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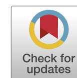
Original article

Molecular authentication of *Anthemis deserti* Boiss. (Asteraceae) based on ITS2 region of nrDNA gene sequence



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ABSTRACT

The dried plant material of medicinally important *Anthemis deserti* Boiss. (family: Asteraceae) especially when it remains in the powdered form often look similar to *Anthemis melampodina* Del.; and therefore, difficult to distinguish, finally lead to chances of adulteration. The adulteration in medicinal plants effects on the efficacy of the drugs. The molecular authentication of herbal plant materials such as based on the internal transcribed spacer 2 (ITS2) sequences of nuclear ribosomal DNA (nrDNA) is considered as more reliable method compared to other the biochemical or histological methods. The present study aims to molecular authentication of*A. deserti* based on molecular phylogenetic analyses of ITS2 gene sequence of nrDNA region. The ITS2 region of nrDNA of *A. deserti* were sequenced, and the molecular phylogenetic analyses were performed together with the GenBank sequences. The Maximum Parsimony tree revealed the close relationships of *A. deserti* with *A. melampodina*; however, the Neighbor-Joining and Maximum Likelihood tree clearly revealed that *A. deserti* is distinct from *A. melampodina*, which is also supported by the differences in nucleotides at five diffident positions (i.e. 22, 28, 87, 175 and 198) in the DNA sequence alignment.

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1. Introduction

The genus *Anthemis* (family: Asteraceae) includes approximately 210 species (Bremer and Humphries, 1993) distributed in Europe, south west Asia, and north and north east Africa, is medicinally important genus as evident from the several pharmacological studies (Quarenghi et al., 2000; Stamatis et al., 2003; Uzel et al., 2004; Akgul and Saglikoglu, 2005; Buruk et al., 2006; Magro et al., 2006; Karioti et al., 2007, 2008, 2009; Papaioannou et al., 2007; Réthy et al., 2007; Collu et al., 2008; Di Giorgio et al., 2008; Hajdú et al., 2010; Vucković et al., 2010; Seol et al., 2010; Kilic et al., 2011; Conforti et al., 2012; Formisano et al., 2012; Samadi et al., 2012). In Saudi Arabia, the genus *Anthemis* is represented by 17 species viz., *Anthemis arvensis* L., *A. bornmuelleri* Stoj. & Acht., *A. cotula* L., *A. deserti* Boiss., *A. dicksoniae* Ghafoor, *A. edumea* Eig., *A. haussknechtii* Boiss. & Reut., *A. hyalina* DC., *A. leptophylla* Eig., *A. melampodina* Del., *A. pseudocotula* Boiss., *A. rascheyana* Boiss., *A. scrobicularis* Yavin, *A. sheilae* Ghafoor & Al-Turki, *A. tenuicarpa* Eig., *A. yemenensis* Podl. and *A. zoharyana* Eig. (Ghafoor and Al Turki, 2000). Among these, the extracts of *A. melanolepis* have been reported to have activity against *Helicobacter pylori* (Stamatis et al., 2003). The flavonoids from *A. cotula* flowers possess antimicrobial activity (Quarenghi et al., 2000). The essential oils of water-distilled vegetative parts of *A. pseudocotula* possess medicinally important molecules such as 1,8-cineole, camphor, artemisiaketone, filifolene (Kilic et al., 2011). *A. arvensis* and *A. cotula* possess pharmacologically important linear sesquiterpene lactones (Vucković et al., 2010).

The difficulties in discrimination of the species of the genus *Anthemis* is due to the diverse variability in morphological characters (Lo Presti et al., 2010). In the field condition, due to overlapping of the morphological characters, the medicinally important *Anthemis* speices need exhaustive taxonomic expertiese for txonomic identification especially to discriminate among *A. cotula*, *A. melampodina*, and *A. pseudocotula*. The dried plant material of *A. deserti* especially when it remains in powdered form often look similar to *A. melampodina*; and therefore, difficult to distinguish; finally lead to chances of adulteration (pers. obs.). The adulteration in medicinal plants effects on the efficacy of the drugs (Jayasinghe et al., 2009). The DNA sequence based molecular authentication of herbal plant materials such as based on the internal transcribed

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spacer 2 (ITS2) sequences of nuclear ribosomal DNA (nrDNA) is considered as more reliable in comparison to the biochemical or histological methods (Zhang et al., 2007; Kiran et al., 2010; Chen et al., 2010; Poczai and Hyvönen, 2010; Yao et al., 2010; Zuo et al., 2011; Ali et al., 2014). Hence, the present study aims to authentication of *A. deserti* based on ITS2 gene sequence of nrDNA region.

2. Materials and methods

2.1. Plant material and sequencing of ITS2 region of nrDNA

The leaves of *Anthemis deserti* were collected from Riyadh region of Saudi Arabia, and fixed in silica gel of 60–120 mesh fine silica gel powder. The herbarium voucher specimens were prepared for the references. The identification was checked by matching the vegetative and reproductive morphological characters of voucher specimen with taxonomic description mentioned in the Flora (Ghafoor and Al Turki, 2000). The voucher specimens were submitted to KSUH (Herbarium, King Saud University, Riyadh, Saudi Arabia) for the record and reference.

The total genomic DNA was isolated, which was then subjected to thermal cycling, the polymerase chain reactions in order to amplification of the nrDNA ITS2 region. The product of polymerase chain reactions was used for DNA sequencing following the method previously described (Ali et al., 2010, 2013a,b, 2014, 2015, 2016; Al-Hemaid et al., 2014, 2015; Choudhary et al., 2011).

2.2. Molecular phylogenetic analyses of nrDNA ITS2 region

The nrDNA ITS2 sequences of 11 species of *Anthemis* (i.e. *A. arvensis, A. bornmuelleri, A. cotula, A. edumea, A. haussknechtii, A. hyaline, A. leptophylla, A. melampodina, A. pseudocotula, A. rascheyana* and *A. zoharyana*) were retrieved from GenBank (Table 1), and trimming at both start and end position were performed according to span in order to include only ITS2 region in the phylogenetic analyses. The ITS2 sequence of *Tripleurospermum transcaucasicum* (GenBank AJ864612) was used as outgroup in the phylogenetic analyses. The DNA sequences were aligned using MUSCLE (Edgar, 2004) and the phylogenetic analyses were performed using Neighbor-Joining (NJ) method (Zuckerkand and Pauling, 1965; Saitou and Nei, 1987; Rzhetsky and Nei, 1992; Dopazo, 1994; Kumar et al., 2016), Maximum Parsimony (MP) method (Felsenstein, 1985; Nei and Kumar, 2000; Kumar et al., 2016) with 100 bootstrap replicates (Felsenstein, 1985) and Maximum Likelihood (ML) method based on the JTT matrix-based model (Jones et al., 1992; Kumar et al., 2016) using the software MEGA X (Kumar et al., 2018).

Table 1
The Genbank accesion number of the taxon included in the molecular phylogenetic analyses.

	Taxon	GenBank accession number
Ingroup		
1.	*Anthemis arvensis* L.	MG218603
2.	*Anthemis bornmuelleri* Stoj. & Acht.	FM957784
3.	*Anthemis cotula* L.	AJ312823
4.	*Anthemus edumea* Eig.	FM957692
5.	*Anthemis haussknechtii* Boiss. & Reut.	FM957658
6.	*Anthemis hyalina* DC.	AJ312808
7.	*Anthemis leptophylla* Eig.	FM957738
8.	*Anthemis melampodina* Del.	AJ312809
9.	*Anthemis pseudocotula* Boiss.	AJ312824
10.	*Anthemis rascheyana* Boiss.	FM957702
11.	*Anthemis scrobicularis Yavin*	FM957755
12.	*Anthemis zoharyana* Eig.	FM957714
Outgroup		
14.	*Tripleurospermum transcaucasicum*	AJ864612

3. Results and discussion

The aligned DNA data matrix of ITS2 region of *A. arvensis, A. bornmuelleri, A. cotula, A. deserti, A. edumea, A. haussknechtii, A. hyaline, A. leptophylla, A. melampodina, A. pseudocotula, A. rascheyana* and *A. zoharyana* and the outgroup *Tripleurospermum transcaucasicum* were 205 base pair long. One out of the six most parsimonious trees (length = 75) showed consistency index (CI) 0.685 and retention index (RI) 0.792. The ITS2 region of *A. deserti* was 202 base pairs (GC content 46%). The MP tree (Fig. 1) revealed that *A. deserti* nested in a clade with *A. melampodina* and *A. zoharyana* with strong bootstrap support (BS 81%). Similar relationship of*A. deserti* with *A. melampodina* and *A. zoharyana* were also recovered in NJ analyses (Fig. 2) and ML (Fig. 3) analysis (BS: 93% in NJ, 87% in ML analysis). The comparison of nrDNA ITS2 sequence of *A. deserti* with *A. melampodina* (Fig. 4) revealed that there are differences in nucleotides at five different position in the DNA sequence alignment (i.e. at the alignment position 22, 28, 87 and 175 there was 'T' in *A. deserti* but 'C' in *A. melampodina*, while at the alignment position 198 there was 'G' in *A. melampodina* but 'R' in *A. deserti.* Morphologically, the key characteristics of *A. deserti* are receptacle hemispherical, achenes obpyramidate, disc corollas inflated towards the base in fruit, which differs from the *A. melampodina*

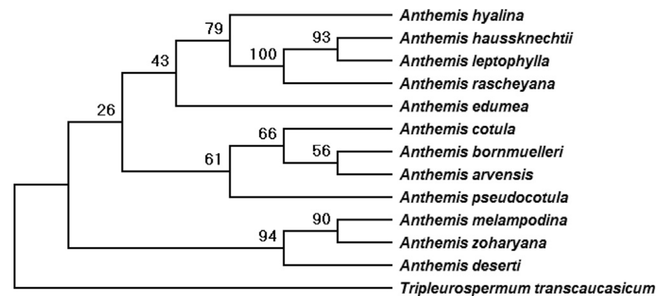

Fig. 1. The maximum parsimony tree [tree #one out of six most parsimonious trees (length = 45), CI: 0.703, RI: 0.822].

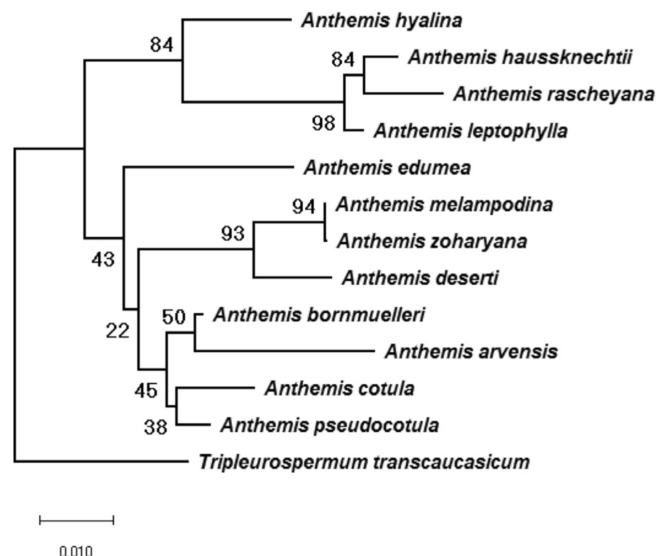

Fig. 2. The Evolutionary relationships of taxa inferred using the Neighbor-Joining method.

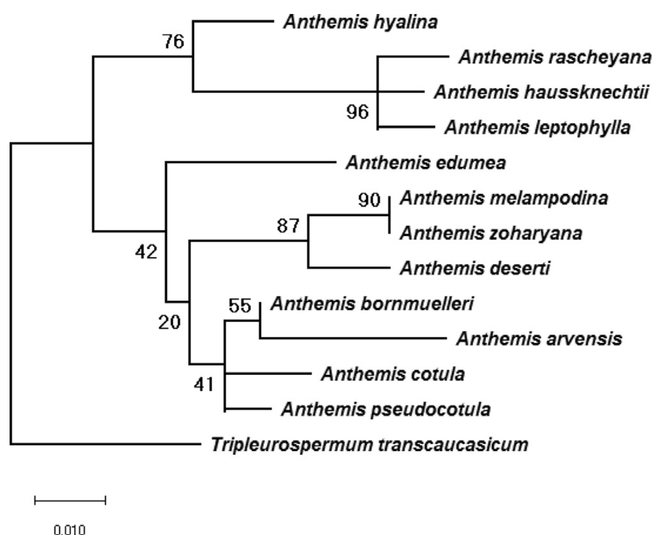

Fig. 3. The Maximum Likelihood tree with the highest log likelihood (-557.00).

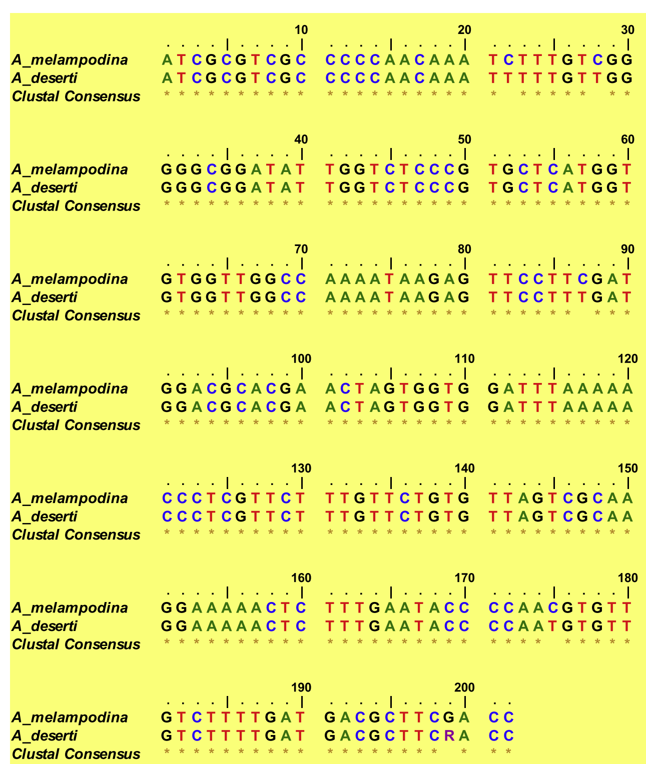

Fig. 4. A comaprision of nueclotide diffeeences in between *A. deserti* and *A. melampodina*.

in having the morphological characteristics disc corollas inflated and indurated in fruit, achenes tuberculate, paleae keeled, not stiffy acuminate.

A number of molecular markers such as Restriction Fragment Length Polymorphisms (RFLPs) have been employed to distinguish *Aegle marmelos*, *Desmodium giganicum*, *Oroxylum indicum*, *Solanum* xanthocarpum, *Solanum indicum*, *Tribulus terresteris* (Biswas and Biswas, 2013), *Boerhavia diffusa* (Biswas et al., 2013) and *Angelica* species (Feng et al., 2010); Amplified Fragment Length Polymorphisms (AFLPs) for American ginseng (Hon et al., 2003), *Capsicum* species (Shirasawa et al., 2013); Randomly Amplified Polymorphic DNA (RAPD) for *Clitoria ternatea* (Ali et al., 2013a,b), *Convolvulus pluricaulis* (Ganie et al., 2015), *Evolvulus alsinoides* (Ganie and Sharma, 2014); Simple Sequence Repeats (SSRs) for *Aloe vera* (Tripathi et al., 2011), *Echinacea* spp (Russi et al., 2009), *Plectranthus* (Passinho-Soares et al., 2006), *Embelica ribes* (Gowda et al., 2010); Inter Simple Sequence Repeats (ISSR) for *Rheum* species (Wang, 2011), *Swertia* (Tamhankar et al., 2009), *Cissampelos pareira* (Vijayan et al. 2014); Sequence Characterized Amplified Regions (SCAR) for *Bacopa monnieri* (Yadav et al., 2012), *Aconitum heterophyllum* and *Cyperus rotundus* (Seethapathy et al., 2014), *Lonicera japonica* (Fu et al., 2013), *Ophiopogon japonicas* (Li and Park, 2012), *Phyllanthus amarus* (Theerakulpisut et al., 2008); Loop Mediated Isothermal Amplification (LAMP) for *Nigella sativa* (Ganie et al., 2013), *Taraxacum formosanum* (Lai et al., 2015), *Zingiber officinale* (Chaudhary et al., 2014), *Curcuma longa* (Sasaki and Nagumo, 2007), *Catharanthus roseus* (Chaudhary et al., 2012), *Panex ginseng* (Sasaki et al. 2008). The latest advancement in the DNA sequencing technology and bioinformatics tools for DNA sequence data analysis lead to the development of DNA barcoding techniques (Hebert et al., 2003, 2004; Hebert and Gregory, 2005; Hebert and Barrett, 2005; Ali et al., 2014) which have revolutionaries the method of the plant taxonomic identification (Poczai and Hyvönen, 2010) using DNA barcoding method especially based on DNA barcode sequence such as nrDNA ITS1, nrDNA ITS2 (Chen et al., 2010; Yao et al., 2010), *rbcL, matK, ycf5, rpoC1, psbA-trnH,* rps16, *trnL-F* and *ndhF* (Ali et al., 2014). DNA barcoding have successfully employed in authentication of *Crocus sativus* (Jiang et al., 2014), *Schisandra chinensis* (Li et al., 2013), *Astragalus* (Gao et al., 2009). The Next Generation Sequencing (NGS) is comparatively new, and have been demonstrated in the authentication of *Costus pictus* (Annadurai et al., 2012), *Aconitum* (Yun et al., 2015), *Dendrobium officinale* (Guo et al., 2013), *Huperzia serrata* and *Phlegmariurus carinatus* (Luo et al., 2010), *Valeriana officinalis* (Pyle et al., 2012), *Hippophae rhamnoides* (Ghangal et al., 2013), *Ocimum sanctum* (Rastogi et al., 2015), *Beta vulgaris* (Dohm et al., 2014), *Panax ginseng* (Jayakodi et al., 2014), *Elaeis guineensis* (Singh et al., 2013), *Curcuma longa* (Annadurai et al., 2013), *Catharanthus roseus* (Van Moerkercke et al., 2013), *Withania somnifera* (Gupta et al., 2013), *Azadirachta indica* (Krishnan et al., 2012), *Cannabis sativa* (van Bakel et al., 2011) and *Populus trichocarpa* (Tuskan et al., 2006). Moreover, Chen et al., (2010) demonstrated the potential use of ITS2 in the DNA barcoding of medicinal plants. The present species specific molecular signature of *A. deserti* nevertheless will be useful in molecular authentication, molecular phylogeny and DNA barcoding of the genus.

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