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COMPARATIVE STUDY OF NORDIHYDROGUAIARETIC ACID EXTRACTION METHODS FROM CREOSOTE BUSH (*LARREA TRIDENTATA*)

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Introduction. Nordihydroguaiaretic acid (NDGA) is a lignan found in a semi-desert plant called creosote bush (*Larrea tridentata*). Several studies have demonstrated that NDGA has important biological activities with a great interest mainly in the health area [1]. Extraction of bioactive compounds from plants is conventionally performed using a heat-reflux extraction method but different techniques have been developed in order to decrease the bioactive compounds extraction time and solvent consumption, as well as to increase the extraction yield and enhance the extracts quality [2]. Therefore, the aim of this study was to evaluate different extraction methods to extract NDGA from creosote bush leaves.

Methodology. Plant material (*Larrea tridentata*) was collected from the semidesert region of Saltillo (North Coahuila, Mexico) during winter season (April, 2008). Leaves were dried in an oven at 60°C for 48 hours and ground to fine powder. **Conventional extraction by reflux (CER)**: twenty-five grams of dried powdered plant were mixed with 100 ml of methanol and digested by heat-reflux in a water bath for 1 hour at 55-60°C; **Extraction by ebullition (EE):** ten grams of dried powdered plant were mixed with 90 ml of methanol and boiled for 10 min on a hot plate; **Ultrasound-assisted extraction (UAE):** one gram of dried powdered plant was placed in a 100 ml glass beaker and 50 ml of methanol were added. The sample was placed in an ultrasonic water bath and subjected to ultrasonic treatment for 20 min at room temperature; **Microwave-assisted extraction (MAE):** one gram of dried powdered plant was mixed with 30 ml of methanol and irradiated for 4 minutes at 800W (70°C). Between each minute of irradiation the sample was allowed to cool at room temperature; **Enzyme-assisted extraction (EAE):** one gram of dried powdered plant was mixed with 40 ml of 0.05M sodium acetate buffer at pH 4.8, and 1% Pectinex Ultra was added. Samples were shacked for 2 hours at 50°C in an incubator at 150 rpm.
Before HPLC analyses all samples were filtered through a 0.45 µm membrane filter. NDGA was quantified by HPLC according to the methodology described by Mercado-Martínez (2008) [3]. All analyses were performed in triplicate.

Results and Discussion. A preliminary study on NDGA liberation from creosote bush leaves during conventional heat-reflux extraction showed that the highest concentration was observed after 1 hour (36.27 mg/g DW). Results showed that NDGA released using MAE and EE techniques was significantly higher (*P<0.05*) compared to CER (Fig. 1), 42.87 and 45.03 mg/g DW, respectively. On the other hand, when UAE and EAE methods were used a significantly lower concentration of NDGA (*P<0.05)* was obtained.

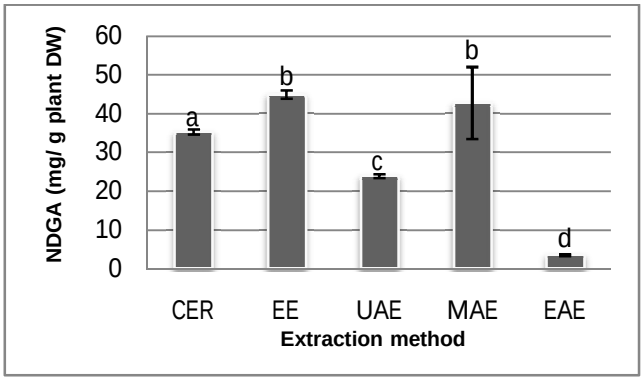

Fig.1. *Effect of different extraction methods on NDGA liberation from creosote bush leaves ([abcd]Values at P<0.05).*

Conclusion. The results of this study showed that MAE and EE were more effective extraction methods of NDGA from creosote bush leaves compared to the conventional technique. These extraction techniques reduced the extraction time to 4 and 10 minutes, respectively. However, MAE has the advantage of using less solvent which decreases the costs of the process. Further research is needed in order to optimize this extraction method.

References.

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