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IDENTIFICATION AND EXTRACTION OF HIGHLY VALUABLE FLAVONOIDS IN *AGAVE LECHUGUILLA* BY-PRODUCT: VALORIZATION OF GUISHE.

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Introduction. *Agave lechuguilla* Torrey (1986) is one of the most abundant key species in arid and semiarid regions of Mexico. The lechuguilla is used to extract Tampico fiber (Ixtle) through manual or mechanical shredding process mainly for export with a price of around 13 Mexican pesos/kg (1). However, fiber recovery is discarded as an industrial residue, the guishe, which represent around 85% of the harvested plant material leading to environmental pollution and toxicity. Thus, for a sustainable development of the productive regions, valorization of the lechuguilla by-product emerged as priority through the last decade. Previous studies have considered the lechuguilla as a source of bioethanol and bioactive compounds for application in agriculture, feed, food and cosmetology (2). Nonetheless, the flavonoid content in guishe was never be precisely screened, even though they present a huge interest due to their wide range of activities (3).
This work claims alternative methods to identify and extract highly valuable flavonoids from guishe to purpose them as new natural bioactive compounds with potential application in industrial sectors.

Methodology. Flavonoids content was predicted by transcriptomic analyses correlating the expression pattern of genes involved in flavonoids biosynthesis and the concentration of these metabolites observed by HPLC-MS (4). Bioactive extract enriched in flavonoids will be obtain by the supercritical fluid extractive technique instead of solvent extraction to provide high quality natural products to industrial sectors (5). Finally, potential bioactivities as antioxidant, antimicrobial and prebiotic will be evaluated through *in vitro* and *in vivo* assays with a focus on aquaculture system.

Results. The achievements in functional annotation of the *de novo* transcriptome of *A.lechuguilla* (255.7 Mpb) results in the identification of 42 genes coding for enzymes and transcription factors involved in the flavonoid biosynthesis. Additionally, the differential expression shows that at least 8 of those genes were overexpressed in guishe compared to complete leaves with a p-value < 0.05 and an FDR < 0.02 (**Table I**). The biochemical screening reveals a concentration of about 500ppm in ethanolic and methanolic extracts which presented antioxidant activities. In the same time, the identification of these enzymes allows prediction the structure and the physico-chemical properties of the produced metabolites which is needed for optimize the supercritical fluid extraction method. Furthermore, HPLC-MS analysis of the flavonoid content depending on the dry method results in that the gas dehydrator is a good alternative to lyophilization as part of extraction optimization process.

Conclusions. Highly valuables flavonoids are produced in the guishe accordingly to transcriptomic prediction validated by HPLC-MS observations. The concentration and the physico-chemical properties of those compounds allow the optimization of their extraction by supercritical fluid technique. Pretreatment and extractive conditions still must be determined specifically for each of the flavonoids that will be extracted.

Table I. Flavonoid biosynthesis enzymes identified as up-regulated in guishe mapped in KEGG database.

KEGG IDs	Enzyme Classification	Name
K00660	EC 2.3.1.74	Chalcone synthase
K00660	EC 2.3.1.170	6'-deoxychalcone synthase
K13065	EC 2.3.1.133	Shikimate O-hydroxycinnamoyltransferase
K10775	EC 4.3.1.24	Phenylalanine ammonia-lyase
K13064	EC 4.3.1.25	Phenylalanine/tyrosine ammonia-lyase
K01188	EC 3.2.1.21	Beta-glucosidase
K12938	EC 2.4.1.-	Anthocyanidin 5,3-O-glucosyltransferase
K12939	EC 2.4.1.238	Anthocyanin 3'-O-beta-glucosyltransferase

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