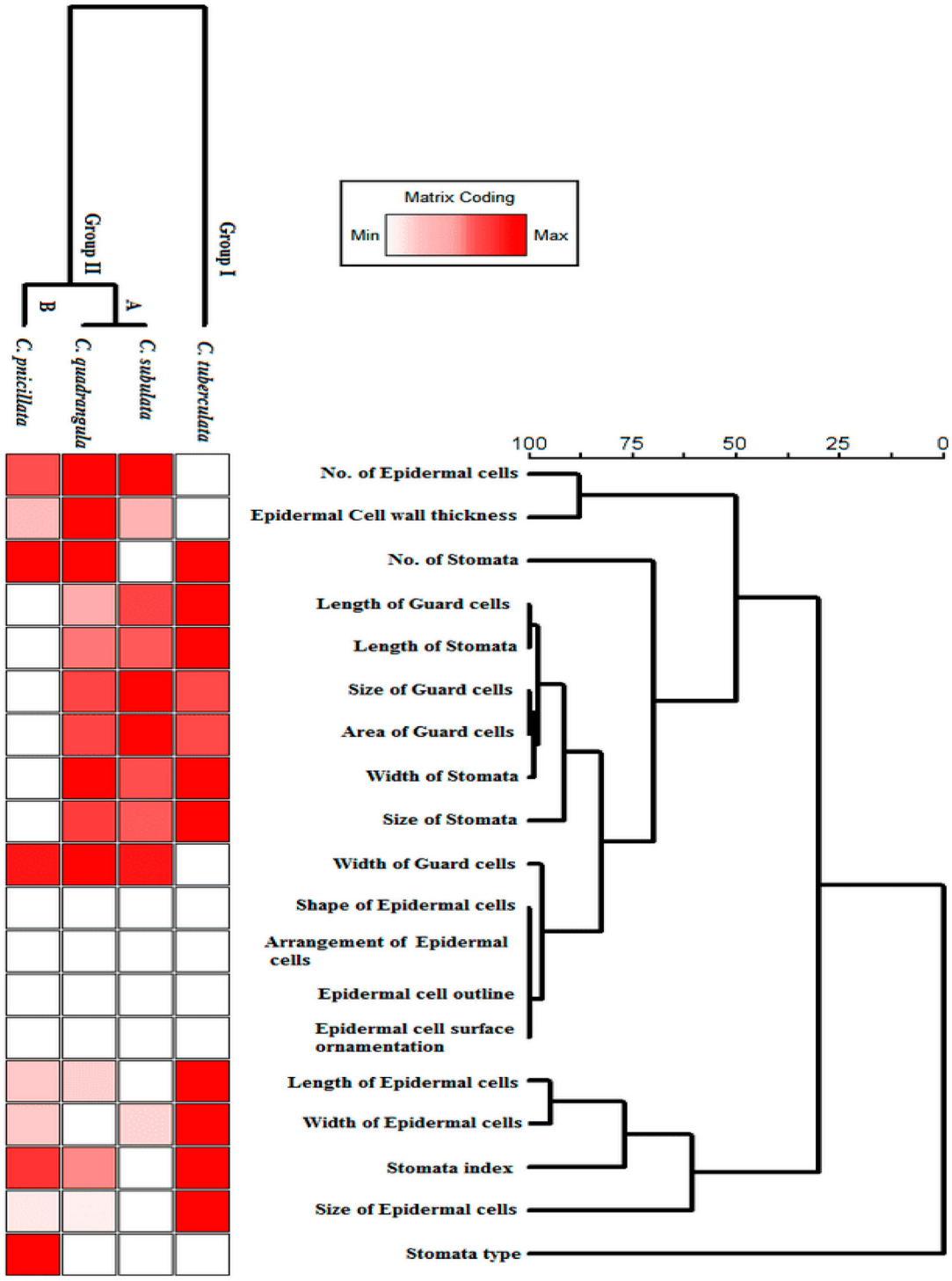

Fig. 6. Cluster analysis illustrates the relationship among the four investigated *Caralluma* species based on 19 (5 qualitative and 14 quantitative) stem epidermal characters by using the Two-Way Cluster Analysis (TWCA) - Group average linkage method.

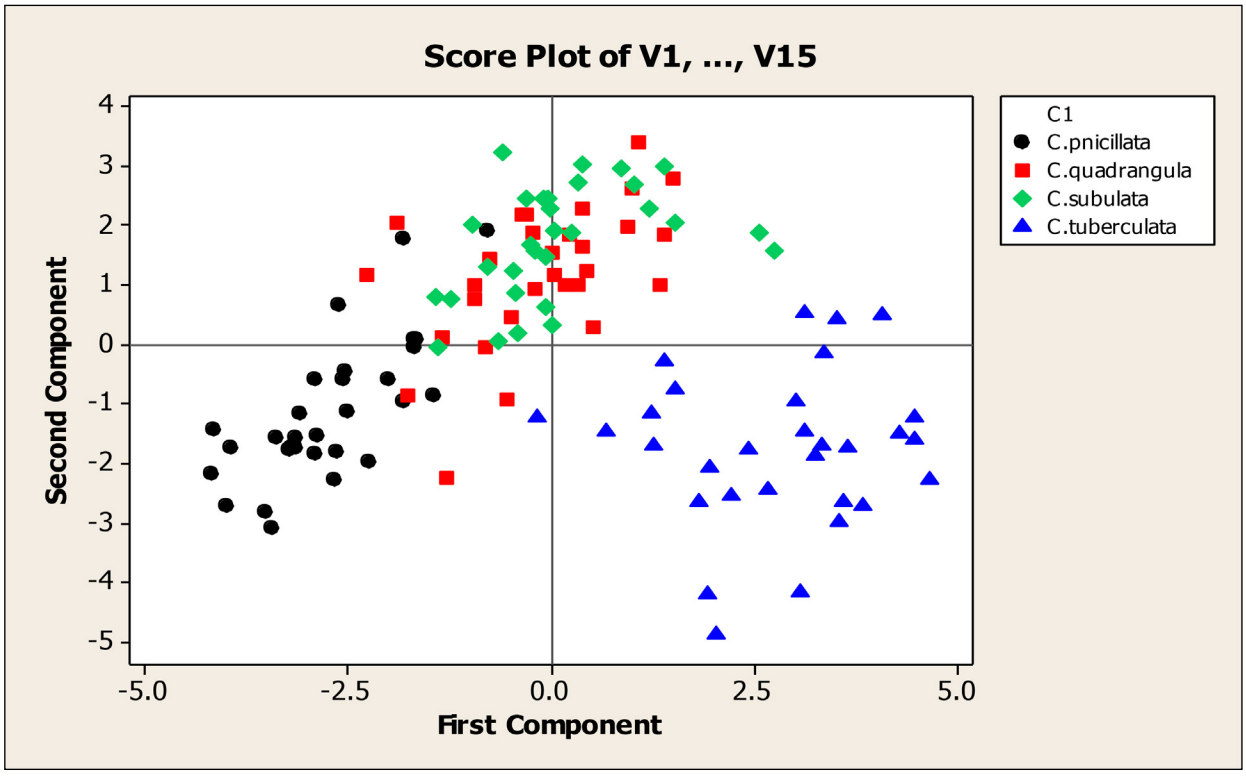

Fig. 7. Principle Component Analysis (PCA) illustrates the relationship among the four investigated *Caralluma* species based on 15 (one qualitative and 14 quantitative) stem epidermal characters.

bers with *C. subulata* and *C. quadrangula* (based on the first component) in PCA. This agrees with the finding of Gilbert (1990), according to him, the four species under study (*C. penicillata, C. quadrangula*, *C. subulata*, and *C. tuberculata*) were classified into four subgenera (*Boucerosia*, *Desmidorchis, Caralluma* and *Urmalcala* correspondingly) under the genus *Caralluma*.

Moreover, the similarity level (95%) between Subgroup A (comprises *C. quadrangula* & *C. subulata*) and Subgroup B (includes *C. penicillata*) in TWCA and the presence of some members of *C. subulata* and *C. quadrangula* with *C. penicillata* (based on the first and second components) in PCA display a relationship between *C. subulata* and *C. quadrangular,* from one side, and *C. penicillata,* from the other side. That result is compatible with the classification of *C. penicillata* (*D. penicillata)* and *C. quadrangula* (*M. quadrangula)* mentioned by Plowes (1995), who placed them in the same group, characterized by flowers in terminal umbels or sub-terminal pseudoumbels and with stem apices that are neither slenderly tapering nor deciduous.

Furthermore, the similarity level (98.23%) between *C. quadrangula* and *C. subulata* in TWCA and the presence of *C. quadrangula* members with *C. subulata* members (based on the second component) in PCA show a relationship between *C. quadrangula* and *C. subulata*. This agrees with the findings of Sharawy & al. (2015).

Conclusion

This study was the first to discuss in detail the properties of the epidermal layer (epidermal cells and stomata properties) of the *Caralluma* succulent stem, and especially the epidermal layer of the four studied *Caralluma* species (*C. penicillata*, *C. quadrangula*, *C. subulata*, and *C. tuberculata*), which exhibits a high taxonomic value in distinguishing between these four often confused species under investigation.

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