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# A SIMPLE, RAPID AND EFFICIENT METHOD FOR THE EXTRACTION OF GENOMIC DNA FROM ZIZIPHORA L. SPECIES (LAMIACEAE).

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**Annotation.** The chemical composition of the plant depends on its growth area. It is important to know the chemical composition of the plant during the preparation of protocol for DNA extraction. In Uzbekistan the STAB method is widely used to extract DNA from plants. However, the STAB method for DNA extraction from medicinal plants containing a lot of essential oils, such as *Ziziphora*, does not give successful results. The article provides conclusions on the use of the STAB and KIT methods and its effectiveness in the case of *Ziziphora*.

**Key words.** *Lamiaceae*, *Ziziphora* L., STAB methods, KIT methods, DNA extraction, nanophotometr.

**Introduction.** Among the *Lamiaceae* family, *Ziziphora* L. stands out for its medicinal properties. The genus *Ziziphora* L. (Lamiaceae) comprises about 30 species widespread over Asia, Africa and Europe [1]. Among the Lamiaceae as a result of our research on the distribution of *Ziziphora* L. species, it was concluded that 6 species and 4 subspecies of this genus are distributed on the territory of the Uzbekistan. They are *Z.clinopodioides* Lam., *Z.pedicellata* Pazij & Vved., *Z.suffruticosa* Pazij & Vved., *Z.capitata* L., *Z.persica* Bunge., *Z.tenuior* L., *Z.clinopodioides* subsp. *interrupta* (Juz.) Sennikov., *Z.clinopodioides* subsp. *clinopodioides*, *Z.clinopodioides* subsp. *bungeana* (Juz.) Rech.f., *Z.clinopodioides* subsp. *pamiroalaica* (Juz.) Sennikov & Lazkov (Fig.1,2). The large number of subspecies within the taxon requires the use of not only morphological methods but also molecular methods for their identification. DNA samples are the basis of molecular analysis. Cloning, sequencing, and efficient PCR amplification depend on the isolation of high-quality purified DNA. DNA quality and

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purity can be affected by a number of factors including: sample size, the presence of contaminants in a sample, nucleic acid degradation during preparation, and the efficiency of lysis during sample preparation.


**Figur 1,2.** Morphology and distribution of the genus *Ziziphora* L (using *Z.tenuior* L as an example).

**Material and methods.** As material, we used dry leaf samples of plants belonging to the Ziziphora L.species. In Kit method buffer G1, mercaptophenol, chloroform:isoamyl (24:1), buffer G2, buffer GD, buffer PW, buffer TE were used, while for the CTAB method the buffer consisted of 2% CTAB (w/v), 100 mM Tris-HCl (pH 7.5), 20 mM EDTA (pH 7.5), 1.4 M NaCl, 1% PVP; chloroform isoamylalcohol (24:1), 70% ethanol, 5 M NaCl, and a TE buffer consisting of 1 M Tris-HCl (pH 7.5) and 0.5 M EDTA were used.

The DNA isolation process was done by following steps:

I. *CTAB method process:* Thirty mg of dry leaf material was weighed and placed in a pre-cooled mortar. The leaf sample was crushed into small pieces with a pestle and added to 800 µl of CTAB extraction buffer. The mixture was incubated for 90 min at 65°C with constant shaking at 10-minute intervals. 600 µl of chloroform-isoamyl alcohol (24:1) was added to the mixture. The emulsion was centrifuged at 12,000 rpm for 10 min. About 400 µl of the supernatant was transferred to a new tube using a micropipette. A second extraction with chloroform-isoamyl alcohol was then performed. The emulsion was centrifuged at 12,000 rpm for 10 min. The supernatant was carefully decanted and transferred to a new tube, then precipitated with 40 µl

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CH3COONa (3 M) and 400 µl isopropyl alcohol, then kept at -20 °C for about 3 h. The precipitated nucleic acids were collected and washed with 900 µl of 70% ethanol. Then centrifuged at 14,000 rpm for 15 min. Alcohol was discarded, the pellets were dried in an incubator at 37 °C for 20 min and resuspended in 30 µl TE buffer to solubilize genomic DNA, and measured with a nanophotometer (Implen 6405 UV/VIS).

II. *KIT method process:* We took 30 mg of dry sample leaves of the *Ziziphora* L. species, placed it in a mortar, and mechanically grind it with a pestle. 800 µl of buffer G1, and 3-5 µl of mercaptophenol was added at the same time. The resulting homogenate was collected in an eppendorf tube and incubated in a thermal shaker water bath (65 °C) for 30 min. After the incubation time was over, 700 µl of chloroform:isoamyl alcohol (24:1) was added and inverted gently by hand, then centrifuged at 14,000 rpm for 10 min. The upper phase was transferred to a new tube and dissolved in 700 µl of buffer G2. We transferred 400 µl of this supernatant to an Eppendorf filter, and centrifuge at 14,000 × g for 30 seconds. We repeated this process again. After repeating the process, the supernatants were discarded. The remaining pellets were dissolved in 400 ml of GD buffer and centrifuged at 12,000 × g for 30 s. Once again, the supernatants were discarded. The remaining pellets were dissolved in 400 ml of PW buffer and centrifuged at 16,000 × g for 30 s. The supernatant was removed and the pellet was transferred to a new tube and dried at room temperature for 30 min, and the DNA was dissolved in 30 ml TE buffer and centrifuged at 12,000 × g for 2 min. DNA diluted with TE buffer is analyzed into nanodrops, then used in PCR (RT-PCR), sequencing, and many other tasks.

**Results.** The DNA samples obtained by both methods were measured using a Jenway 6405 UV/VIS nanophotometer at a wavelength of 260 nm. The DNA purity was determined by calculating the absorption coefficient A260/280. Pure DNA has a ratio of 1.8 ± 0.1 [2]. Polysaccharide contamination was estimated by calculating the absorption coefficient A260/230 [3]. According to the results of the STAB method, the DNA concentration of Z. tenuior was 68.000 ng/mL. The purity ratio was Z. tenuior 1.317 at 260/280 nm. Then, we re-measured the purity using a wavelength of 230/260

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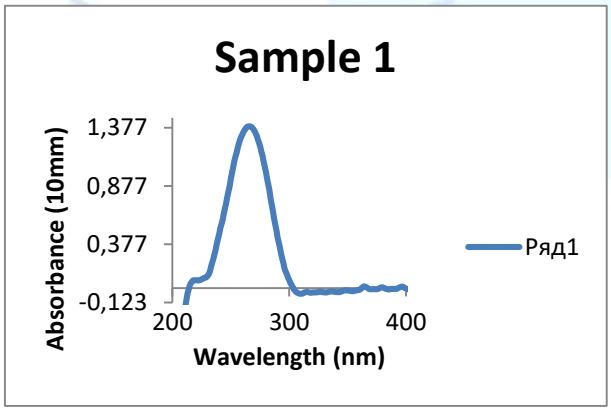
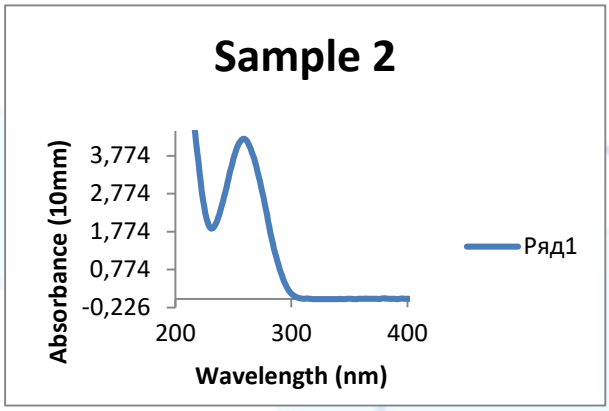
nm. Because there may be contaminants that do not absorb at 280 nm, it was Z. tenuior 0.995, indicating good DNA purity (Fig. 1). According to the results of the KIT method, the DNA concentration of Z. tenuior was 210.95 ng/mL. The purity ratio was Z. tenuior 2.055 at 260/280 nm. Then, we re-measured the purity using a wavelength of 230/260 nm. Because there may be contaminants that do not absorb at 280 nm, it was Z. tenuior 2.049, indicating good DNA purity (Fig. 2).




**Figur 1. Nanodrop analyses by CTAB methods; 2. Nanodrop analyses by KIT methods.**

**CONCLUSION.** We selected the species *Z. tenuior* for DNA analysis of *Ziziphora* L. Because *Z. tenuior* is spread over a wide area and grows in a temperate climate region. Among the species in the category, it is distinguished by the large amount of essential oils in its composition (0.6–10%) [4]. As we know, extracting DNA from essential oil plants is quite complicated. As a result of our research, it was possible to extract high-quality DNA using the KIT method. In our opinion, it was useful to use more buffers in the KIT method. In comparison, the SATB method uses fewer buffers. Because the function of purifying essential oils is performed more by the buffer.

According to our conclusion, it is better to use the KIT method when extracting DNA from *Ziziphora* L.

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**References.**

1. Srivedavyasasri R. et al. Phytochemical and biological studies on *Ziziphora bungeana* //Chemistry of natural compounds. – 2018. – Т. 54. – С. 195-197.

2. Joyce, A.C. (2014). Genetic relationship of Elymus alaskanus, E. caninus, E. fibrosus, and E. mutabilis revealed by chloroplast DNA sequences.

3. Wilson IG (1997) Inhibition and facilitation of nucleic acid amplification. Appl. Env. Microbiol, 63: 3741-3751

4. Юзепчук С.В. Зизифора – *Ziziphora* L. / С. В. Юзепчук // Флора СССР. – М.: Л.: Наука, 1954. – Т. 21. – С. 381–411.