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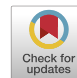
Original article

Gene-targeted molecular phylogeny, phytochemical profiling, and antioxidant activity of nine species belonging to family Cactaceae



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ABSTRACT

Cactaceae plant family comprises over 130 genera and 2000 species of succulent flowering plants. The genera *Mammillaria* and *Notocactus* (*Parodia*), which have medicinal and nutritional applications as well as aesthetic appeal, are considered to be among the major genera of the family. Several species of both genera show morphological and chemical similarities and diversities according to environmental conditions and genotypes. Here, we assessed the genetic relationships of nine species belonging to two major genera *Mammillaria* and *Notocactus* under the family Cactaceae, using two modern gene-targeting marker techniques, the Start Codon Targeted (SCoT) Polymorphism and the Conserved DNA-Derived Polymorphism (CDDP). Besides, we screened the various phytochemicals and evaluated the antioxidant activities of the nine species of cacti. Five out of the 10 SCoT and eight CDDP primers used to screen genetic variations within the nine species yielded species-specific reproducible bands. The entire 156 loci were detected, of which 107 were polymorphic, 26 were monomorphic, and 23 were unique loci. The nine species were categorized into two groups based on the dendrogram and similarity matrix. Phytochemical profiling revealed that sterols, triterpenes, flavonoids, and tannins were found in all the tested species. Additionally, two *Notocactus* species (*N. shlosserii* and *N. roseoluteus*) and one *Mammillaria* species (*M. spinosissima*) revealed a considerable antioxidant activity. Our results demonstrated that gene-targeting marker techniques were highly powerful tools for the classification and characterization of the nine investigated species, despite displaying high similarities at both morphological and phytochemical levels.
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1. Introduction

The family Cactaceae includes approximately 130 genera and 2000 species that were originally native to the New World. Cacti are the best representatives of plants that are good improved toward dry lands and a variety of weathers. Cacti remain famous for their ability to not only grow, but also thrive under stressful environments in numerous parts worldwide, including Australia, India, Mediterranean basin, Middle East, and South Africa. As they do not need much water, they exhibit unusual physiological and morphological characteristics (Osuna-Martínez et al., 2014; Abdelaziz et al., 2019). Different trends in the adaptation of the plants to conditions of water deficit have been identified. Although

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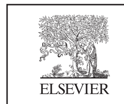

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they are mainly cultivated for their edible fruits, dissimilar parts of these plants are used in the nutrition and cosmetic manufacturing in some countries, thus giving this family a strong economic relevance. They have been globally commercialized as ornamental plants (Hernández-Hernández et al., 2011; Boshara, 2014). Cacti are known to contain several chemical compounds with nutritionally and medicinally desirable properties (Solórzano et al., 2014). In Mexico, Cuba, Colombia, and the United States, the fruits of cacti are used as human food, and some species have a cultural value. In certain rural communities, cactus is predominantly utilized as folk medicine to treat illnesses such as the flu, infections, worms, and urethral problems. Cacti possess a therapeutic effect against cancer cells (Boshara, 2014). The family Cactaceae consists of two major genera, the *Echinocactus* that represents distinctive cacti of the deserts of Mexico and the USA, and *Mammillaria*, which is among the largest genera of North American cacti. They are little-rising, usually globose, and have tuberculate shoots. Together these genera were preciously enclosed in the core classification systems of the 19th century (Vázquez-Sánchezet al., 2013).

The genus *Mammillaria*, which constitutes about 168–171 species, is mostly endangered at the global level. Some species of this genus have a small range of distribution and correspondingly a small population size (Butterworth, 2003; Solórzano et al., 2014). These cacti are fairly small, usually elongated or globular, and profusely flowering. They have colored spines that vary significantly in figure, size, and color. The flowers are typically short and arranged abundantly in rings round the apex of the shoot like a garland. They are famous for their flat, juicy, club-shaped berries, typically of bright red color, which gives a colorful vision during fall. Therefore, *Mammillaria* represents a permanent source of admiration for collectors as well as nature lovers (Mattagajasingh et al., 2006). This appearance can be attributed to their abundant content of anthocyanin pigments. Some species of *Mammillaria*, such as *M. spinosissima, M. theresae*, and *M. flavicentra*, are rich in flavonoids such as quercetin, luteolin, and kaempferol and their glycosides (Cecília et al., 2006; Formagio et al., 2014). Most species of *Mammillaria* are reported to be nontoxic and are used to treat earaches, dysentery, and as insecticide, poison (either as poison or for treatment against poisoning), purgative, pulicide, and snake repellent. *Notocactus* or *Parodia* (the type genus of the family) is a genus of flowering plants in the family Cactaceae native to the uplands of Argentina, Bolivia, Peru, Colombia, Uruguay, and Brazil. *Notocactus* was included in *Parodia* by the International Organization for Succulent Plant Study at the end of the 1980s, a decision which is still controversial even today (Machado et al., 2008). The traditional taxonomy of plants is based on shared morphological, biochemical, cytological, and ecological characteristics/traits. In recent years, several novel molecular marker approaches have been developed and applied in numerous plant genetic studies. The development of these methods is mainly as a result of fast growing in genomic study and has initiated a new tendency away from arbitrary markers toward the development of gene-targeting markers (Osuna-Martínez et al., 2014). As a result of rapidly increasing availability of genomic databases, the progress of efficient markers positioned within or near certain genes has become relatively easier (Hernández-Hernández et al., 2011; Atia et al., 2020). These gene-targeting markers have many other applications, such as in studies on genetic variability and diversity, phylogenetics and systematics, molecular ecology, conservation biology, and developmental biology. At the end of the 2000s, Collard and Mackill successfully developed two of the most effective novel gene-targeting marker systems in plants. These marker systems were named the Start Codon Targeted (SCoT) Polymorphism and Conserved DNA-Derived Polymorphism (CDDP). The principle of the SCoT marker system depended on the small, conserved region flanking the ATG start codon in plant genes. SCoT markers were found to be highly reproducible, which could be due to the use of a single 18-mer primer with an annealing temperature of 50 °C (Butterworth, 2003). SCoT is a dominant marker similar to the random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR). It was successfully used in numerous genetic analyses, mapping of quantitative trait loci (QTL), and in studies on bulk segregation analysis. SCoT markers yielded tremendous success in fingerprinting and diversity analyses in peanut, potato, grapes, and several medicinal plants (Solórzano et al., 2014). On the other hand, the CDDP approach for producing DNA markers in plants was settled grounded on data mining for small conserved amino acid sequences that were found in proteins. It used a single (15 to 19-mer) primer with a fixed annealing temperature of 50 °C for PCR amplification (Cecília et al., 2006) Indeed, gene-targeted fingerprinting techniques such as SCoT and CDDP were developed to combine the well-established practice of arbitrary marker techniques with new procedural novelties, through the combination of gene or promoter in their primers (Mokhtar and Atia, 2019). These features provided the advantages of good reproducibility and improved resolution to gene-targeted markers, by the simultaneous occurrence of dominant and co-dominant bands. Therefore, this study aims to perform a molecular characterization and fingerprinting of nine species of cacti belonging to two genera; Notocactus and Mammillaria, by using two novel gene-targeting markers (SCoT and CDDP) in order assess their genetic relatedness. Additionally, a preliminary screening of their phytochemical constitutions and evaluation of their antioxidant activities was carried out to obtain baseline information on their pharmacological activities.

2. Materials and methods

2.1. Plant material

All plants were obtained from Private Cactus Farm in Shibin El Qanater (Qalyubia, north of Cairo in the Nile Delta region), Egypt. The plants were collected in the flowering stage in the middle of August 2018. Voucher specimens of altogether cactus plants were placed in the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt. Facts about the names of collected samples, their respective codes and voucher numbers are presented in Table 1 and Fig. 1.

2.2. Molecular analysis

2.2.1. Extraction of plant DNA

Genomic DNA was isolated from plants (100 mg) of all the nine-plant species *via* a DNeasy Plant Mini Kit (QIAGEN, Santa Clarita, CA), rendering to the manufacturer's protocol. The extracted DNA concentration was measured using a Qubit® 3.0 Fluorometer (Thermo Fisher Scientific Inc.). The DNA concentrations were adjusted to 10 ng/μL in all samples for subsequent molecular analyses.

2.2.2. Analysis of SCoT polymorphism

PCR-based amplification of SCoT polymorphism was done as outlined by the technique described by Collard and Mackill (2009a). A set of 10 SCoT primers was screened against the nine species of cacti. PCR was carried out on reaction mixtures of 25 μL comprising 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM of each dNTPs, 1 μM of primer, 1U Go-Taq Flexi polymerase (Promega), and 25 ng of genomic DNA. The PCR amplification was set at 94 °C for 5 min for preliminary denaturation, trailed by 35 cycles (94 °C for 1 min, 50 °C for 1 min, and 72 °C for 90 s), and a final elongation at 72 °C for 7 min. The amplification fragments were

Table 1
The cactus species, code numbers, and voucher specimen code.

Name of cactus species	Code Number	Voucher Specimen Code
M. hahniana Werderm	1	14816 II
M. spinosisima Lem.	2	14816 IV
M. supertexta Hort.	3	14816 V
M. polythele Mart.	4	14816 VI
M. springlei J.M.Coult	5	14816 VII
N. shlosserii Vliet	6	14816 III
N. magnificus F.Ritter	7	14816 IX
N. roseoluteus Vliet	8	14816 VIII
N. Leninghausii A.Berger	9	14816 I

resolved using electrophoresis in 1.5% agarose gel comprising ethidium bromide (0.5 μg/mL) in 1× TBE buffer. A 100 bp DNA ladder was used. The PCR products were visualized in UV light and photographed.

2.3. Analysis of CDDP

PCR-based amplification of CDDP was done as described by Collard and Mackill (2009b). A set of eight CDDP primers were screened for the nine cacti species. The amplification reactions were done on 25 μL containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM of each dNTP, 1 μM of primer, 1U Go-Taq Flexi polymerase (Promega), and 25 ng of genomic DNA.

A PCR cycle was done: a preliminary denaturation at 94 °C for 3 min, and then 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; the final elongation was carried out for 5 min. All the PCR magnification fragments were electrophoresed on 1.5% agarose gels in 1X TBE buffer stained with ethidium bromide. A 100 bp DNA ladder was used as a DNA marker. The PCR products were visualized on UV light and photographed.

2.4. Data analysis

For the analysis of molecular data, the amplified bands were scored visually. To reduce errors, only clear and distinguishable bands were scored. The fragments were counted as present (1) or absent (0) to generate a binary data set. Percentage of polymorphism was calculated by dividing the sum of amplified polymorphic bands by the entire quantity of amplified fragments by the same primer or primer mixture. To estimate the genetic similarity, Jaccard's coefficient (Jaccard, 1908) was used. A dendrogram was produced by cluster analysis using the unweighted pair group technique of the arithmetic averages (UPGMA) for all the marker systems. Principal component analysis (PCA) was conducted using a D center module (Jaccard, 1908).

2.5. Phytochemical screening

Phytochemicals of all the samples under investigation were screened to identify the main secondary metabolites, such as alkaloids, tannins, saponins, anthraquinones, flavonoids, sterols and/or triterpenes, and cardiac glycosides (Cecília et al., 2006).

2.6. Extraction procedure

The plants were cut into small pieces and extracted with 70% ethanol for three days. The solvent was evaporated using a rotating evaporator (Buchi®R-300, USA) at 45 °C, and kept at 20 °C till used for phytochemical analysis.

2.7. Determination of phenolic content

The entire phenolic contented of cactus extracts was estimated using Folin–Ciocalteu's reagent Machado et al., 2008. Briefly, 1 mg/mL of each cactus extract was liquified in methanol and used as the stock solution. An aliquot of 100 μL of each extract was mixed with 0.5 mL of Folin–Ciocalteu's reagent, after which 1.0 mL of distilled water and supplemented with1.5 mL of 2% aqueous sodium bicarbonate. The reaction combination was left for 30 min with frequent shaky. Finally, the absorbance of the mixture was calculated at 765 nm. All the experiments were repeated three times and the readings were stated as milligrams of gallic acid correspondent (GAE) per gram of dry plant extract.

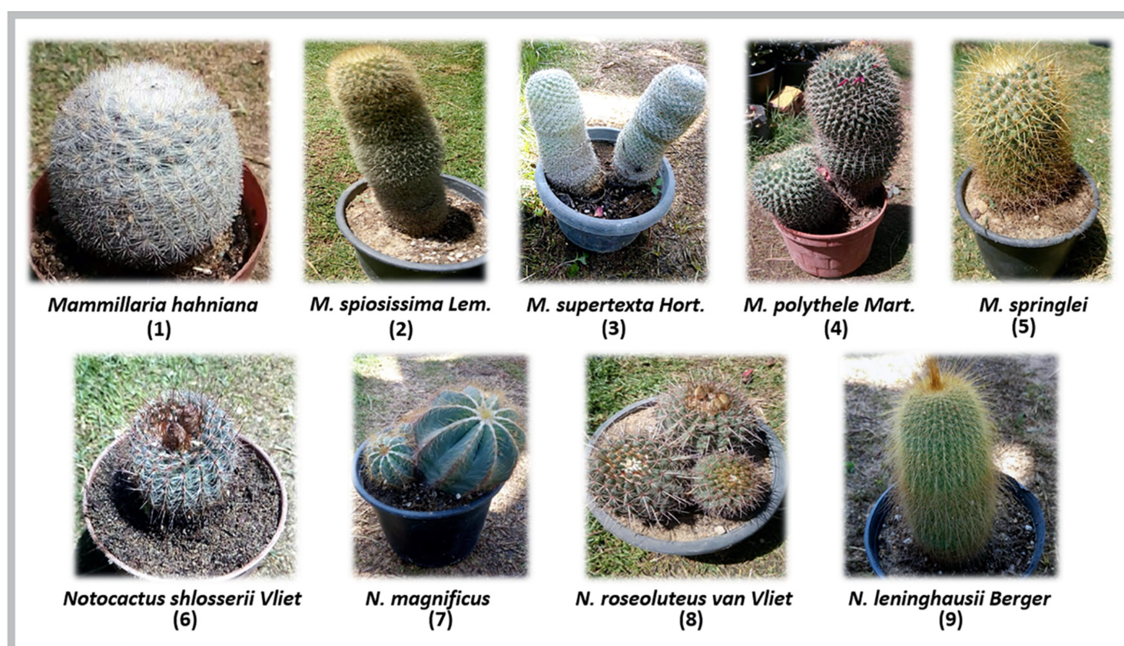

Fig. 1. The cactus species; 1- *Notocactus Leninghausii* A. Berger; 2- *N. shlosserii* Vliet; 3- *N. roseoluteus* Vliet; 4- *N. magnificus* F. Ritter; 5- *Mammillaria spinosisima* Lem;6- *M. supertexta* Hort.;7- *M. polythele* Mart.;8- *M. springlei* J.M. Coult; 9- *M. hahniana* Werderm.

2.8. Estimation of total flavonoid and flavanol contents

The entire flavonoid contents were estimated using 10% aluminum trichloride hexahydrate in methanol (Machado et al., 2008). Briefly, each extract (500 μL) was diluted with 2.80 mL of distilled water and allowed to react with 0.1 mL of $AlCl_3$ and 0.1 mL of 1 M sodium acetate. The reaction mix was kept for 40 min, after which its absorbance was measured at 415 nm. On the other hand, the flavanol content was measured spectrophotometrically using 2% $AlCl_3$. Of each extract, 2 mL was mixed with 2 mL of $AlCl_3$ and 3 mL sodium acetate (50 g/L) at 20 °C. The resulting mixture was kept for 2.5 h and the absorbance was measured at 440 nm using quercetin as standard. Both flavonoids and flavanols were stated as milligrams of quercetin equals per gram of dry weight (mg QE/g extract).

2.9. Antioxidant activity

2.9.1. Estimation of DPPH free radical scavenging activity

Free radical scavenging capacities of the tested extracts were determined using steady DPPH (Harborne et al., 1984; Hwang et al., 2014). The final concentration of DPPH was 200 μM and the total reaction was 3.0 mL. After 60 min of incubation in dark conditions, the absorbance was calculated at 517 nm compared to pure methanol as the blank. The percentage of inhibition of free DPPH radicals was measured *via* the equation: Inhibition (%) = 10 0 × [(Control – Sample)/Control]. The standard curve was prepared using Trolox as positive control. Results were stated as milligrams of Trolox equivalents per gram of sample (mg TE/g sample).

2.10. Determination of ABTS free radical scavenging activity

The standard resolution of ABTS reagent was prepared by adding equal amounts of a 7 mM aqueous resolution of ABTS with 2.45 mM potassium persulfate and incubating the mixture for 16 h at (25 °C) in the shady. The final resolution was then set by water down using 1 mL of ABTS resolution with 60 mL of ethanol: water (50:50, v/v) to get an absorbance of 1.0 ± 0.02 units at 734 nm. The extracts (50 μL) permitted to react with 4.95 mL of the ABTS resolution for 1 h in shady. The absorbance was then calculated at 734 nm (Harborne et al., 1984). Percentage of inhibition of the ABTS free radical was measured using the equation: Inhibition (%) = 100 × [(Control – Sample)/Control]. The standard curve was arranged using Trolox as positive control. The results were stated as milligram of Trolox equivalent per gram of sample (mg TE/g sample).

3. Results

3.1. Analysis of CDDP and SCoT

In order to investigate the genetic diversity and evaluate the degree of polymorphism between the nine species of cacti (four of *Notocactus* and five of *Mammillaria*), 10 SCoT and eight CDDP primers were used (Table 2). For CDDP analysis, CDDP sequences from the nine species of cacti were amplified using eight CDDP primers (Fig. 2). A total of 190 amplification products were obtained in the form of scorable bands, all of which were polymorphic (100%) (Table 2). The number of amplified DNA fragments per primer ranged from 19 (primer CDDP–8) to 27 (primer CDDP–2), and the percentage of polymorphism for all the used primers was 100%. For SCoT analysis, SCoT sequences from species of both the genera were amplified using 10 SCoT primers (Fig. 3). A total of 179 amplification products were obtained in the form of scorable bands, out of which 175 (98.0%) were found to be polymorphic, with an average 17.9 bands/PC (Table 2). The number of amplified DNA fragments per primer ranged from 13 (primers SCoT–2 and SCoT–9) to 26 (primer SCoT–1), and the percentage of polymorphism ranged from 86% (primers SCoT–7) to 100% (primers SCoT–3, 4, 5, 6, 8, 9, and 10).

3.2. Analysis of molecular phylogeny

Dendrograms based on UPGMA analysis of SCoT, CDDP, and combined data were constructed for the nine species of cacti (Figs. 4–6). For CDDP, the dendrogram comprised two main clusters. The first cluster successfully grouped all *Mammillaria* species as two species (3 and 1) that were the most genetically similar and grouped them with species no. 4 in one sub-cluster, while the second sub-cluster comprised two species (2 and 5) (Fig. 4). On the other hand, the second cluster grouped four *Notocactus* species as two species (6 and 8) that were the most genetically similar and grouped the species 7 and 9 in the second sub-cluster. Furthermore, the PCA analysis of CDDP data revealed highly similar results that were comparable to the cluster analysis (dendrogram). The PCA results indicated that the grouping remained similar to that shown by the cluster analysis (Fig. 4 and Table 3). For SCoT, the dendrogram comprised two main clusters; the first cluster was further divided into two sub-clusters, the first of which grouped all the *Mammillaria* species as two species (2 and 3) that were the most genetically similar, followed by species 4 and 5, and finally the species no. 1, which was the most diverged among all the tested *Mammillaria* species (Fig. 5). The second sub-cluster grouped two *Notocactus* species as 7 and 9, while the rest of the *Notocactus* species were grouped as species 6 and 8. Furthermore, PCA analysis of the SCoT data exhibited consistent results, as they showed comparable groups to the ones obtained from the cluster analysis (dendrogram) (Fig. 5 and Table 4). For the combined data, the cluster analysis of the nine species of cacti using the above marker systems revealed two dendrograms exhibiting unique topology with some similarities. The data scored from SCoT and CDDP were combined and analyzed to generate deeper relationships based on the wider and more versatile genome coverage. The combined dendrogram comprised two main clusters with a high topology that matched with the SCoT dendrogram. The first cluster was further divided into two sub-clusters; one grouped all the *Mammillaria* species in addition to two *Notocactus* species (no. 7 and 9). On the other hand, the rest of the *Notocactus* species (no. 6 and 8). Furthermore, PCA analysis of the combined data exhibited consistent results that were comparable with the grouping obtained from cluster analysis (dendrogram) (Fig. 6 and Table 5).

3.3. Preliminary phytochemical screening

The results of the phytochemical screening are presented in Fig. 7. The presence of sterol, triterpene, tannin, and flavonoids was observed in all samples. Saponins were present in all the extracts, except in that of *N. magnificus*. On the other hand, alkaloids were abundant in *N. leninghausii*, *M. hahniana* Werderm, and *N. magnificus* F. Ritter, present in traces in *N. shlosserii*, *M. supertexta* Hort., and *N. roseol*, and absent in *M. spinosis, M. polythele* Mart., and *M. spriglei*. Anthraquinones and cardiac glycosides were not present in any of the specimens under investigation.

3.4. Determination of the total phenolic, total flavonoid, and total flavanol contents

The total phenolic contents of different species of cacti were determined spectrophotometrically using Folin–Ciocalteu's reagent and are presented in Table 6. The total phenolic content ranged from 16.58 ± 0.14 to 87.15 ± 0.01 μg/g of plant extract. *N.*

Table 2
Primer code, primer sequences, number of total bands, polymorphic bands, and percentage of polymorphism in the CDDP and SCoT primers.

Code	Primer Sequence	Number of bands		% of polymorphism
		Total	Polymorphic	
CDDP				
CDDP-1	TGGCGSAAGTACGGCCAG	25	25	100
CDDP-2	GTGGTTGTGCTTGCC	27	27	100
CDDP-3	GCCCTCGTASGTSGT	20	20	100
CDDP-4	GGCAAGGGCTGCCGC	23	23	100
CDDP-5	GGCAAGGGCTGCCGG	24	24	100
CDDP-6	CACTACCGCGGSCTSCG	23	23	100
CDDP-7	GCSGAGATCCGSGACCC	22	22	100
CDDP-8	TGGCTSGGCACSTTCGA	19	19	100
Total		190	190	100
Average		19	19	
SCoT				
SCoT-1	CAACAATGGCTACCACCA	26	25	96
SCoT-2	CAACAATGGCTACCACCC	13	12	92
SCoT-3	CAACAATGGCTACCACCG	16	16	100
SCoT-4	CAACAATGGCTACCACCT	19	19	100
SCoT-5	CAACAATGGCTACCACGA	23	23	100
SCoT-6	CAACAATGGCTACCACGC	21	21	100
SCoT-7	CAACAATGGCTACCACGG	14	12	86
SCoT-8	CAACAATGGCTACCACGT	18	18	100
SCoT-9	CAACAATGGCTACCAGCA	13	13	100
SCoT-10	CAACAATGGCTACCAGCC	16	16	100
Total		179	175	98
Average		17.9	17.5	

leninghausii yielded the highest phenolic content, while *M. supert* and *N. magnificus* F. Ritter displayed comparable amounts of the compound (58.64 ± 0.73 μg/g and 58.93 ± 0.14 μg/g, respectively). *M. spriglei* yielded the lowest phenolic content among the cactus species. In contrast, *N. leninghausii* and *N. magnificus* F. Ritter showed the least flavonoid and flavanol content among the species of cacti (Table 6).

3.5. Antioxidant activity

The generation of reactive oxygen species (ROS) in our body is widely known to play a significant role in the progression of several oxidative stress-related diseases (Mosmann, 1983; Elaasser et al., 2011). We determined the capacities of different cactus extracts to scavenge the free radicals, using ABTS and DPPH methods, against Trolox (water-soluble analog of vitamin E) as the positive control. The ABTS assay showed a higher percentage of inhibition, ranging from 40.324 ± 0.295 mg/g to 17.783 ± 0.145 mg/g sample, compared to the DPPH assay. The data presented in Table 6 indicated that *N. roseol* exhibited the highest percentage inhibition of ABTS solution, while *N. magnificus* F. Ritter and *N. leninghausii* exhibited nearly similar values (31.663 ± 0.096 and 31.333 ± 0.079 mg/g sample, respectively). *M. polythele* Mart. and *M. supert* showed moderate inhibition capacity, while *M. hahni* displayed the least inhibition capacity against ABTS solution (17.783 ± 0.145 mg/g sample). In contrast, all the cactus extracts exhibited a low percentage of inhibition against the DPPH solution. The antioxidant capacity of the species was in the order: *M. spinosis* > *N. shlosserii* > *N. roseol* > *M. supert* > *N. leninghausii* > *M. polythele* > *M. spriglei* > *N. magnificus* > *M. hahni*.

4. Discussion

To the best of our knowledge, this study is the first to investigate and analyze the genetic diversity within and between the genera *Mammillaria* and *Notocactus* using modern approaches involving functional markers such as SCoT and CDDP, in addition to the preliminary screening of their phytochemical constitution and antioxidant activities that represent an initial image of their pharmacological activities. Genome-conserved regions across different plant species have facilitated the development of molecular markers such as SCoT and CDDP. These markers utilize longer primers with higher annealing temperatures, which makes them more reliable, reproducible, and easier to design than other arbitrary markers such as RAPD or DAF. Moreover, they focus on gene regions, which makes them preferable to random markers in QTL mapping applications (Collard and Mackill, 2009a; de Lucena et al. 2013). Collard and Mackill (2009b) exploited conserved DNA regions within the selection of well-known plant genes that were mainly involved in response to biotic and abiotic stresses or plant development to design CCDP primers. Finally, it was recommended that since SCoT and CDDP markers were generated from the functional region of the genome, the genetic analyses utilizing them would be highly useful for crop improvement programs, such as QTL mapping, assessment of genetic diversity, construction of linkage maps, and identification of different genotypes (Zibaee et al., 2011). We applied the SCoT and CDDP techniques for the first time to analyze the genetic diversity between nine species belonging to the genera *Mammillaria* and *Notocactus* (*Parodia*), which are considered to be major genera of the family Cactaceae. Both the markers were able to differentiate between the nine species, although the percentage of polymorphism for SCoT and CDDP was different (98% and 100%, respectively). This indicated that CDDP was more capable of discriminating between different genotypes, which is in agreement with the findings of a study on chickpea, that also reported that CDDP was more effective than SCoT and SSR markers in showing the diversity patterns across different genotypes (Hahm et al., 2010; Ezzat et al., 2016). Our results also indicate that CDDP has a higher rate of reproducibility than SCoT, although fewer primers were used for CDDP (eight primers) compared to SCoT (10 primers). Poczai et al., (2013) reported that the reproducibility of CDDP was higher than that of traditional arbitrarily amplified DNA markers and that the technique could easily generate functional markers (FM) related to a given plant phenotype. Furthermore, this study is the first to highlight the major secondary metabolites of these species of cacti. Several phytoconstituents, such as sterols, triterpenes, tannins, flavonoids, and saponins were detected in these species, except for *N. magnificus.*

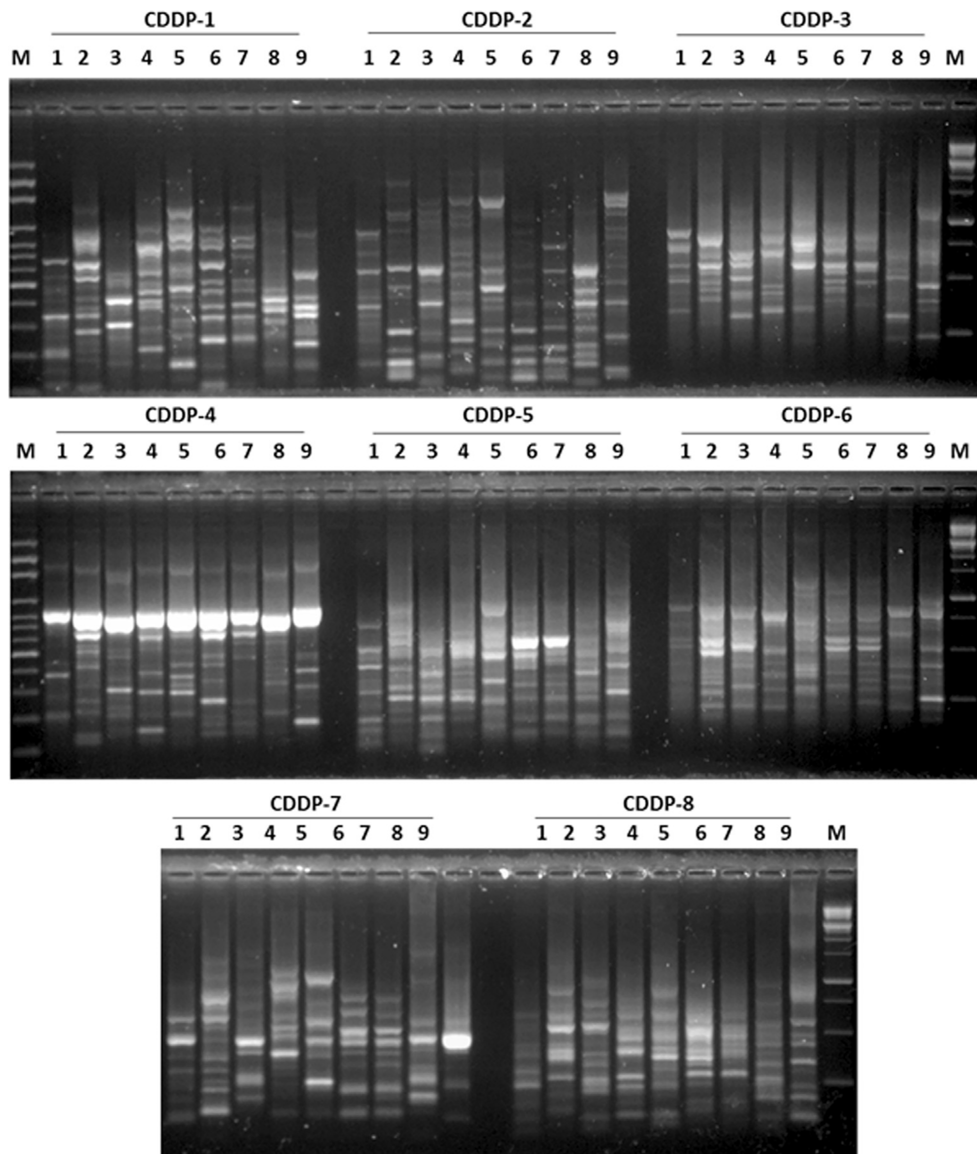

Fig. 2. Comparison of the eight CDDP-PCR profiles of the four *Notocactus* species and five *Mammillaria* species. Lane on both, the right and left side, is the DNA size marker (100 bp plus). Samples order (1 to 9; left to right).

On the other hand, alkaloids were only observed in two species of *Notocactus* (*N. leninghausii* Berger *and N. magnificus*) and one of *Mammillaria* (*M. hahniana* Werderm). *M. supertexta* Hort. and *N. Leninghausii* Berger showed the highest phenolic content. However, they exhibited the lowest content of total flavonoids and flavanol. Recently, a similar finding was reported by Figueroa-Pérez et al., (2018), who observed a considerable amount of saponin in cladodes of medium age, which decreased with age. Our results agreed with those of with Dib et al. (2013), who reported a moderate number of flavonoid compounds in *Opuntia ficus-indica*. L. To the best of our knowledge, there are no reports regarding the quantitative determination of polyphenolic content in these cactus species. Several epidemiological studies have established an inverse relationship between the intake of dietary antioxidants and the incidence of several diseases such as cancer, Alzheimer's, inflammation, and cardiovascular diseases (Li et al., 2009; Bhattacharyya et al., 2013; Atia et al., 2016). Therefore, the antioxidant activity of different cactus species was evaluated using two complementary methods (ABTS and DPPH). Both the reagents are known to be stable against free radicals and the resulting colors show characteristic absorptions at 734 and 519 nm, respectively (Figueroa-Pérez et al., 2018). The scavenging activity of DPPH depends mainly on proton donation to produce reduced DPPH, which can be evaluated by a decrease in its absorbance (Dib et al., 2013; Shalaby et al., 2013). The inhibition potential in DPPH assay can be attributed to the level of flavonoids and tannins in several cactus species (Abdel-Hameed et al., 2009). Among the two methods, the ABTS method appeared to be more sensitive than DPPH, as it gave reproducible results at several pH levels (unlike DPPH, which was affected by acidic media). In addition, ABTS solution reacted rapidly to the sample, as compared to the slow reactivity of DPPH solution (Bendary et al., 2013; Ammar et al., 2015). Among the nine investigated species, *N. roseoluteus* exhibited the strongest antioxidant potential according to ABTS method. Other phytoconstituents such as alkaloids and saponins may participate in higher antioxidant activity, as measured using the ABTS method. Future studies in the identification and isolation of the active compounds from cacti are recommended. Further pharmacological studies are required to explore the medicinal uses of cacti in more detail.

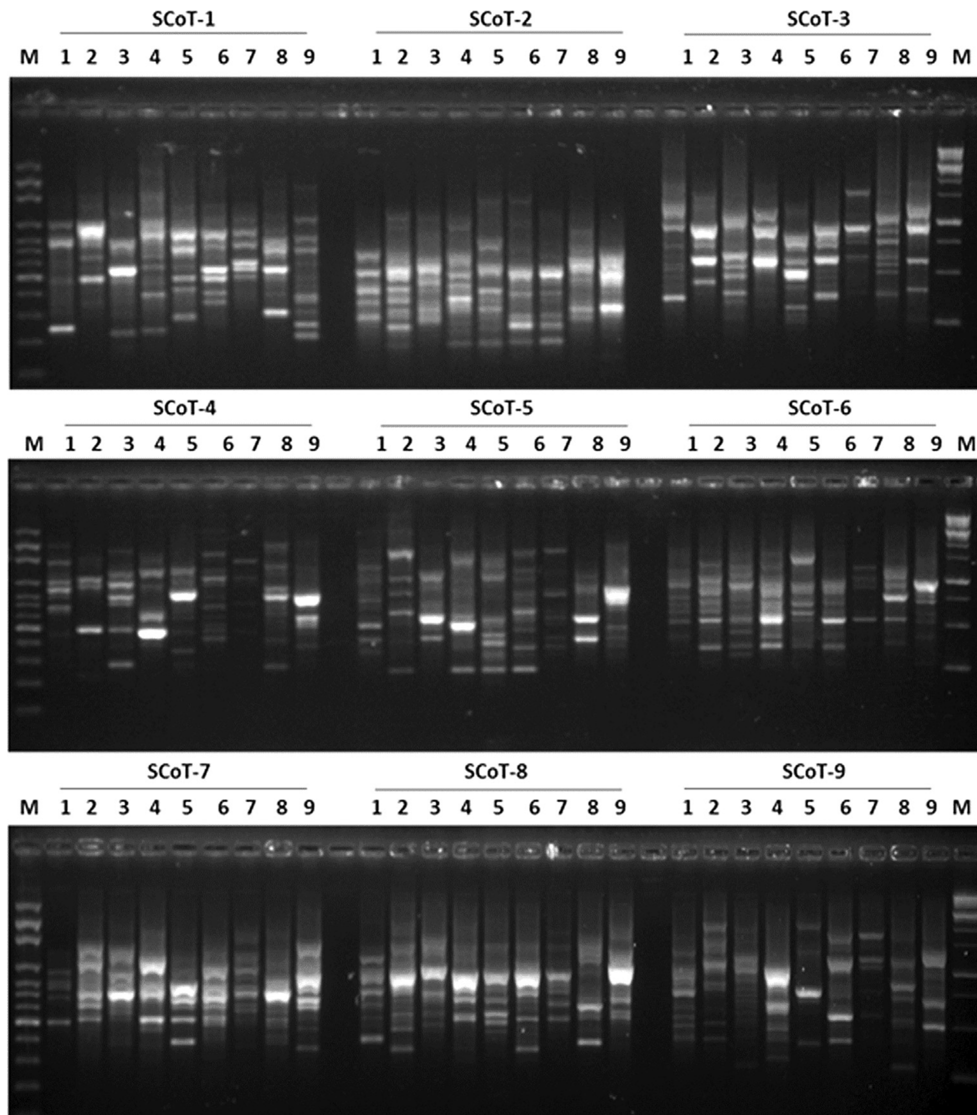

Fig. 3. Comparison of the nine SCoT-PCR profiles of the four *Notocactus* and five *Mammillaria* species. Lane on both the right and left side is the DNA size marker (100 bp plus). Samples order (1 to 9; left to right).

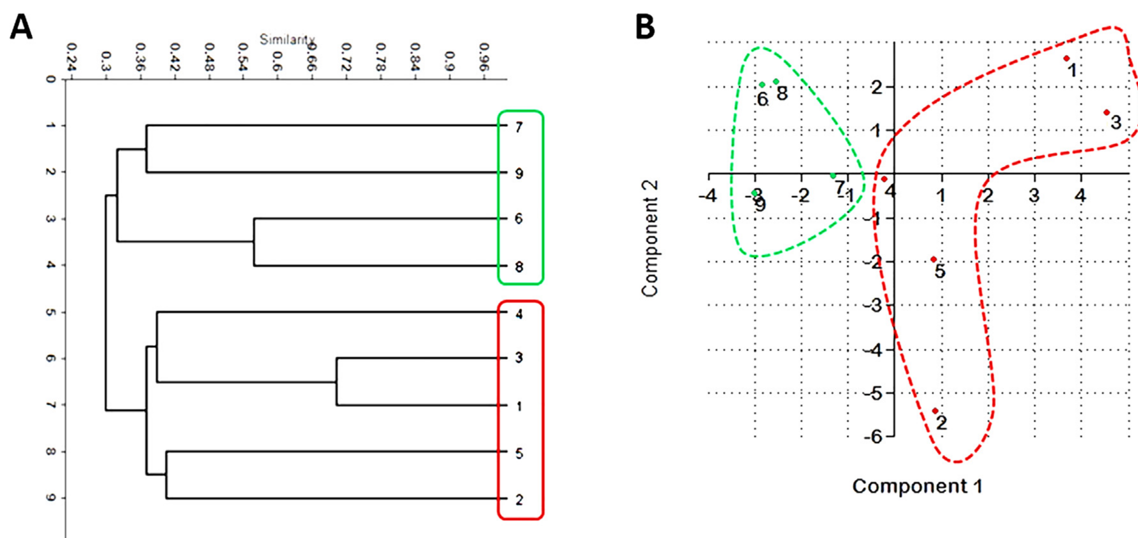

Fig. 4. (A) UPGMA cluster analysis based on Jaccard's similarity coefficient of CDDP analysis of the four *Notocactus* species and five *Mammillaria* species. (B) Principal Component Analysis (PCA) of the CDDP-PCR data of the four *Notocactus* species and five *Mammillaria* species showing the two-dimensional (PC1 and PC2) plot.

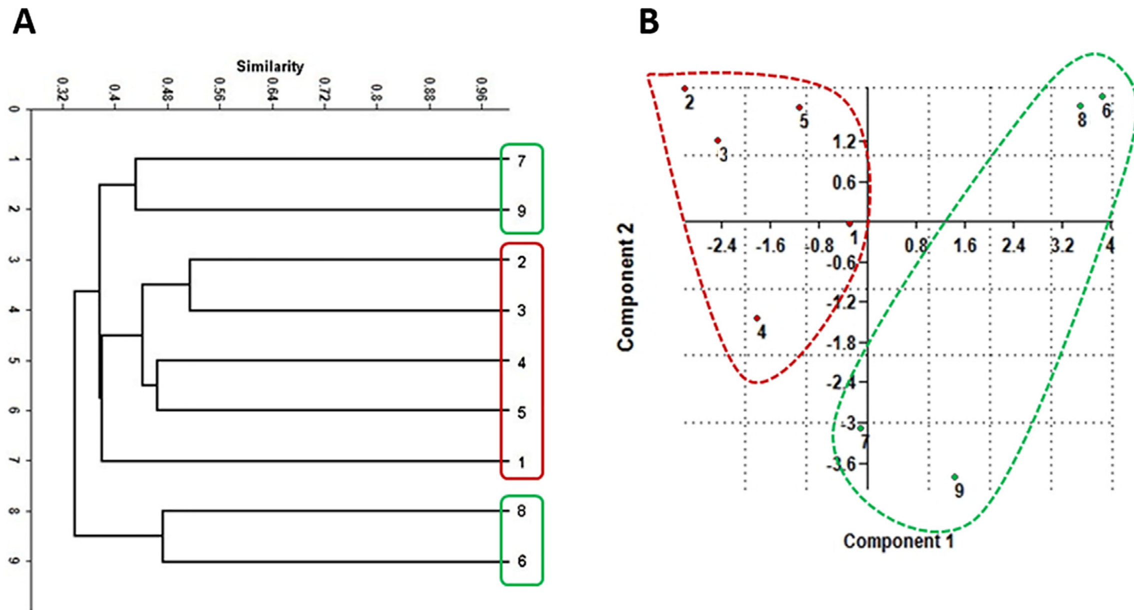

Fig. 5. (A) UPGMA cluster analysis based on Jaccard's similarity coefficient of SCoT analysis of the four *Notocactus* species and five *Mammillaria* species. (B) Principal Component Analysis (PCA) of the CDDP-PCR data of the four *Notocactus* species and five *Mammillaria* species showing the two-dimensional (PC1 and PC2) plot.

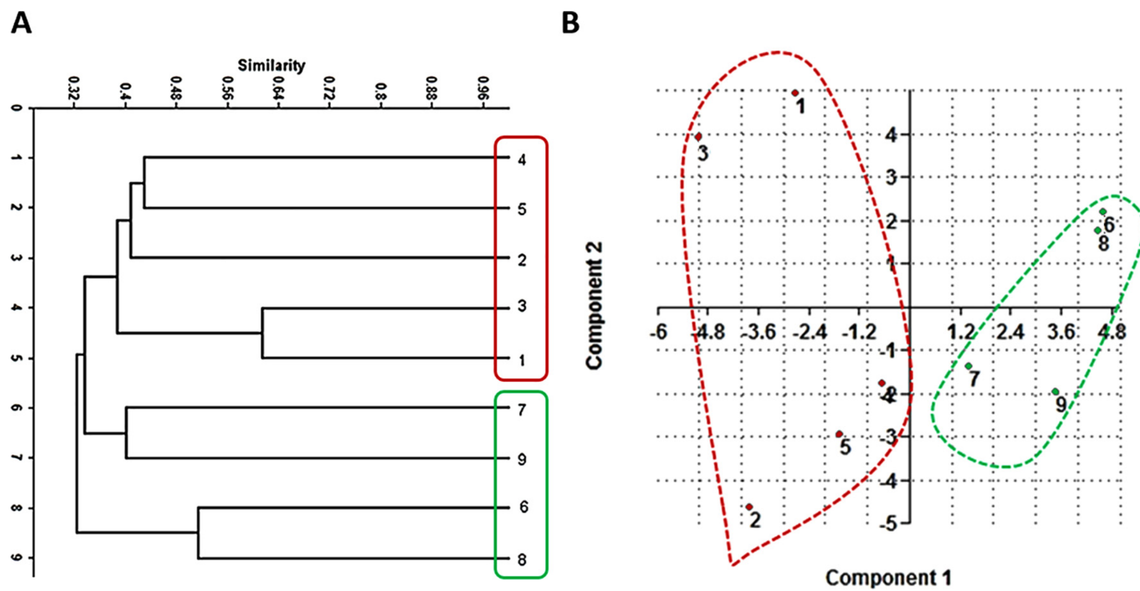

Fig. 6. (A) UPGMA cluster analysis based on Jaccard's similarity coefficient of combined analysis (CDDP + SCoT) of the four *Notocactus* species and five *Mammillaria* species. (B) Principal Component Analysis (PCA) of the combined data (CDDP + SCoT) of the four *Notocactus* species and five *Mammillaria* species showing the two-dimensional (PC1 and PC2) plot.

Table 3
Jaccard's similarity matrix based on the CDDP analysis of the four *Notocactus* species and five *Mammillaria* species.

	Sample-1	Sample- 2	Sample-3	Sample-4	Sample-5	Sample-6	Sample-7	Sample-8	Sample-9
Sample-1	100%								
Sample- 2	30%	100%							
Sample-3	27%	27%	100%						
Sample-4	39%	38%	39%	100%					
Sample-5	34%	40%	30%	39%	100%				
Sample-6	70%	41%	31%	39%	38%	100%			
Sample-7	30%	29%	30%	24%	31%	28%	100%		
Sample-8	35%	31%	56%	35%	22%	27%	25%	100%	
Sample-9	25%	34%	38%	38%	28%	28%	37%	34%	100%

Table 4
Jaccard's similarity matrix based on the SCoT analysis of the four *Notocactus* species and five *Mammillaria* species.

	Sample-1	Sample- 2	Sample-3	Sample-4	Sample-5	Sample-6	Sample-7	Sample-8	Sample-9
Sample-1	100%								
Sample- 2	35%	100%							
Sample-3	33%	25%	100%						
Sample-4	35%	43%	30%	100%					
Sample-5	31%	42%	34%	46%	100%				
Sample-6	51%	51%	35%	46%	44%	100%			
Sample-7	40%	38%	27%	46%	38%	40%	100%		
Sample-8	33%	37%	47%	31%	39%	37%	41%	100%	
Sample-9	32%	29%	33%	40%	33%	39%	43%	36%	100%

Table 5
Jaccard's similarity matrix based on the combined analysis (CDDP + SCoT) of the four *Notocactus* species and five *Mammillaria* species.

	Sample-1	Sample- 2	Sample-3	Sample-4	Sample-5	Sample-6	Sample-7	Sample-8	Sample-9
Sample-1	100%								
Sample- 2	32%	100%							
Sample-3	30%	26%	100%						
Sample-4	37%	40%	34%	100%					
Sample-5	32%	41%	32%	43%	100%				
Sample-6	61%	46%	33%	43%	41%	100%			
Sample-7	35%	33%	28%	35%	34%	34%	100%		
Sample-8	34%	34%	51%	33%	31%	32%	34%	100%	
Sample-9	28%	32%	36%	39%	30%	33%	40%	35%	100%

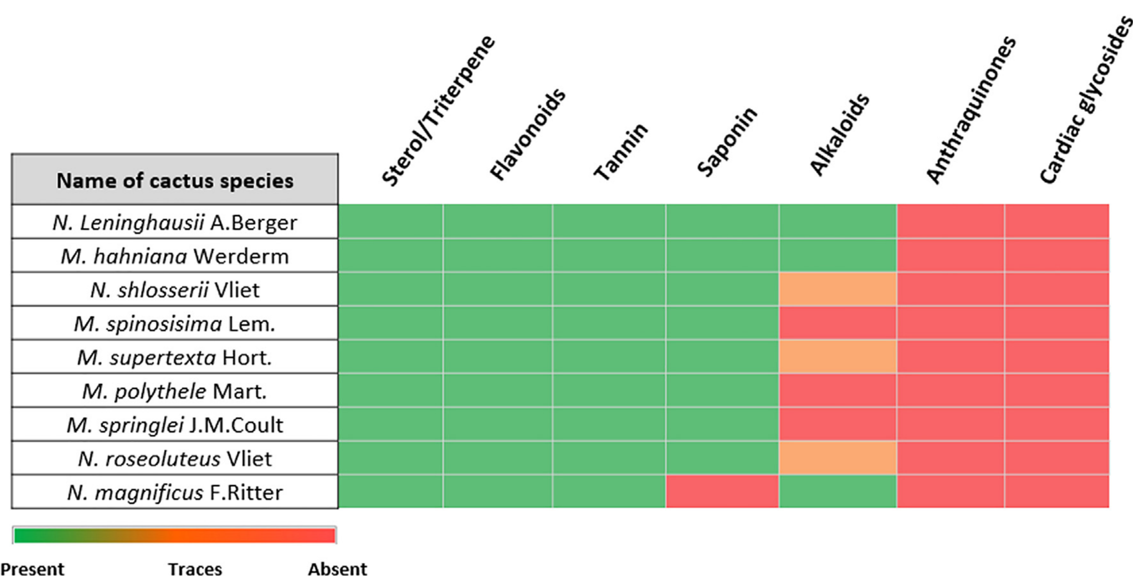

Fig. 7. Heatmap representing the identified levels of phytochemical screening within the four *Notocactus* species and five *Mammillaria* species.

Table 6
The concentration of total phenolic compounds, total flavonoids, and total flavanol along with the estimation of the antioxidant activity of the cactus species determined by the ABTS and DPPH methods.

Name of species	Conc. of total phenolic compounds	Conc. of total flavonoid	Conc. of total flavanol	ABTS	DPPH
N. Leninghausii A.Berger	87.15 ± 0.01	5.71 ± 0.01	5.63 ± 0.01	31.333 ± 0.079	7.158 ± 0.230
M. hahniana Werderm	36.43 ± 0.11	26.41 ± 0.01	19.80 ± 0.01	17.783 ± 0.145	4.849 ± 0.213
N. shlosserii Vliet	33.73 ± 0.01	11.83 ± 0.01	9.21 ± 0.01	29.443 ± 0.081	9.591 ± 0.248
M. spinosisima Lem.	41.05 ± 0.01	19 ± 0.01	12.40 ± 0.09	30.935 ± 0.082	10.600 ± 0.036
M. supertexta Hort.	58.64 ± 0.73	29.11 ± 0.01	6.88 ± 0.01	27.762 ± 0.208	7.232 ± 0.219
M..polythele Mart.	27.39 ± 0.01	11.04 ± 0.01	7.18 ± 0.04	28.622 ± 0.291	7.157 ± 0.154
M. springlei J.M.Coult	16.58 ± 0.14	8.55 ± 0.01	3.92 ± 0.01	21.411 ± 0.524	6.408 ± 0.177
N. roseoluteus Vliet	36.74 ± 0.14	6.79 ± 0.01	5.68 ± 0.05	40.324 ± 0.295	9.518 ± 0.288
N. magnificus F.Ritter	58.93 ± 0.14	5.19 ± 0.01	5.15 ± 0.01	31.663 ± 0.096	5.552 ± 0.112

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