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Investigation on *Commiphora gileadensis* (syn. *Commiphora opobalsamum*) (Burseraceae) Plant Species in a Saudi Arab Desert

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Commiphora gileadensis **is a plant species belongs to Burseraceae family that was cultivated in ancient times. The plant is also known as balsam, was renowned for the expensive perfume that was produced from it, as well as for exceptional medicinal properties that were attributed to its sap, wood, bark, and seeds. The aim of this study was to evaluate the variants for assessing genetic relationships and diversity in *Commiphora gileadensis* species collections. Selected plant species were collected from Al-Asir and Al-Makkah region from Kingdom of Saudi Arabia. DNA was extracted from the selected plant species using the phenol-chloroform method and polymerase chain reaction (PCR) was performed with the specific primers chosen from ITS2 gene. Later on, DNA sequencing was performed to find out the presence of mutation. In this study, we did not find any mutation after performing the PCR and DNA sequencing. In conclusion, we cannot find any mutation from the plant species B and J from Saudi Arabia.**

Key words: *Commiphora gileadensis,* Al-Asir, Al-Makkah, PCR-DNA Sequencing.

Commiphora gileadensis (syn. *Commiphora opobalsamum*) (syn. *Commiphora opobalsamum*) (BURSERACEAE) communally referred to as Balm of Mecca, belongs to the Burseraceae family, and is widely known in the Mediterranean Basin, within the dry stony hills around the Red Sea, especially within the borders of Saudi Arabia, Yemen, Oman, and Eritrea[1]. The perfume was widely known in the Mediterranean Basin because balsam was cultivated exclusively in the land of Israel or, more precisely, in the oases of the Dead Sea Basin, Ein Gedi and Jericho. It is also known as balsam, and well known for the expensive perfume, produced from it, as well as for exceptional medicinal properties that were attributed to its sap, wood, bark, and seeds. It was recognized in ancient times, along with myrrh and frankincense, as a perfume and incense plant that grows in areas with very specific ecological conditions[2]. It yields a fragrant of oleo-gumresin, following the damage of the bark. In the middle ages, balsam cultivation shifted to Egypt for approximately one thousand years[3-5], but its importance has declined over the last few centuries. The crude methanolic extract of *Commiphora* show a significant anti mycobacterial activity, with a minimum inhibitory concentration of 62.5 µg/ml[6]. *C. gileadensis* was also active against *E. coli*, and *Bacillus cereus*[5]. The aim of this study was to evaluate the variants for assessing genetic relationships and diversity in *C. gileadensis* species collections. To our knowledge, this is the first study carried out in Saudi Arabian region.

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METHODS

Collection

In this study, *C. gileadensis* species were collected from Asir Area (Sample named as A) and Makkah Area (Sample named as M) from the Saudi region. The collected plants identification took place in our laboratory. Stem parts of collected plants were washed separately with distilled water, and air dried at room temperature.

DNA and PCR amplification

DNA was separated from A and M DNA was isolated by standard phenol-chloroform method as described by Natoli *et al.*,[7]. The DNA isolation technique was adopted from molecular genetic analysis of population. The quality of DNA was quantified with Nanodrop 2000 spectrophotometer. DNA was stored in the freezer for further analysis. The Internal transcribed spacer (ITS2) gene region was amplified using the following universal primers: ITS2F: ATGCGATACTTGGTGTGAAT, and ITS2R: GACGCTTCTCCAGACTACAAT[8]. Primers were synthesized from Applied Biosystems, Saudi Arabia. Polymerase chain reaction (PCR) amplification was implemented using approximately 30 ng genomic DNA as a template in a 25 mL reaction mixture as described by Gu *et al.*,[8]. The initial denaturation was performed with 94 °C for 5 min and denaturation with 94 °C for 45 sec and 40 cycles, 55 °C for 45 sec, and 72 °C for 45 sec, followed by 72 °C for 30 min. After the reaction has been completed, gel run was carried out with PCR products (Figure1) and DNA sequencing was carried out.

RESULTS AND DISCUSSION

PCR amplification success rate

After the separation of genomic DNA, PCR was carried out to analyze the results by DNA Sequencing. The PCR amplification rate in ITS2 gene sequences from medicinal plants of *Commiphora Gileadensis* was 100%, and the DNA sequencing success rate was 100%. The amplified sequence length was 378 bp. After removing the conserved 5.8S and 28S rRNA sequences, the lengths of the ITS2 sequences used in the analyses ranged from 145 to 189 bp, with an average length of 162 bp. The mean GC content was 56% and ranged from 46% to 67%. Therefore, the length and GC content.

DNA genotyping

We have performed the DNA sequencing analysis for the selected plants i.e. Sample A and M. None of the mutations were included in this study for the Sample A and M plants, which indicates there is no harm and it can be used in the medicine preparations (Figure 2).

To the best of our knowledge, this is the first study carried out in Saudi Arabia. In this study, we used the ITS2 region of nuclear ribosomal DNA to test 2 samples of *Commiphora Gileadensis* species. Our results did not highlighted the advantages of using the ITS2 region as a DNA barcode; which may include good universality, small intraspecific variation but high interspecific divergence, and a small fragment length, approximately 200 bp. Indeed, this lead to easy amplification and sequencing in one Sanger reaction. In addition, both the primary and secondary sequence structures of ITS2 perform

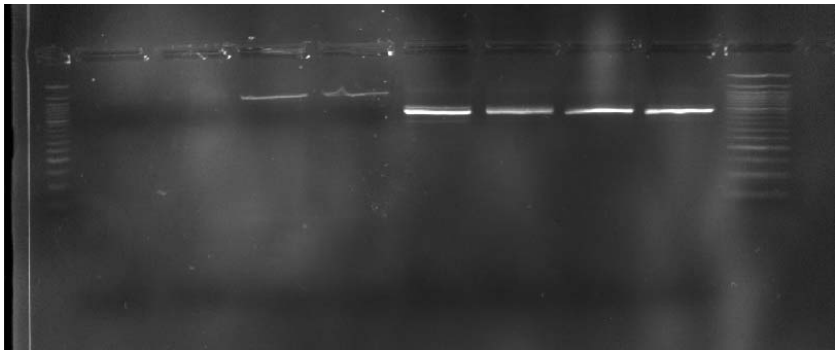

Fig. 1. Representation of agarose gel to identify the bands

well and provided sufficient molecular morphological characteristics to distinguish the *Commiphora Gileadensis* species.

The use of plants, their extracts inclusive for secondary bioactive metabolites (tannins, terpenoids, alkaloids, and flavonoids), in traditional medicine, increased significantly[9-10]. The flexible, strong, young stems or roots of *Salvadora persica* (miswak) and *Commiphora gileadensis* (Balasm) are common in the Saudi Arabian region, and the Middle East. They are inexpensive, and traditionally used to clean teeth[1]. *Commiphora gileadensis* were collected, and extracted with phenol chloroform method and screened for the analysis of the mutations.

ITS2 is considered to have evolved in concert, which leads to a homogenization of all the copies of this gene throughout the genome and in

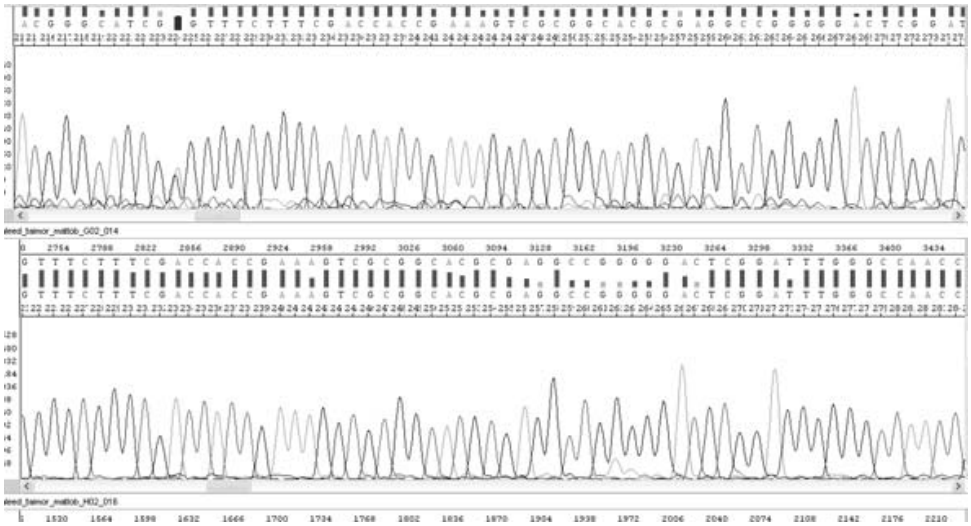

Fig. 2. Sequencing analysis used for the detection of the mutation

most organisms ITS2 was treated as a single locus. Thus, the ITS2 region might be a suitable marker for taxonomic classification[11-13]. Recently, ITS2 has been suggested as a useful barcode for medicinal plants[14-18], as a universal DNA barcode to identify plants and as a complementary locus of CO1 to identify animals[19]. The China Plant Barcode of Life Group considered ITS2 to be a useful alternative to internal transcribed spacer (ITS) because it is more easily amplified and sequenced[20]. In addition, the secondary structure of ITS2 was shown to be an efficient tool for biological species identification[21-22]. One of the main problems with Evidence-Based Complementary and Alternative Medicine compounds used for chemotherapy is that they are nonselective, namely, they kill normal cells as well as cancer cells[23]. Our study concludes that none of the mutation were identified from the plant species B and J from Saudi Arabia.

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