Barcode and Phytochemical Analysis of Invasive Alien Arundo Donax L (Poaceae) in Abha Area, Saudi Arabia

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Abstract: Despite its current status as one of the most notorious invasive species worldwide, Arundo donax (Poaceae) has been utilized extensively by humans across Eurasia for thousands of years. This study utilized DNA barcoding of two chloroplast genes (matK and ITS) to establish a scientific foundation for identifying Arundo species. Genomic DNA extraction was performed, followed by PCR amplification using designated primers and subsequent sequencing via Sanger sequencing. Comparative analysis of nucleotides demonstrated that the obtained sequences were suitable for analyzing phylogenetic relationships. It was demonstrated that Arundo donax had been accurately identified and showed close relatedness to other specimens of the same species within the identical cluster when using the ITS primer. Chemotypic identification of Arundo donax leaf extract by GC-MS analysis found that a total of 16 compounds were detected, among which Z-10-Pentadecen-1-ol (C15H30O) was screened as a characteristic compound. These findings underscore the efficacy of DNA barcoding for precise and consistent identification of A. donax. Moreover, investigating Arundo donax chemotypes carries substantial theoretical and practical implications for its utilization and classification in practice.

***Keywords*: Arundo donax L., invasive species, DNA barcoding, GC-MS**.

1. INTRODUCTION

The Poaceae or Gramineae family of flowering plants is one of the most important groups in terms of economics, as 70% of all crops belong to it. Ten thousand species from six hundred to seven hundred genera have been documented worldwide [1]. Grass has distinct structural characteristics that are typically lacking in other plant species, and its relatively small flower makes it difficult to recognize. Additionally, Poaceae terminology differs from those of other plant families, whereas some plants, such as sedges and rushes, superficially resemble grasses. It can occasionally be difficult to distinguish actual grass species, which may lead to confusion [2]. To monitor grassland habitat and use native or invasive grass species in ecological restoration projects, accurate identification of grass species is essential. Currently, a distinct method is required to accurately identify each plant species, particularly grasses.

One of the most aggressive plant species in subtropical and temperate wetlands is *Arundo donax (Poaceae*). Herrera et al. [3] reported that this gigantic cane is tall (up to 6 m) and rhizomatous and invasive in many warm locations, including Oceania, Africa, and the Americas. *Arundo donax*, commonly known as giant reed, is a robust perennial grass species native to the Mediterranean Basin. Its towering stature and rapid growth make it a prominent component in various ecosystems.

Arundo donax was originally believed to be native to subtropical Asia, which includes the Mediterranean Basin, the Middle East, northern India, and East Asia, and to be invading aquatic habitats continuously [4]. Historically, it has been utilized for a myriad of purposes, ranging from traditional crafts to biofuel production. However, its aggressive spread and invasive tendencies have raised concerns among environmentalists and land managers worldwide. Despite its ecological drawbacks, ongoing research seeks to explore its potential benefits while addressing its negative impacts on native flora and fauna.

Giant cane has been used by humans for a variety of purposes since ancient times, including fire, agriculture, building, fodder, fishing, hunting, music, erosion control, and medicine [5, 6, 7]. A growing body of research on the genetic characterization of this plant has resulted from its status as one of the most promising biomass plants for the generation of biofuel [8, 9].

Due to its elevated position, the Abha area in Saudi Arabia encounters a chilly, semi-arid climate, with temperatures seldom rising above 35°C year-round. Meanwhile, *Arundo donax* plants are extensively found across nearly all wetlands and barren areas in the region. Because DNA barcoding is a commonly used method, quickly and more precisely identify plant species based on nucleotide diversity of short DNA segments [10]. The application of DNA barcoding methodologies is anticipated to lead to the precise identification of *A. donax*. Moreover, delving into its chemotypes is expected to offer significant insights into the practical utilization and taxonomic classification of this species. In this study, DNA barcoding techniques were employed to molecularly identify *Arundo donax found* in the Abha region, Saudi Arabia [11]. Secondly, the current investigation was dedicated to the analysis of phytoconstituents in the derivatized leaf extract of *Arundo donax* using GC-MS. This approach aimed to establish chromatographic

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fingerprinting and integrate it with DNA barcoding to enhance the accuracy and efficiency of plant species identification.

2. MATERIALS AND METHODS

Specimen Collection and Identification

Field trips were organized to collect *Arundo donax* plants from Abha area, Saudi Arabia, with the following locations: 18° 13' 42" N, 42° 31' 2" E at an elevation of 2.191 meters and 18° 13' 4" N, 42° 30' 21" E at an elevation of 2.205 meters (Fig. 1 A and B). All the specimens were deposited in the herbarium of the Biology Department, Faculty of Science, King Khalid University. They were subsequently examined and identified after being added to the scientific collections to verify the preliminary field identification that was made in the field.

DNA Barcoding

Genomic DNA from healthy fresh leaves was extracted using a Qiagen DNAeasy Plant Mini kit. The maturase K gene was sequenced by the forward primer matK-F (5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3') and the reverse primer matK-R (5'-CGTACAGTACTTTTGTGTTTACGAG -3') and the ITS1/2 gene region (internal transcribed spacers 1, 2) was sequenced by the forward primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and the reverse primer ITS2 (5'-TCCTCCGCTTATTGATATGC-3'). 1× PCR buffer, 0.5 mmol/L dNTPs, 0.25 µmol/L per primer, 1 U Taq polymerase, and 5–50 ng template DNA were the components of the PCR reaction mixture. Thermal cycling conditions were maintained at 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 48°C for 40 s, 72°C for 1 min, and a final extension at 72°C for 10 min. The results were confirmed by electrophoresing the PCR products in 1% agarose gels stained with ethidium bromide. Using an ABI3730XL automated sequencer (Applied Biosystems), Macrogen Inc. (Seoul, South Korea) carried out the sequencing. Obtained sequences from the matK and ITS regions were compared to other relevant sequences using BLAST (Basic Local Alignment Search Tool in https://www.ncbi.nlm.nih.gov/). Close sequences were downloaded, aligned, and further analyzed by MEGA6 software [12, 13].

Preparation of Volatile Derivatives

Arundo donax dried leaves extract was diluted in 20 µl of pyridine and then derivatized for 60 minutes at 70°C using 50 µl of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA). The derivatized extract was injected into the GC/MS[14].

Gas Chromatography–mass Spectrometry Analysis (GC-MS)

The GC-MS system (Agilent Technologies) was outfitted with a mass spectrometer detector (5977A) and a gas chromatograph (7890B) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness) was fitted to the GC. Helium was used as the carrier gas for the analyses, with a split ratio of 20:1, a flow rate of 1 ml/min, an injection volume of 1 µl, and the following temperature program: 50°C for one minute, then rising to 300°C at an 8°C/min hold for 20 minutes. At 250°C and 300°C, respectively, the injector and detector were held. Electron ionization (EI) at 70 eV with a spectral range of m/z 30-700 was used to acquire mass spectra. By comparing the spectrum fragmentation pattern with those found in Wiley and NIST Mass Spectral Library data, chemicals were identified.

3. RESULTS AND DISCUSSIONS

Based on surveying Abha area and sampling, it was documented that the expanded range of *Arundo donax,* which invaded many other regions worldwide (Fig. 1 A and B). The species *Arundo donax has* been reported to be highly productive and easily transplantable, with culms that are exceptionally strong, light, and flexible [5]. Given its wide naturalized distribution, the invasiveness of *Arundo donax* may be considered one of the first invasive plant species in wetland areas across many locations. In Abha region, it forms pure stands, contributing to the record of historical incursions from Southwest Asia to the Mediterranean. [15].

Fig. 1. Arundo donax growing in Abha area, Saudi Arabia, in various locations. A; growing in 18° 13' 42" N, 42° 31' 2" E at elevation 2.191 meters; B; growing in 18° 13' 4" N, 42° 30' 21" E, at elevation 2.205 meters.

The ITS region has been shown to be a useful variable marker, and the conserved plastid matK gene is the most frequently explored marker in many studies [16]. Therefore, the aim of this study was to evaluate, for the first time, how effective these markers were in barcoding Arundo donax from the Abha region of Saudi Arabia. The results showed that the two primers used in the amplification process of the genetic markers under study gave nucleotide sequences for *Arundo donax* as expected. The results demonstrate that the use of the two markers matK and ITS identified *Arundo donax* growing in Abha as a species closely related to other species of Arundo. Clustal W was used in the MEGAX tool to align the sequences, and bootstrap 1000 replicates were used to generate the evolution trees. Within the established phylogenetic trees, the plant was compared in each case with the neighboring species.

The first phylogenetic tree was constructed using 29 matK sequences from various plant species, in addition to the matK sequence (863 bp, Fig. 2) from *Arundo donax* isolated from Abha. The results of the matK tree showed that *Arundo donax* isolated from Abha region clustered with a bootstrap value of 53 with several species of the

Arundo genus as well as species from other genera, including *Sorghum, Phragmites, Molinia*, and *Hakonechloa* (Fig. 3).

The second phylogenetic tree was constructed using 27 ITS sequences from different species, in addition to the ITS sequence of Arundo donax isolated from Abha (610 bp, Fig. 4). Results of the ITS tree showed that *Arundo donax* isolated from Abha clustered—with a higher bootstrap value of 59—in a single clade with one other species of the *Arundo genus* (Arundo donax voucher Columbus 32, accession no. DQ172077.1) (Fig. 5).

This pattern of genetic diversity observed in *Arundo donax* from Abha and its closely related species shows the genetic diversity present in plastid and nuclear markers for such a widespread plant species. While invasive species with minimal genetic diversity, polyploid genomes, and clonal reproduction, such as Spartina anglica and Pennisetum setaceum (Poaceae), showed patterns of genetic uniformity [17, 18]. Low genetic diversity has been found in Arundo donax in previous research carried out over large geographic areas. Ahmed et al. [19] used sequence-related amplified polymorphism and transposable element-based molecular markers to assess 185 potential clones of Arundo donax from the southern USA. They identified just one prevalent genotype that was comparable to four populations from southern France. The reason the plant is dominant in many locations and forms pure stands is that its dispersal and invasive ability are supported by this degree of genetic variety.

Furthermore, this study reaffirms the phytochemical profile of *Arundo donax* (Abha population) leaf extract using gas chromatography and mass spectroscopy (GC-MS) (Fig. 6), supporting its potential use in chemotaxonomic classification. GC-MS analysis separated (identified) 16 chemical compounds. Each chemical compound was characterized by its chemical formula, peak area percentage, and retention time (Table 1). Among the identified phytocompounds, Z-10-Pentadecen-1-ol was the most abundant compound (36.36%), followed by D-(-)-Tagatofuranose, pentakis(trimethylsilyl) ether (isomer 2) (13.85%), while the remaining 14 chemicals ranged from 7.58% to 0.68%.

In particular, the diverse chemical composition of *Arundo donax* from the Abha region presents a valuable opportunity for the isolation of bioactive compounds, which could have potential applications in chemotaxonomy and chemotherapy. However, further research is needed to investigate the biological activity of many of these compounds. For instance, no biological activity has been reported for Z-10-Pentadecen-1-ol [20], which may serve as a chemotypic marker for *Arundo donax* growing in the Abha area. Previous studies have also shown that stem extracts of Arundo donax L. contain high levels of phenolic acids, indicating promising pharmacological potential and supporting the advancement of circular economy initiatives across key industrial sectors [21].

5’-
GAAATCTTGGTTCAACTCCTTGAATACCGGATCCAAGA
TGTTCCATCTTTGCATTTATTGCGATTCTTTCTCAACTAT
TATTCGAATTGGAATAGTCTTATTACTTCAATGAAATCG
ATTTTTCTTTTGAAAAAAGAAAATAAAAGACTATTTCG
ATTCCTATATAACTCTTATGTATCAGAATATGAATTTTT
CTTGTTGTTTCTTCGTAAACAATCTTCTTGCTTACGATT
AACATCTTCTGGAACCTTTCTGGAACGAATCCACTTTTC
TAGGAAGATGGAACATTTGGGGGTAATGTACCCAGGGT
TTTTTCGGAAAACCATATGGTTCTTTATGGATCCTCTTA
TGCATTATGTTCGATATCAAGGAAAGGCAATTCTTGCA
TCAAAAGGAACTCTTCTTTTGAAGAAGAAATGGAAATC
TTACCTTGTCAATTTCTCGCAATATTTTTTCTCTTTTTGG
ATTCAACCGCAAAGGATCTGTTTAAACCAATTAACAAA
CTCTTGCTTCGATTTTCTGGGGTACCTTTCAAGTGTACC
AATAAATACTTTGTTAGTAAGGAATCAAATGCTGGAGA
ATTCTTTTCTAATAGATACTCGAATGAAAAAATTTGAT
ACCACAGTCCCCGCGATTCCCCTCATTGGATCCTTATCA
AAAGCTCAATTTTGTACTGGATCGGGGCATCCTATTAG
TAAACCTGTTTGGGCCGATTTATCAGATTCGGATATTCT
TGATCGCTTTGGTCGGATATGTAGAAATCTTTTTCATTA
TCATAGTGGATCTTCGAAAAAACGGACTTTGTATCGAC
TAAAATATATACTTCGACTTTCATGTGCTAGAACTTTAG
CTCGTAAACACAA-3’

Fig. 2. Arundo donax growing in Abha, Saudi Arabia, obtained sequence for matK.

Fig. 3 Phylogenetic tree of Arundo donax using matK markers. The phylogenetic tree was inferred by using the Maximum Likelihood method and Tamura 3-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The

tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 29 nucleotide sequences of the maturase K gene. There were a total of 860 positions in the final dataset. Evolutionary analyses were conducted in MEGA software.

5-

TGCGGAGGATCATTGCCGTGACCCTTGACCAAAACAGACCGCGCACGCGTCATCCATGCCGCCGGGTGTCGGGGC
TTGCCCCGCCACCCGGCTCAGGCCCCCGACCTCCGCGAGGAGGGGAGGGGCCGCAACAGAACCCACGGCGCCGA
ACGGCGTCAAGGAACACCGTTATTGCCTGGCGCGGGGGGCGGCCGGCCCGCCGGTCGCCCCCGAGCCAGCGATGC
TATCATAACCACACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGATAC
CTGGTGTGAATTGCAGAATCCCGCGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAGGCCTTCTGGCCGAGG
GCACGCCTGCCTGGGCGTCACGCCAAAAGACGCTCCCGAACCCCTCCTCGGGTCGGGTCGGGACGCGGTGTTTGG
CTCCCCGCGCCGCAGGGCGCGGTGGGCCGAAGTTGGGGCTGCCGGCGTACCGTGCCGGGCACAGCACATGGTGGG
CGACACAAGTTGTTCTCGGTGCCGTGCCTCGGCTCGCAGCGGGCGTGAGGGCCCGAAGGACCCATCGTTCGGACC
GCGACCCCAGG-3’

Fig. 4. *Arundo donax* growing in Abha, Saudi Arabia, obtained sequence for ITS1/2.

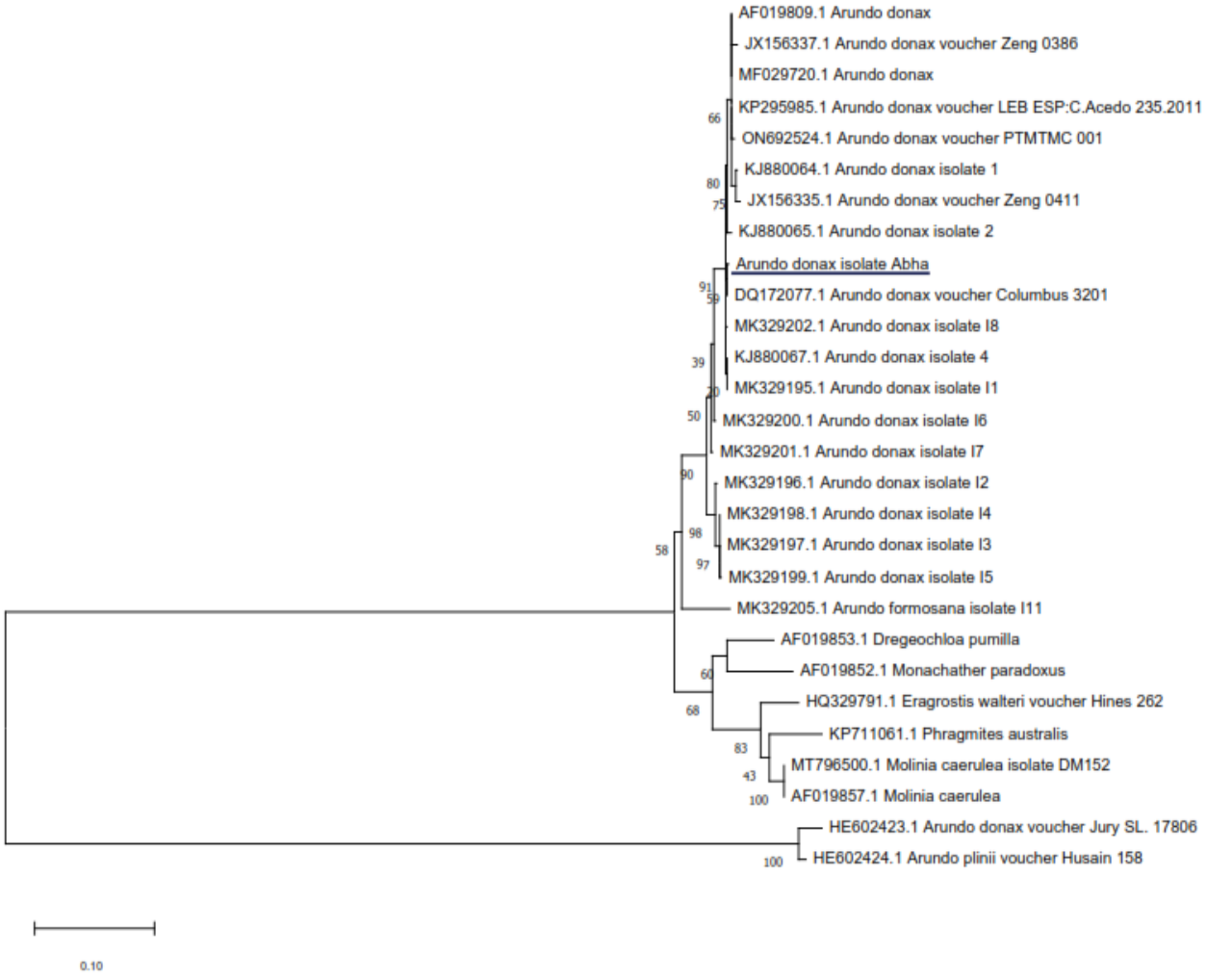

Fig. 5. Phylogenetic tree of Arundo donax using ITS markers. The phylogenetic tree was inferred by using the Maximum Likelihood method and Tamura 3-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. This analysis involved 28 nucleotide sequences of the ITS region. There was a total of 615 positions in the final dataset. Evolutionary analyses were conducted in MEGA software.

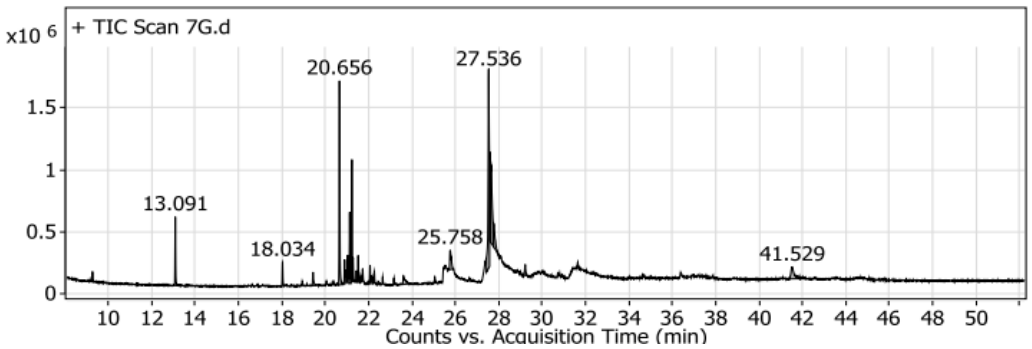

Fig. 6 GC-MS chromatogram for leaf plant extract of *Arundo donax growing in Abha area, Saudi Arabia.*

TABLE 1. GC-MS ANALYSIS OF DRY LEAVES EXTRACT OF ARUNDO DONAX GROWING IN THE ABHA AREA, SAUDI ARABIA.

Peak	RT	Name	Formula	Area	Area Sum %
1	9.27	Lactic Acid, 2TMS derivative	$C_9H_{22}O_3Si_2$	161994.35	0.68
2	13.091	Glycerol, 3TMS derivative	$C_{12}H_{32}O_3Si_3$	1043194.39	4.37
3	18.034	D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 1)	$C_{17}H_{42}O_5Si_4$	402773.43	1.69
4	19.435	Butylated Hydroxytoluene, TMS derivative	$C_{18}H_{32}OSi$	317866.29	1.33
5	20.656	D-(-)-Tagatofuranose, pentakis(trimethylsilyl) ether (isomer 2)	$C_{21}H_{52}O_6Si_5$	3242071.78	13.58
6	20.89	D-Erythro-Pentofuranose, 2-deoxy-1,3,5-tris-O-(trimethylsilyl)-	$C_{14}H_{34}O_4Si_3$	367351.69	1.54
7	21.131	L-(-)-Sorbofuranose, pentakis(trimethylsilyl) ether	$C_{21}H_{52}O_6Si_5$	1188056.45	4.98
8	21.229	D-(-)-Fructopyranose, pentakis(trimethylsilyl) ether (isomer 1)	$C_{21}H_{52}O_6Si_5$	1810799.8	7.58
9	21.282	Gluconic acid, .gamma. -lactone, 2-methoximine, tris(O-trimethylsilyl)-	$C_{16}H_{35}NO_6Si_3$	413266.84	1.73
10	21.515	2-Deoxypentofuranose, 3TMS derivative	$C_{14}H_{34}O_4Si_3$	397847.47	1.67
11	22.065	5-Dimethyl(trimethylsilyl)silyloxytridecane	$C_{18}H_{42}OSi_2$	310799.51	1.3
12	23.602	2-Pentadecyn-1-ol	$C_{15}H_{28}O$	504691.91	2.11
13	25.758	2-Pentadecyn-1-ol	$C_{15}H_{28}O$	760628.73	3.19
14	27.536	Z-10-Pentadecen-1-ol	$C_{15}H_{30}O$	5668773.33	23.74
15	27.596	Z-10-Pentadecen-1-ol	$C_{15}H_{30}O$	2103805.09	8.81
16	27.649	Z-10-Pentadecen-1-ol	$C_{15}H_{30}O$	2975555.83	12.46
17	27.777	Z-10-Pentadecen-1-ol	$C_{15}H_{30}O$	908717.16	3.81
18	29.216	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	278900.83	1.17
19	41.529	9-Octadecen-12-ynoic acid, methyl ester	$C_{19}H_{32}O_2$	1022215.1	4.28

4. CONCLUSION

DNA barcoding and phytochemical analysis were performed for *Arundo donax* growing in the Abha region, Saudi Arabia. Both the matK and ITS barcodes yielded positive results for Arundo species identification and for constructing phylogenetic relationships, especially ITS barcodes that resolved all other A. donax. GC-MS analysis was used to identify the phytochemicals that found 16 different compounds, all of which need to be isolated for chemotaxonomic significance and for fruitful biological application.

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