Maestría en Ingeniería en Diseño de Bioprocesos

Obtaining Kaempferitrin with regulatory activity on adipogenesis: therapeutic approach for obesity

Jerson López Pérez

Fabiola Domínguez Avilés

Pilar Nicasio Torres

Leticia Ramírez Castillo

Patricia Jaramillo Quintero

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Jerson Lopez Perez[1], Fabiola Domínguez Avilés[2], Pilar Nicasio Torres[3], Leticia Ramirez Castillo[4] and Patricia Jaramillo Quintero [5].

Master in engineering in bioprocess design

Jerson.lopez4506@uppuebla.edu.mx

[1,4]Tercer Carril del Ejido Serrano S/N, San Mateo Cuanalá, Juan C. Bonilla, Puebla, México.
[2]Centro de Investigación Biomédica de Oriente, IMSS, Atlixco Puebla, HGZ No. 5 Km 4.5 Carretera Federal Atlixco.
[3]Centro de Investigación Biomédica del Sur, IMSS, Xochitepec Morelos. Republica de Argentina No.1 Col. Centro.
[5]Universidad Autónoma de Tlaxcala, Carretera Apizaquito S/N, San Luis Apizaquito, C.P. 90401, Apizaco, Tlaxcala, México.

1. Introduction

Justicia spicigera is an evergreen shrub with tubular orange flowers that grows in warm climates, native to Mexico and South America [1]. This plant is widely distributed in the states of Veracruz, Morelos, San Luis Potosí, Yucatán, Hidalgo and Puebla.

Previous phytochemical studies have shown that the ethanol extracts of the leaves of *J. spicigera* show the isolation of the flavone kaempherol-3,7-bisrhamnoside (kaempferitrin) active compound of this plant [2]. Also, the effects of ethanolic extracts of *J. spicigera* on the glucose uptake in insulin-sensitive and insulin-resistant murine 3T3-F442A and human subcutaneous adipocytes was evaluated [3].

That is why *J. spicigera* is a valuable natural resource, therefore, for future use, there is a great need for conservation, cultivation, production of metabolites and the sustainable use of this medicinal plant. Tissue culture techniques have been reported for the conservation, propagation and production of compounds of industrial interest of various medicinal plants.

In view of its potential pharmacological benefits, the objectives of this study It will be: *J. spicigera* cellular systems, producers of the active compound Kaempferitrin and evaluate its activity on adipocyte cells as the primary mechanism to its antidiabetic effect, already reported, in order to find a new compound with activity on obesity.

In this report we only report the first specific objective that is obtaining cell lines from *J. spcigera*

2. Objectives

2.1. General objective

To produce the active compound Kaempferitrine from to cell lines of the medicinal plant *J. spicigera* for future therapeutic applications in obesity.

2.2. Specific objectives

- To establish *in vitro* cell cultures of *J. spicigera* in solid medium, to obtain cell calluses.
- To Induce the production of kaempferitrin in cell lines, through biotechnological strategies.
- To Determine the activity of kaempferitrin on adipose differentiation and lipid accumulation of 3T3-L1 cells.

3. Methods

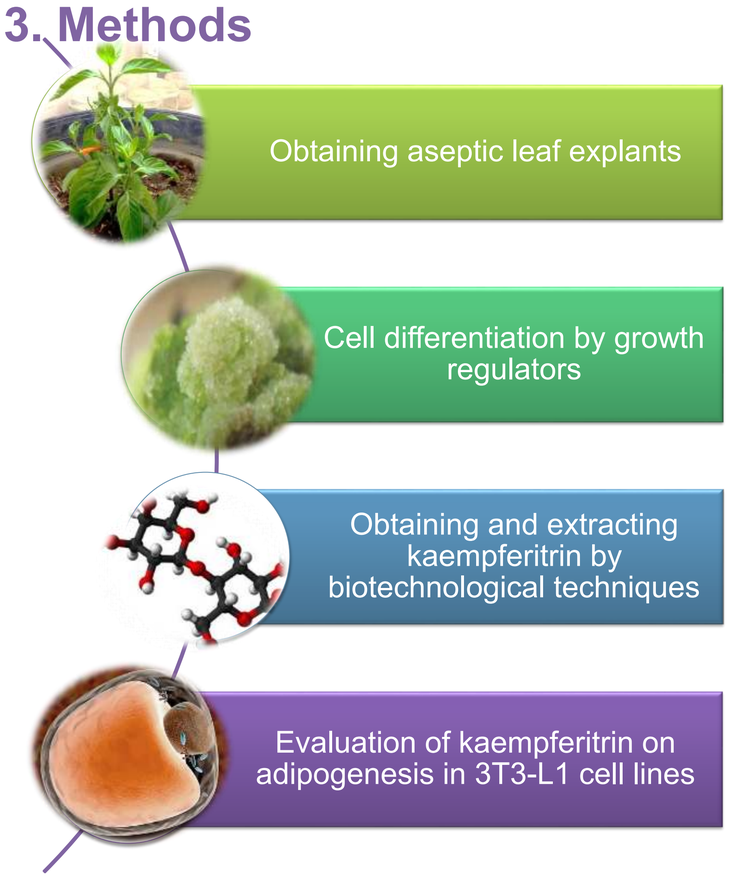

Figure 1. Methodology used in this research.

Figure 2. J. spicigera, plant used in this research project

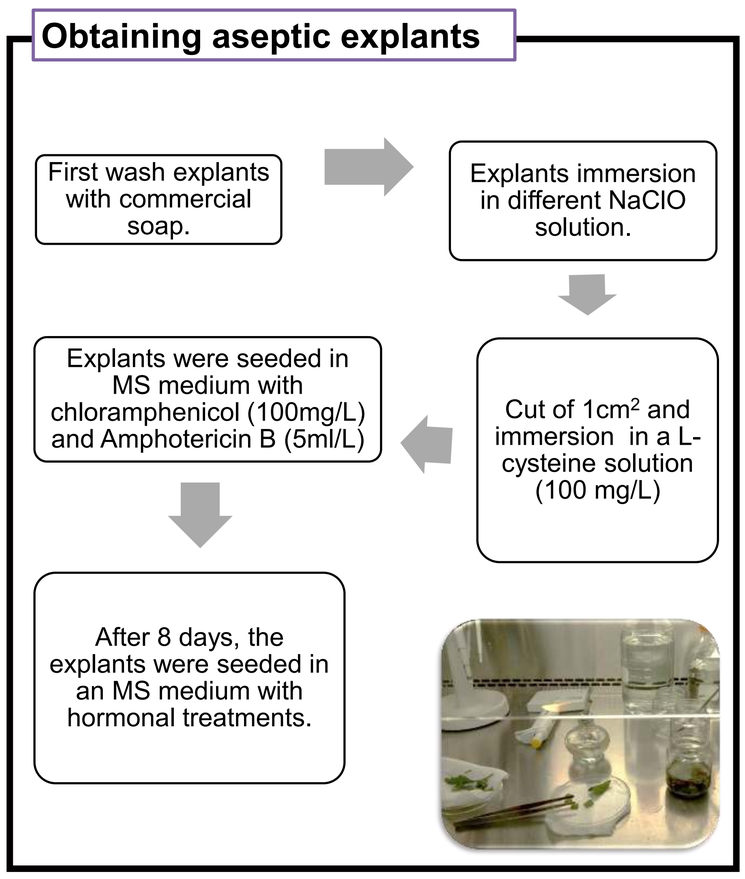

Figure 3. Flow diagram for obtaining aseptic explants

Hormonal treatments

Table 1. Hormonal treatments for the induction of calluses in for J. spicigera .

Treatment	Naphthalene acetic acid (NAA) mg/L	Benzylamino purine (BAP) mg /L
T1	1	0,5
T2	1	1
T3	0,5,	0,5
T4	0,5	1

4. Results

The experimental results showed that the asepsis of *J. spicigera* explants is fundamental for a viable crop, in this work the best concentration of sodium hypochlorite was 1.1% during 10 minutes. However, this was not enough to eliminate the endogenous contamination of the explants. Antibiotics were also used in the culture medium for better asepsis, such as chloramphenicol at a concentration of 100mg / L and Amphotericin B at 5ml / L.

The use of hormonal treatments showed a significant effect in the formation of calluses. T4 and T3 proved to be the most efficient with 20% and 43% induction with the characteristics of being a yellow and white friable callus; however they also exhibited a high percentage of oxidation above 40% (Table 2 and Figure 4).

Otherwise T1 and T2 had low induction percentages, between 3% and 12% with very poorly defined characteristics such as a compact white callus without mentioning that the percentage of oxidation was less than 30%.

Table 2. Morphological characteristics of callus of J. spicigera

Treatments	PGR mg/L		Callus morphological characteris	
	Auxin	Cytokinin	Type	Coloration
	NAA	BAP		
T1	1	0,5	CC	Y
T2	1	1	CA	-
T3	0,5	0,5	CF	W,Y
T4	0,5	1	CF	W,Y

PGR= Plant regulator grow.

Callus absence =CA , compact callus =CC, friable callus =FC, green =G, yellow =Y , transparent callus = TC and white =W

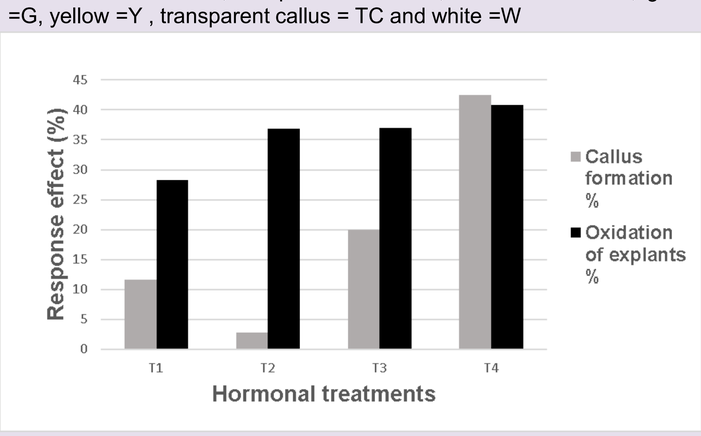

Figure 4. Percentage of callus response and oxidation of the explant using L-cysteine in the medium.

Figure 4 Callus formed at 30 days from sheets of J. spicigera with treatment 4.

5. Conclusion

According to preliminary results we can conclude that T4 hormone treatment has the ability to proliferate a viable cell line however it is recommended to use polyvinylpyrrolidone to avoid oxidation instead of l-cysteine. On the other hand, this thesis project has achieved a strong advance in the experimetal part in the obtaining of acpetic explants and callus induction, thus achieving 40% of the total thesis.

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