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Phytochemical Analysis in *Capsicum spp*

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ABSTRACT

Chili is one of the most consumed vegetables worldwide, after tomato, because of its exquisite flavor and high nutritional value. The genus Capsicum represents a potential source of capsaicinoids. The capsaicinoids are a set of secondary metabolites with different therapeutic activities. In this research, analysis of secondary metabolites by qualitative evidence of terpenoids, determination of vitamin C, total acidity and extraction of DNA in various species of chili were performed. The results demonstrate that DNA extraction was effective and efficient. The identification of the vitamin C is based on the organ of each species of Capsicum. The analysis of the identification of secondary metabolites from acetone extracts of Capsicum shows the presence of a higher content of sterols and lower content of steroids. There was a higher percentage of citric acid, malic acid and tartaric acid in Capsicum tissues without presence of oxalic acid.

KEYWORDS *Capsicum,* phytochemical, DNA extraction, secondary metabolites, vitamin C.

INTRODUCTION

Traditional medicine is an important and often underestimated part of health services. In some countries, traditional medicine or conventional medicine is often called complementary medicine. Historically, traditional medicine has been used to maintain health and prevent and treat diseases, particularly chronic diseases (OMS, 2013). The genus *Capsicum* comprises more than 26 species, of which only 12, including some varieties are used by humans. Only five species have been domesticated and cultivated. Today are grown in regions as China, Spain, India, Turkey, Nigeria and Mexico, where they occupy a high production of great economic importance. Mexican traditional medicine attributes to chili properties as: irritating, laxative, rubefacient and expectorant. It is used to treat certain cultural conditions as "bad air" evil eye, skin diseases such as erysipelas, rashes, infected external wounds and sores. Other uses are as an aphrodisiac, anti-diarrhea, anti-inflammatory, antirheumatic, antiseptic, emmenagogue, in treatment of kidney disease and hemorrhoids. The biggest part of consumption is mainly the ripe fruit, and route of administration is oral preferably as an infusion, although the ancient Mexicans used it as a gargle, ointments, ground and mixed with honey or infused with another plant, for the treatment of asthma, sore throat, cough, bronchitis and other respiratory problems (López-Riquelme, 2003; Ranajit *et al*., 2013). The objectives of this article are: extracting DNA from five species of *Capsicum*, characterizing the presence of terpenoids, vitamin C content and total acidity in the genus *Capsicum*.

MATERIALS AND METHODS

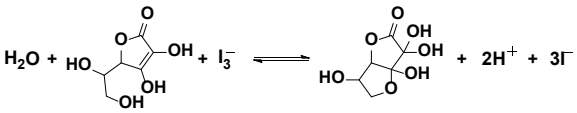
Obtaining extracts. 3 applications for each type of chili species were performed, separating the fruit, petiole and seed. It was prepared the necessary jars with acetone (25 ml) each. Samples of 1 g of fruit, petiole and seeds were weighted, each by triplicate for each species of chili, and were added to the jars with acetone. They allowed to stand for 7 days..

DNA extraction. Seeds, petioles and fruit of chilies: jalapeño, bell pepper, poblano, dried jalapeño, de arbol, were placed; immediately was added 200 mL of cold distilled water, 0.5 g of sodium chloride and liquefied at maximum speed for 15 sec. Then the mixture was filtered and measured. It was added 1/6 volume of the enzyme mixture (approx. 30 mL) shacked and allowed to stand 10 min. Subsequently the mixture was passed to test tubes, filling up to 1/3 of the tube. 0.5 g of enzyme was added to each tube. It was then shaken gently to avoid breaking the DNA. Alcohol was added slowly to the tube over the walls until two equal layers were formed. DNA was observed in the superior part from the mixture until the layer of ethyl alcohol.

Free terpenoids analysis: To the acetone extracts were added the Liebermann-Burchard reagent (ethanolic solution of acetic anhydride in the presence of sulfuric acid) and subjected to heating (110 ° C) during 10 min. It was considered as positive test if under these conditions typical red, blue or green hue spots appear. It is used for observation of various organic compounds such as: triterpenes (presented by revealing purple colour) and steroids, whose colour varies over time from pink to green and then to brown.

Vitamin C. It was prepared, an indicator solution of lugol. Re-

acting the amylose-iodine complex with vitamin C, present in beverages (ascorbic acid), the indicator solution loses colour. This is because vitamin C (1) is oxidized by a mild oxidant such as iodine solution, to result dehydroascorbic acid (2) and iodide ions according to the reaction:



(1) (2)

Total acidity. Total acidity or tritrable acidity, is defined as the sum of all organic acids found in foods that can be neutralized by titration with a strong base, usually sodium hydroxide 0.1 N or 1 N. The result was expressed referring to acid present in major proportion. Determining the total acidity is based on the fact that the fruits contain citric, tartaric and malic acid which react with strong alkali to give the corresponding sodium salts. The following formula is used:

$$\%\ acidity = \frac{(ml\ of\ solution)(miliequivalents\ of\ acid)(N\ of\ NaOH)100}{sample}$$

RESULTS AND DISCUSSION.

DNA extraction of the species *Capsicum spp*, was efficient, and can be isolated by this practical and economical method (Figures 1-3). DNA appeared like, white and stringy mucus.

Figure 1: DNA extraction from fruits of: a) Jalapeño, b) Poblano, c) Bell pepper, d) Dried jalapeño, e) De arbol.

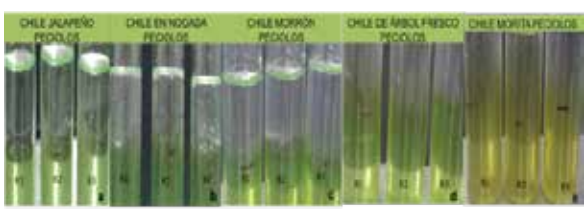


Figure 2: DNA extraction from petioles of: a) Jalapeño, b) Poblano, c) Bell pepper, d) De arbol, e) Dried jalapeño.

Figure 3: DNA extraction from seeds of: a) De arbol, b) Bell pepper, c) Jalapeño, d) Poblano, e) Dried jalapeño.

Although there are various methods of extracting DNA from plant tissues, Glick and Thompson, 1993; Ifeoma *et al*., 2014, Kumari *et al*., 2012 the method we present is easy and inexpensive.

For test of Libermann, specific staining reagents were used for functional groups, of the Merck Company. The identification of sterols is observed blue, green, red and orange, after 15 min there was the presence of a yellow color, locating the methyl group at C-14 and an immediate yellow coloration indicating unsaturation methyl group at C-7, this indicate the presence of saturated triterpenes; in triterpenes, a purple coloration is observed, and in the case of steroids: pink, green and brown colors (Figure 4).

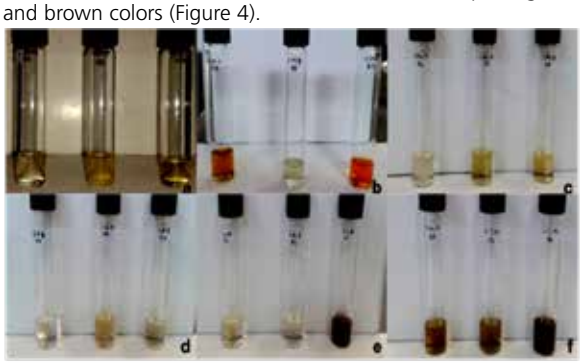


Figure 4: Test Liebermann-Buchner in extracts of *Capsicum*. a) Jalapeño, b) Bell pepper, c) Dried jalapeño, d) Poblano, e) De arbol, f) Grind chili.

Afrodet, (2006), add 1 ml of the Liebermann-Burchard reagent in 1 ml of chilli extract, showing the greenish-blue colour indicating the presence of oil in the fruits of *Capsicum*.

The analysis for the identification of vitamin C in acetone extracts of *Capsicum* are presented in Table 1. The reducing ability of vitamin C, reduces iodine to iodide and makes the starch, purple in the presence of iodine, colourless in presence of iodide.

The identification of the vitamin C is based on the organ of each species of *Capsicum*. The acetone extracts of seeds and petioles of jalapeño, contain medium amount of vitamin C, the fruit and petiole of dried jalapeño contain an intermediate amount of vitamin C, extracts of bell pepper seeds contain high amounts of vitamin C; extracts from fruit, seed and petiole of poblano and de arbol, is characterized by containing vitamin C; the acetone extracts of powder chilies had vitamin C except from dried jalapeño. The presence of vitamin C acts as an antioxidant; since it has groups in the molecular structure which are oxidizable.

Table - 1. Identification of oxidation in vitamin C.

Chili	Organ	Minor	Medium	Mayor
Jalapeño	F	++	-	-
	P	-	-	++
	S	-	++	-
Dried jalapeño	F	-	-	++
	P	++	-	-
	S	++	-	-
Poblano	F	-	-	+++
	P	-	-	+++
	S	-	-	+++
Bell pepper	F	-	++	-
	P	-	++	-
	S	-	-	+++
De arbol	F	-	-	+++
	P	-	-	+++
	S	-	-	+++
Powder jalapeño	F	-	-	+++
Powder bell pepper	F	-	-	+++
Powder Poblano	f	-	++	-

(+): Presence of secondary metabolites. F: Fruit, P: Petioles, S: Seed.

The indicator presence of vitamin C is presented in Figure 5.

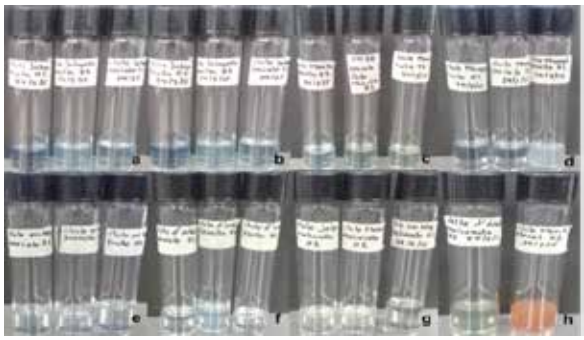


Figure 5: Determination of vitamin C in *Capsicum* species. a) Jalapeño, b) Jalapeño, c) Dried jalapeño, d) Bell pepper, e) Poblano, f) De arbol, g) Jalapeño and h) Dried jalapeño.

Identification analysis of secondary metabolites from the acetone extracts of *Capsicum*, shows the presence of more sterols and lower content of steroids (Table 2). No presence of triterpenes was observed in any species of *Capsicum*.

Table - 2. Identification of sterols, steroids and triterpenoids, by Liebermann´s test, for different types of species selected chilies. F: Fruit, P: Petiole, S: seed.

Chili	Organ	Sterols	Steroids	Negative: Precipitate
Jalapeño	F	Green	-	-
	P	-	-	-
	S	-	-	Precipitate
Bell pepper	F	Yellow	-	-
	P	Yellow	-	Precipitate
	S	-	Brown	-
Poblano	F	Green	-	-
	P	Yellow	-	-
	S	-	-	-
De arbol	F	Yellow	-	-
	P	Green	-	-
	S	-	-	-
Dried jalapeño	F	Yellow	-	-
	P	Green	-	-
	S	Green	-	-
Bell pepper	F	Yellow	-	-
Jalapeño	F	-	-	Negative
Poblano	F	-	-	Negative
De arbol	F	-	Pale brown	-
Dried jalapeño	F	-	-	Precipitate

For percentages of total acidity, the highest percentage of tartaric acid was found in jalapeño (88%), bell pepper (88%) and poblano (84%). As malic acid, the highest percentage was found in the petiole and seeds of jalapeño (72%) and seeds of the poblano (72%). Other species of *Capsicum* had 69% citric acid. It has been reported that the titratable acidity in fruit chili 'poblano' is mainly attributed to ascorbic acid content, being a fruit rich in vitamin C and as the fruit become senescent without showing discoloration, degradation of this acid is higher (Hernández-Fuentes *et al*., 2010).

CONCLUSIONS

Here, we report a practical and economical method of extraction and isolation of DNA from species *Capsicum spp*. The identification of the vitamin C is based on the organ of each species of *Capsicum*. The analysis of the identification for secondary metabolites from the acetone extracts of *Capsicum* shows the presence of highest content of sterols and lower content of steroids. There was a higher percentage of citric acid, malic acid and tartaric acid, no presence of oxalic acid. An efficient and practical method for characterizing phytochemical analysis was developed through this study to other plant species of international interest.

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