Chemical biodirected determination of *Ricinus communis* and *Argemone mexicana* extracts

Hebert Jair Barrales-Cureño¹, Jesús Eduardo Zaragoza Ruiz¹, César Reyes Reyes¹, Petra Andrade Hoyos², Alfonso Luna Cruz³, Leticia Mónica Sánchez Herrera⁴, Juan Antonio Cortes Ruiz⁵, María Carmina Calderón Caballero⁶, Salvador Chávez Salinas¹ and Luis Germán López Valdez⁷

¹(Departamento de Ingeniería en Biotecnología. Universidad Politécnica del Valle de Toluca. Carretera Toluca-Almoloya de Juárez Km. 5.6 C.P. 50904. Santiaguito Tlalcilalcali, Almoloya de Juárez, Estado de

México)

²(Ingeniería en Innovación Agrícola Sustentable. Instituto Tecnológico Superior de la Sierra Norte de Puebla) ³(Facultad de Ciencias Agropecuarias. Universidad Michoacana de San Nicolás de Hidalgo)

⁴(Universidad Autónoma de Nayarit, Unidad de Tecnología de Alimentos) ⁵(Instituto Tecnológico de Mazatlán, Sinaloa, México)

⁶(Instituto Tecnológico Superior de Uruapan)⁷(Preparatoria Agrícola. Universidad Autónoma Chapingo).

Abstract: In human pharmacology, Ricinus communis and Argemone mexicana are two medicinal plants with great therapeutic potential. Identification of therapeutic secondary metabolites was performed in this research from Ricinus communis and Argemone mexicana aqueous, chloroform and ethanol extracts. Argemone mexicana flavonoids were identified through the Shinoda, gaseous ammonia and base action on aqueous extracts tests. Moreover, flavonoids analysis was performed using Ricinus communis and adding gaseous ammonia and base. Presence of sterols was also identified via the Liebermann-Burchard test; saponins recognition by way of the dihydrocodeine test; leucoanthocyanidins detection was conducted by using the acidity test; meanwhile coumarins identification was made through applying UV light on Ricinus communis extracts. In addition, works revealed acidity and presence of vitamin C, and included DNA extraction from different organs of the Ricinus communis plant.

Keywords – Flavonoids, Leucoanthocyanidins, Saponins, Secondary metabolites, Vitamin C.

I. INTRODUCTION

Argemone mexicana plant belongs to Papaveraceae family. It is used to treat different respiratory diseases such as asthma. Furthermore, Argemone mexicana helps stimulating diuresis and eliminates renal calculus, hydrops, cataracts and eye infections and fever [1]. Nowadays, malaria is treated with the use of the plant in various countries. It is also an important compound in the production of pesticides for organic agriculture. Finally, it is regarded as a potentially bioenergetics plant [2].

On the other hand, *Ricinus communis* belongs to *Euphorbiaceae* family. The plant is grown in many parts of the world and is largely used. For instance, stems are used for papermaking and leaves as a pharmaceutical product. In their own, seeds are relevant in the production of castor oil –a compound that has laxative properties, used in aircrafts and as an important material in the manufacturing of soaps and tinctures, among others. *Ricinus communis* has been used in the last 4000 years in order to cure digestive disorders, flu, uterus inflammation, stomach pain, injuries, swollenness, abscesses and rheumatism. It also has antioxidant, anti-inflammatory, antidiabetic, central analgesic and antitumor properties. Moreover, it causes inhibition of adult insect's bites and serves as larvicide. Caution is advised in the use of the plant and its derivatives due to their content of alkaloids (alocryptopine, berberine, codeine, copticine, dihydrosanguinerine, morphine, and sanguinarine), a highly poisonous compound family [3]. Ricin is a phytotoxin with cytotoxic activity present in castor plant (*Ricinus communis* L.) [4].

Geographical distribution of the *Argemone mexicana* plant ranges from North America and Central and South America to Argentina, Cuba and the Antilles. On its own, *Ricinus communis* is found in Mexico, Colombia, Venezuela, India, China, Brazil and Peru. On a general manner, extracts are substances integrated by a great array of multivariate chemicals compounds, which are obtained using solvents from natural sources. In this regard, different therapeutic properties have been found at vegetables in the past years. This has conducted to an increase in the scientific research activity, mainly at a greater knowledge and tests carried out at Homeopathy and Phytotherapy. Moreover, identification of secondary metabolites by phytochemical analysis allows a better knowledge of new biotic health products for humans in order to conduct deeper studies on the therapeutic potential of plants around the world. This research was focused in the chemical analysis of the main secondary metabolites contained in *Ricinus* communis and *Argemone mexicana*.

II. MATERIAL AND METHODS

Flavonoids identification through the Shinoda test. *Argemone mexicana* and *Ricinus communis* leaves were dried and macerated. Subsequently, 300 mL of reagent grade ethanol were added to the organic material. Ethanolic extracts were filtered and cooled. Later, 10 mL of magnesium chips were added jointly with concentrated hydrochloric acid.

Flavonoids identification through gaseous ammonia. *Ricinus communis* and *Argemone mexicana* leaves were dried and 100 g were macerated for the further addition of 400 mL of distilled water to form an aqueous extract. Later, 400 mL of ethanol were incorporated. Mixtures were filtered for their placement in a vessel containing 300 mL of ammonia.

Flavonoids identification through base action. 100 g of *Argemone mexicana* and *Ricinus communis* leaves were dried, and then macerated in a porcelain mortar. Subsequently, 500 mL of distilled water was used to prepare the aqueous extracts. The mixture was filtered. Then 150 mL were used to add 20 mL of 0.1 N sodium hydroxide to each sample. Four replications were carried out.

Sterols identification through the Liebermann-Burchard test. 100 mL of chloroform were added to 10 g of *Ricinus communis* material. Subsequently, extracts were filtered and added 3 drops of Liebermann reagent to the sample.

Saponins identification test. Fresh *Ricinus communis* leaves were used in the test. 50 g were placed in a beaker and added 300 mL of ethanol. The extract was filtered through a gauze. Subsequently, 40 mL of the extract were placed in a test tube and vigorously stirred. Foam presence indicates the existence of saponins. The compound represents a water-soluble, foam-producing group of oily glycosides.

Leucoanthocyanidins identification. 50 g of dry *Ricinus communis* material was weighed for a later addition of 100 mL of ethanol. Subsequently, the mixture was filtered and added 50 drops of concentrated hydrochloric acid for a later water bath.

Ricinus communis acidity identification. 100 mL of *Ricinus communis* aqueous, ethanolic and chloroform extracts were prepared. Subsequently, 50 mL of each extract were measured in a 500 mL volumetric flask with distilled water and the addition of 0.1 N sodium hydroxide.

Coumarins identification through UV light. 50 mL of ethanolic extract were transferred to a beaker on which a filter paper was placed. Later, 5 drops of ethanolic extract were added on the paper. Subsequently, the beaker was heated on an electric grill until boiling. Finally, observation of the paper through UV light was carried out.

III. RESULTS

Argemone mexicana analysis. Chemical bio directed Shinoda test on Argemone mexicana showed flavone-type flavonoids in ethanolic extracts of leaves and fruits (Fig. 1). Chalcones and aurones were detected in the aqueous extract through the gaseous ammonia test, as well as flavones and flavonols in the ethanolic extract (Fig. 2). As for the base action test, flavonal and flavonols were detected in stems, flavonols in petals, chalcones in flowers, and flavonols in pistils from their aqueous extracts (Fig. 3).





Figure 1. Flavonoids identification through the Shinoda test in *Argemone mexicana*.

Figure 2. Flavonoids identification through gaseous ammonia in Argemone mexicana.



Figure 3. Flavonoids identification through base action in Argemone mexicana.

Ricinus communis analysis. Presence of flavones and flavonols in leaves, chalcones and aurones in aqueous extracts, flavones and flavonols in ethanolic extracts, flavones and flavonols in the stem, were detected using the gaseous ammonia test (Fig. 4).



Figure 4. Flavonoids identification through gaseous ammonia in Ricinus communis.

As for the base action test in *Ricinus communis*, it gave positive due to the presence of flavones and isoflavones in flowers and flavonols in leaves, stems and fruits (Fig. 5).



Figure 5. Flavonoids identification through base action in *Ricinus communis*.

Moreover, the Liebermann-Burchard test identified sterols in *Ricinus communis*, giving positive for leaves, fruits and stems from chloroform extracts. In addition, it tested positive for the flower; however, a yellow color was obtained indicating that the sterol contained a methyl group (CH₃) in C_{14} and an unsaturated group in C_7 (Fig. 6).



Figure 6. Sterols identification through the Liebermann-Burchard test in Ricinus communis.

On their own, saponins were identified in the biological material prepared. Saponins identification test in aqueous extracts was positive, revealing an increased presence of saponins in *Ricinus communis* fruits (Fig. 7). The leucoanthocyanidins identification test was positive in *Ricinus communis* ethanolic extracts, with a greater concentration of leucoanthocyanidins in leaves (Fig. 8). As for the acidity, measurements were 0.025, 0.05 and 0.05 % in chloroform, ethanolic and aqueous leaves extracts, respectively. Similarly, acidity percentages from stem extracts were 0.025, 0.025 and 0.051 % in chloroform, ethanolic and aqueous extracts, respectively. Similarly, acidity percentages from stem extracts were 0.025, 0.025 and 0.051 % in chloroform extracts of fruits. Meanwhile, coumarins were detected by applying UV light on leaves, but not on the stem. Regarding Vitamin C, the nutrient was found in *Ricinus communis* leaves, flowers, fruits and stems from their aqueous extracts, with a greater concentration of the vitamin in the stems. Efficient proof of isolated DNA was also detected (Fig. 9).



Figure 7. Saponins identification test in Ricinus communis.



Figure 8. Leucoanthocyanidins identification in *Ricinus communis*.



Figure 9. Acidity, vitamin C, DNA extraction and coumarins phytochemistry analysis.

IV. DISCUSSION

Secondary metabolism is defined as the biosynthesis, transformation and degradation of endogenous compounds by specialization proteins, derived from differentiation processes and classified according to their biological significance and role in the producing cell [5].

Flavonoid analysis in *Argemone mexicana* matches that reported by various authors with a color analysis and preparative chromatography from ethyl acetate extracts. Analysis outcome of flavonoids and saponins presence in *Ricinus communis* is comparable to that reported by various authors, using a thin-layer and preparative chromatography analysis. It was observed that *Argemone mexicana* ethanolic extracts reveal antibiotic activity against bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* [6]. Acetone and aqueous extracts exhibit antibiotic activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Major metabolites isolated from *Argemone mexicana* are four quaternary isoquinoline-like alkaloids, namely dihydrocodeine, jatrorizine, columbamine and oxyberberine. On its own, berberine is used as an antibiotic. The two toxic alkaloids produced by these major metabolites are sanguinarine and dihydrosanguinarine. Furthermore, main *Ricinus communis* constituents reported are rutin, gentisic acid, quercetine, gallic acid, kaempferol 3-O-beta-rutinoside, tannins, ricin A, B and C [7]. A new benzylisoquinoline alkaloid, argemexirine, together with two known protoberberine alkaloids, dihydrocoptisine and di-tetrahydrocoptisine, have been isolated from the methanolic extract of the whole plant of *Argemone mexicana* [8].

V. CONCLUSION

The phytochemical analysis of *Ricinus communis* and *Argemone mexicana* allowed the actual identification of the main secondary metabolites synthesized in different organs of both plants using standardized methods.

VI. ACKNOWLEDGEMENTS

The authors would like to thank the Universidad Politécnica del Valle de Toluca and Rector Dr. Luis Carlos Barros González, Consejo Nacional de Ciencia y Tecnología (CONACYT) and PRODEP.

REFERENCES

- [1] HS Waizel and B.J. Waizel, Algunas plantas utilizadas en México para el tratamiento del asma. *Anales de Otorrinolaringología*, 54(4), 2009, 145-171
- [2] M Krishna, T. Khemchandani and R. Balaji, Extraction of a novel biopesticide obtained from agricultural weeds useful for medicinal plants, *Journal of Medicinal Plants Research*, 7(30), 2013, 2236-2242.
- [3] LP Iaxmikant and K.R. Bhimashankar. *Ricinus communis* (Castor): An Overview. *International Journal of Research in Pharmacology & Pharmacotherapeutics*, 3(2), 2014, 136-144.
- [4] R Pita, A. Anadón and L.M.R Martínez, Ricina: una fitotoxina de uso potencial como arma. *Revista de Toxicología*, 21(2), 2004, 51-63.
- [5] Y Lezcano, M. Soca, L.M. Sánchez, F. Ojeda, Y. Olivera, D. Fontes, I.L. Montejo and H. Santana, Caracterización cualitativa del contenido de metabolitos secundarios en la fracción comestible de *Tithonia diversifolia* (Hemsl.) A. Gray, *Pastos y Forrajes*, 35(3), 2012, 283-291.
- [6] A Osho and T. Adetunji, Antimicrobial activity of the essential oil of *Argemone mexicana* Linn. *Journal* of *Medicinal Plants Research*, 4(1), 2010, 19-22.
- [7] G Brahmachari, D. Gorai and R. Roy, *Argemone mexicana*: chemical and pharmacological aspects. *Brazilian Journal of Pharmacognosy*, 23(3), 2013, 559-575.
- [8] S Singh, T.D. Singh, V.P. Singh, V.B. Pandey. A new benzylisoquinoline alkaloid from Argemone mexicana, Natural Product Research, 24(1), 2010, 63-67.