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Cocoa materials of pharmacological interest, (*Theobroma cacao* L.)

Orlando López Báez [1]
olopez@unach.mx • olopez_baez@hotmail.com

Mario Noel Ballinas Gómez [2]
noel.gomez10@yahoo.com.mx

1 Autonomous University of Chiapas, 2 University of Science and Arts of Chiapas.

ABSTRACT

The purpouse of this reseach was study of some metabolites of pharmacological importance in the cocoa beans (*Theobroma cacao* L.), which they have been used since pre-Columbian times to make chocolate (Miranda, 1962, Motamayor *et al*., 2002 a, b) With this, it seeks to create an interest in its propagation and sustainable use, as it has been doing AUDES Cacao - Chocolate Department at Universidad Atonoma de Chiapas in the states of Tabasco and Chiapas in Mexico (López-Báez y Ramírez-González, 2009).

Keywords: Metabolites, alkaloids, flavonoids, anthocyanins, antioxidants.

The states of Chiapas and Tabasco, in southeastern Mexico, are the largest domestic producers of cocoa, with 83,350 hectares (Lopez-Baez and Ramirez-Gonzalez, 2009). In both entities, cocoa production has been decreasing due to the age of plantations, low agronomic quality genetic material, pests (*Phytophthora spp, Moniliophthora roreri* and *Ceratocystis fimbriata*),devaluation of the product, an excess of intermediaries, lack of credit and dependence on international prices (González Lauk, 2005 and Ramirez Gonzalez et al., 2014). All of these issues have generated a crisis in families that depend on their crop.

This paper aims to present several metabolites which are present in cocoa beans that are of pharmacological importance, in order to develop an interest in their preservation, propagation and sustainable use in such a manner that its cultivation offers a new social , economic and environmental perspective for all Mexicans. Cacao tree plantations are agro-ecosystems similar to tropical ecosystems, and thereby promote the conservation of natural resources in the tropics. Cocoa, being cultivated together with other species of agronomic interest, provides shade and soil protection, generates oxygen, sequesters carbon and protects biodiversity.

The propagation and sustainable use which involve cocoa producers and researchers is developing in the state of Chiapas and Tabasco by the University Agency for Cocoa-Chocolate Development of the Autonomous University of Chiapas (UNACH) (Lopez-Baez and Ramirez-Gonzalez, 2009).

EL CACAO

Theobroma cacao L. is an evergreen tree that produces a fruit which is the capsule for the seeds. The seeds are separated from the pulp, and are washed and dried or fermented to improve their sensory properties. The dried seeds are roasted, hulled and ground into cocoa paste (Murray, 2007; Clark, 2008;. Romero - Cortes, et al, 2013). This paste can be unpleasant, hence it is preferred to be mixed with sugar, milk and other components, which is commonly known as chocolate (Table No. 1).

From the seed, cocoa butter and chocolate or cocoa powder is obtained. Its uses include gastronomical to chemical, pharmacological and in cosmetics (Bridges, et al . , 2009). The shell of the fruit contains pectin, pigments (poliflavono glucoside) and chitosan for food use. The fruit pulp in some regions is eaten fresh or juiced, and also to produce vinegar with variable flavors and aromas depending on their aging process (Dominguez, 2012; Villagomez and Arguello, 2013). In Table # 2 the composition of the cocoa bean is shown.

Table 1. Names of chocolate in Mexico.

Producto	Manteca de cacao total	Cocoa desgrasada totalmente	Sólidos totales de cacao
Chocolate	> 18.0	> 14.0	> 35.0
Chocolate amargo scuro	> 22.0	> 18.0	> 40.0
Chocolate semi amargo	> 15.6	> 14.0	> 30.0

Source: (MEXICO, 2013)

Table 2. Composition of washed and dried cacao seed.

Componente	%	Componente	%
Grasa	48 – 57	Teobromina	0.8 - 1.4
Humedad	2 - 5	Cafeina	0.1 - 0.07
Proteina	10 – 16	Flavonoides y Procianidinas	1.6
Carbohidratos	27 – 30	Cenizas	2.6 - 4.2
Fibra	15 – 17		

Source: (Garti and Widlak, 2015)

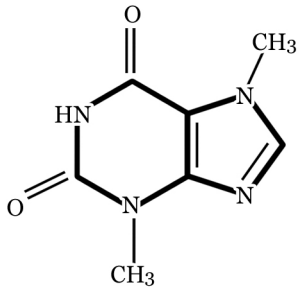
XANTHINES

Xanthines are plant alkaloids found in cocoa, chocolate, mate, guarana, etc.. The main ones are theobromine, caffeine and theophylline (Lorenzo *et al.*, 2009).

Theobromine (Figure No. 1). Theobromine is a nitrogenous substance of the class of methylxanthines (Crozier, *et al.*, 2012). In cocoa paste it is found in concentrations around 1.2% and in a cup of cocoa there is usually approximately 0.1 grams of theobromine (Gil, 2010).

During fermentation the content decreases in part by grain exudation, which is released by the acetic acid produced in the process. It has also been found that it influences the time and temperature of fermentation and in the final theobromine content in the seed ((Korolkovas and Burckhalter, 1983 and Portillo, *et al.*, 2011).

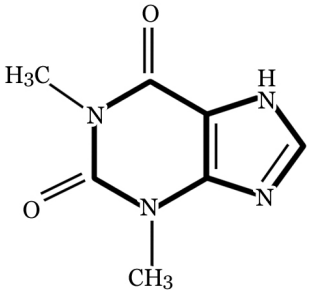
Figure 1. Theobromine (3, 7 - dimethyl - xanthine).



Theobromine is used as a diuretic, cardiac stimulant, vasodilator and muscle relaxant. Its effect on the nervous system is lower than that of caffeine (Velayos, 2009;. Medrano, 2010 and Martinez-Pinilla, et al, 2015).

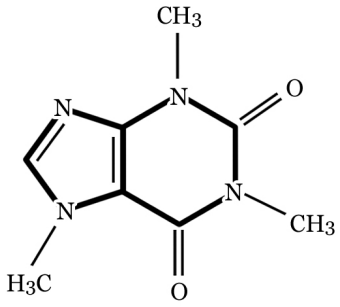
Theophylline (Figure No. 2). It is especially found in tea. In cocoa beans in it has been reported in trace amounts (Harue Wakao, 2002 and Brunetto, et al., 2007). This substance has been used for some time in asthma treatments. It stimulates the central nervous system and increases renal blood flow, among others (Barnes, 2003).

Figure 2. Teofilina (1, 3 – Dimetil - Xantina).



Caffeine (Figure No. 3). Caffeine is found mostly in coffee, tea and cocoa (Lorenzo et al., 2009). Its main effects are as respiratory, musculoskeletal and cardiovascular psychostimulants (Alvarez Garcia et al., 2007).

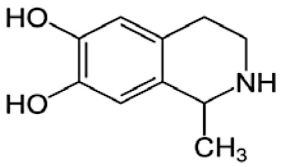
Figure 3. Cafeína (1, 3, 7 Trimetil - Xantina).



Cocoa beans contain between 0.6-0.8% caffeine and in a cup of chocolate there are approximately 4 mg. The caffeine content in the cocoa bean decreases in the fermentation process (Brunetto, et al., 2007; Lorenzo et al, 2009 and Crozier, et al, 2012..).

Salsolinol (Figure No. 4). Through liquid chromatography techniques and electrochemical detection, salsolinol has been identified in cocoa powder in levels of 40 ± 4 mg / g (Riggin and Kissinger, 1976). This substance is related to the addition to chocolate (Melzig, et al., 2000). Currently the salsolinol plays an important role in the treatment of Parkinson's disease. Some foods that contain it are dried bananas, cheese, and wine, etc. (Naoi, et al . , 1997; Morris and Taren 2005 and Xie, et al, 2012.).

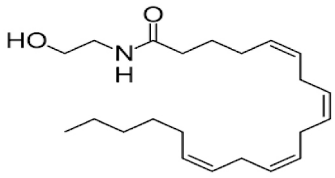
Figure 4. Salsolinol.



Anandamide (Figure No. 5). The anandamide is present in chocolate in amounts of about 0.05 µg/g with a bioavailability of only 5% (Watson et al., 2012). This substance causes relaxing sensations, and may even induce sleep (Morales J, et al., 2012 and Garcia Sanchez, et al., 2013). Current studies on

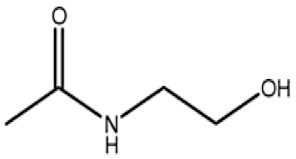
its anti - cancer effects are made on skin cells (Adinolfia, et al, 2013.) and for breast cancer (Laezzaa, et al, 2010;. Mayorga Niño and Torres Vidales, 2014).

Figure 5. Anadamina (arachidonyl - ethanolamide).



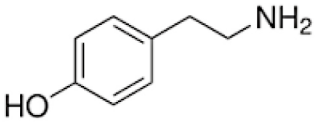
N-acetylethanolamine, N-linoleiletanolaminas and N-oleoiletanolaminas (Figure No. 6). These substances have recently been discovered in chocolate powder. Medical researchers are seeking therapeutic strategies for their use as neuro protectors (Fowler, 2003;. Capasso, et al, 2011 and Morales J, et al, 2012.)

Figure 6. N-acetylethanolamine.



Tyramine (Figure No. 7). Tyramine is present in chocolate, cocoa, wine, processed meat, dried fish, papaya, beans, peanuts, etc. (Vasudevan and Sreekumari, 2012). Various studies have been done related to the increase of cardiac activity and blood pressure, as well as the satiation of appetite (Alkema, et al. (2005).

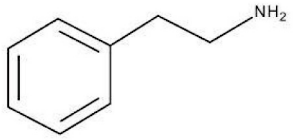
Figure 7. Tyramine.



Phenylethylamine (PEA) (Figure No. 8). This is a common compound in foods such as wine and cheese, and in chocolate it is found at levels of 3.5 to 8.02 g / g (Watson et al., 2012).Several studies have shown that its effects influence mood, therefore it is important in the therapeutic field for

its antidepressant action, among others (Greenshaw, 1989; Shimazu and Miklya, 2004).

Figure 8. Phenylethylamine.

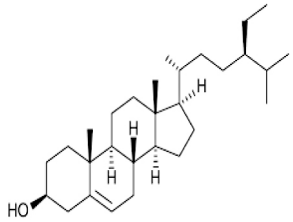


PHYTOSTEROLS

Phytosterols, or plant sterols, are found in seeds, cereals and vegetable oils, avocado, cocoa, chocolate, etc. These have a similar structure to cholesterol (Hung et al., 2008). Cocoa is about 55% lipids. Of these, 0.3% are unsaturated and are comprised of more than 30 phytosterols which content is approximately 230 mg / 100 g, of which the most abundant are Beta-sitosterol, stigmasterol and campesterol (Gil, 2010).

Beta-sitosterol (Figure No. 9). Its structure is similar to cholesterol, but differs by an ethyl group at the C24 chain. Therefore, this compound can inhibit the absorption of cholesterol in the body and reduces levels of it in blood plasma. It also aids in the regulation of liver functions and prevention of cardiovascular disease (Ikeda, et al . , 1989; Plaza, 2001 and Kakade and Magdum, 2012).

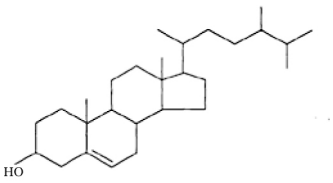
Figure 9. β - sitosterol (3β) -Stigmast-5-en-3-ol.



Existen diversos estudios de los efectos del sitosterol en el tratamiento del cáncer de próstata y colon (Awad, *et al.*, 1996; Dutta, 2004 y Jourdain, *et al.*, 2006).

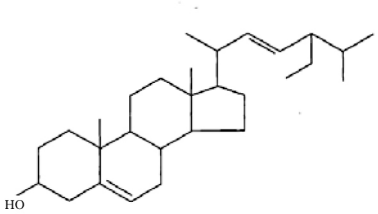
Stigmasterol (Figure No. 10). Among its pharmacological properties are reducing cholesterol absorption in the intestine, prevention of some cancers and it contributes to anti-inflammatory processes. Currently, foods are formulated with phytosterols with the intention of lowering cholesterol in the population (Ju, et al, 2004, Polagruto, et al . , 2006. Gabay, et al, 2010 and EFSA, 2012.).

Figure 10. Stigmasterol [(3β) -Stigmasta-5,22-dien-3-ol].



Campesterol (Figure No. 11). Campesterol β-sitosterol differs from a methyl group in place of the ethyl group. It is an aid in lowering cholesterol and preventing cardiovascular risk (Ikeda, et al . , 1989; Plaza, 2001 and Kakade and Magdum, 2012).

Figure 11. Campesterol [(3 β) -Ergost-5-en-3-ol].



The ratio of stigmasterol / campesterol content is used to determine the adulteration in cocoa butter (Jee, 2002) (See Table No. 3). It has been determined that cocoa roasting conditions influence the content of phytosterols (Oracz, *et al.*, 2014).

Table 3. List of stigmasterol / campesterol in cocoa butter.

Esteroles gr/kg	% de Estigmastero / % de Campesterol
1.8	2.8 - 3.5

Source: (Belitz and Schierberle, 2009)

POLYPHENOLS

Polyphenols are characterized by having phenolic rings. These bioactive substances have antioxidant capacity due to its molecular structure (Giacometti et al., 2015). The content of antioxidants found in cocoa beans is higher than in wine and tea (Rogers and Alipo, 2008). Some polyphenols identified in cocoa beans and its products are catechins, flavonoids, anthocyanins and procyanidins (Hammerstone et al., 1999).

It has been found that the polyphenol content and antioxidant activity tends to decrease in the processes of roasting cocoa as a function of time and temperature (Ioannone, et al, 2015; McFarlina, et al, 2015), therefore there is surely a higher antioxidant capacity in ground cocoa and less roasted cocoa (Chavez and Rivera Ordoñez, 2013; Zapata Bustamante and Tamay, 2013).

In vitro studies have shown that chocolate polyphenols have the ability to control LDL oxidation reactions or oxidative DNA damage (Maydata Gutierrez, 2002).

Flavonoids: The most abundant polyphenols in the cocoa bean are flavonoids, which are between 6 and 8% dry weight (Grassi et al . , 2008). Some of them are (+) catechin, (-) epicatechin, (+) gallocatechin, (+) - epilagocatequine (Rimbach, et al . , 2009). Polyphenols are beneficial in preventing cancer, vascular health, cardiovascular diseases, and cell damage (Manach and Donovan, 2004). Nehlig (2013) mentions that consumption of flavonoid-rich cocoa can help improve vascular health and increase cerebral blood flow. They also inhibit neuronal death by apoptosis induced by neurotoxins. Other foods containing flavonoids are fruit skin and red wine (Hara et al. 1995), soy, tea (Lopez et al., 2001).

Procianidinas: By using HPLC techniques and mass spectrometry on cocoa seeds, various procyanidins such as B1, B2, B3, B4, B5, C1 and D were found (Hammerstone et al, 1999 and Marcano and Hasegawua . , 2002).

The effect of these substances is of interest for their potent biological activity, antioxidant capacity and reduced risk of cardiovascular disease and blood clots. Some can also be found in cinnamon, grape seed, blueberries, etc. (Santos-Buelga and Scalbert, 2000, Rios, et al . , 2002; Rusconi, et al, 2013.).

Anthocyanins: Cyanidin 3-α - L arabinose and cyanidin 3-α - L – galactose can be found in cocoa. They are water - soluble compounds and responsible for the red, blue, and purple colors of plants and fruit. When the glucoside group is missing they are called anthocyanins (Taiz and Zeiger, 2006). Analysis of anthocyanins (cyanidin-3-glucoside) by UV spectrophotometry,

made by Chavez and Rivera Ordoñez (2013) showed that whole cacao seeds have a content of 1.490 ± 0.043 mg / g.

Generally anthocyanins have an industrial interest for their ability to color foods, as well as in medicine as they are potent antioxidants, anti-diabetics, anti-carcinogenic and can improve visual acuity (Shipp and Abdel-Aal, 2010). Other food sources of anthocyanins are purple and blue corn, berries, red grapes, etc. (Aguilera Ortiz, et al., 2011).

Resveratrol: This compound has been reported to have antioxidant, anti-cancer and cardio protective properties (Vidavalur et al . , 2006). It has been found in grapes, wine, grape juice, blueberries, etc. (Wang et al., 2002). Counet, et al. (2006) reported that through HPLC , trans resveratrol have been identified in cocoa liquors (0.4 ppm) and in dark chocolate (0.5 ppm) in samples from the Ivory Coast.

CONCLUSIONS

Research in cocoa beans have demonstrated that they contain substances with important nutritional and pharmacological potential, which are not found in the same proportions and with the same biological activity in derivatives and which are lost during processing. Its removal and purification is necessary. Many of these substances are also found in other foods such as the resveratrol in grapes, and theophylline in tea. Learning about these properties is of interest to promote cacaos conservation and sustainable use in southeastern Mexico, which has a long chocolate tradition.

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