EXTRACTION, ISOLATION AND CHARACTERIZATION OF *SALVIA HISPANICA*: MUCILAGE, SEED OIL AND PHARMACOLOGICAL PROPERTIES-A REVIEW

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I. ABSTRACT

Chia seeds also known as “Salvia Hispanica” belongs to the mint family and are native to Central America. These are black and white tiny seeds with 1mm diameter and are used as energy boosters. These are highly rich in fibre, omega-3 fatty acids, polyunsaturated fatty acids(PUFA), calcium and some other minerals and hence they are known as “Power House of Nutrients”. They are highly nutritious. They have many health benefits as they prevent cardiovascular diseases, inflammatory diseases and high blood pressure problems. The seeds and the oil extracted is highly rich in antioxidants (tocopherol, carotene, phytosterol) and various phenolic compounds like kefic acid and chlorogenic acid. The solvent extraction, soxhlet extraction and screw pressing methods are being used for the extraction of oil. The mucilage of the seed as a variety of functional properties. The mucilage is being extracted by hot and cold methods.

KEYWORDS: Salvia hispanica, mucilage, PUFA, solvent extraction, soxhlet extraction, pressing method, phenolics, oil extraction.

II. INTRODUCTION

Chia seeds belong to the family of Lamiaceae. Salvia is the known genera of the seed and hispanica is the species to which the seed belongs. Salvia hispanica belongs to the Plantae kingdom with a subkingdom of Tracheobionta (Loreto A. Munoz,2013). The seeds are an excellent source of nutrition. They are enriched with protein, omega-3 fatty acids, dietary fibres, vitamins and minerals, total fat and antioxidant (Bartosz Kulczyn ski,2019). The nutritional composition of the seed is highly recommended for its 15-25% (protein content), 26-41% (carbohydrate content), 30-33% (fat content), 18-30% (dietary fibres), and a surplus amount of antioxidants (Maira Rubi Segura-Campos,2014). The seed is rich in PUFA, Polyunsaturated fatted acids and is uniquely rich in a huge percentage of omega-3 Alpha-linolenic acid (ALA) (Ahmed Saad Noshe,2017).

***Table 1*: Nomenclature of Chia seeds** (Munoz, 2013)

FAMILY	***Lamiaceae***
GENERA	*Salvia*
SPECIES	*Hispanica*
KINGDOM	*Plantae* *Subkingdom (Tracheobionta)*

Table 2: Nutrition content of Chia seeds

NUTRIENTS	AMOUNT
Carbohydrates	42%
Proteins	16%
Cholesterol	0%
Fat	31%
Saturated fat	3.3g
Polyunsaturated fat	24g
Monounsaturated fat	2.3g
Trans fat	0.1g

Table 3: Amino acids present in Chia seeds (Suri, 2016)

AMINO ACIDS	AMOUNT(g/kg)
Arginine	2.14
Lysine	0.97
Cystine	0.41
Valine	0.95
Glycine	0.94
Glutamic acid	3.50
Histidine	0.53

The seeds are a natural source of major food components with an extremely effective level of health benefits. The seeds control diabetes by improving the glucose tolerance and sensitivity of insulin in the body. Hence, they possess anti-diabetic activity. They are antianxiety and antidepressant, also, they reduce the cholesterol level. (Food Funct.,2019). The PUFA content of the seed oil possesses

Anti-cancer property and thus, cures human cancer and its various forms. The seed oil and powder also inhibits anti-inflammatory property by the consumption of PUFA in the diet. The seed also possesses antioxidant activity as the chia seed oil is rich in a variety of antioxidants likewise, phenolics, tocopherol, myricetin and carotenoid compounds. (Ramzi Abdulrashed Abdulkhaleq gazem,2016).

The mucilage extraction of *Salvia hispanica* is a several step method, that is, first extraction, second extraction and third extraction methods. The mucilage has a major industrial application as a stabilizer and emulsifying agent in various ice cream industries. The different methods of extraction of mucilage describe its functional property and there are various factors on which the mucilage content depends. One of the most important factors in this aspect is the temperature. (Ruaa Naif Abdullah,2017).

The study aims to cover a wider aspect of the extraction of the seed's mucilage content and also the extraction and isolation of the seed oil. The seeds play a major role in health and have an extensively greater extent of pharmacological and therapeutic properties. According to various studies its been illustrated that the seed, its powder and the seed oil possess numerous health benefits (Ramzi Abdulrashed Abdulkhaleq gazem,2016).

III. STRUCTURE AND CLASSIFICATION

The chia seeds are tiny, smooth, levelled and oval. The structural description of the seeds includes length (2.0-2.5mm), breath (1.2-1.5mm) and thickness (0.8-1.0mm). The seeds are generally classified into two categories, that is, white and black chia seeds. This is a classification of the seeds based on the colour attributes. With varying colour, the seeds also vary in their nutritional composition. They vary in size, shape, thickness and nutritional composition. Black seeds have maximum moisture content than the white seeds. While the white seeds have maximum oil yield (Dr Sukhneet Suri,2016). The seeds are also classified as ground milled, white, black, pre-hydrated seeds.

Figure 1: Classification of Chia seeds

IV. NUTRITIONAL COMPOSITION

Chia seeds are known to be very significant as per its nutritional composition. Many studies have evaluated the significant view of the seed's composition. With the varying varieties, the seeds vary in the nutritional composition, that is in terms of, protein content, oil yield, dietary fibres and fatty acids (Dr Sukhneet Suri,2016).

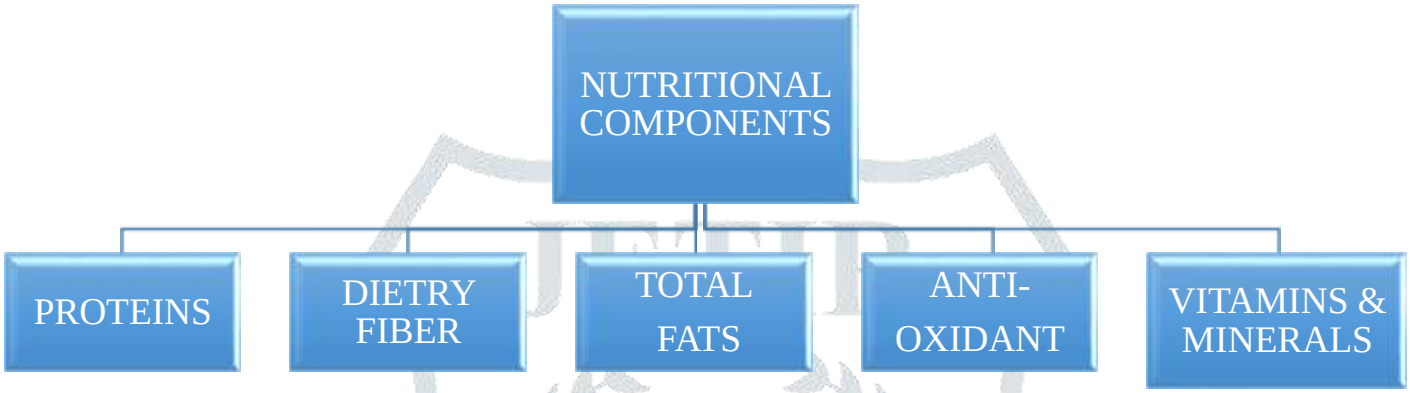

Figure 2: Nutritional composition of Chia seeds

i. PROTEIN

Salvia hispanica varies in its protein content based on the condition of the growth and the geographical growth location of the chia crop (Loretz A. Munoz,2013). In comparison to the grains like barley, rice and wheat, chia seeds have maximum protein content. The seeds are rich in numerous amino acids such as arginine, valine, alanine, serine, lysine, tryptophan, and many more (Dr Sukhneet Suri,2016).

ii. ANTIOXIDANTS

The antioxidant activity of the seeds is higher than the other food grains. The potential of an antioxidant compound is high in the chia seeds (Rafaela da Silva Marineli,2014). Phenols and its compounds(myricetin, chlorogenic acid and quercetin) are the most important antioxidant found in the seeds. The compounds are effective than various vitamins and flavonoids (Loreto A. Munoz,2013). The

antioxidant property of this seed paly a major role in fighting the diseases like cancer and diabetes (Dr Sukhneet Suri,2016).

iii. DIETARY FIBER

The fibre content of the seeds is evaluated as a protective component against the diseases like obesity, stroke and hypertension. Based on the chemical and physical properties, soluble and insoluble fibres are found in the seeds. the action of the bulk is initiated by the insoluble fibres while the colon fermentation is inhibited by the soluble fibres (Dr Sukhneet Suri, 2016).

Hence, it can be sated that chia seeds have excellent nutritional attributes. The seeds have an effective functional and pharmacological properties. The seeds have both traditional and commercial use.

V. EXTRACTION OF CHIA SEEDS—OIL EXTRACTION

The oil is extracted by various methods of extraction from the chia seeds to obtain a maximum percentage of oil being extracted. The methods of extractions used for the extraction of oil are as shown below:

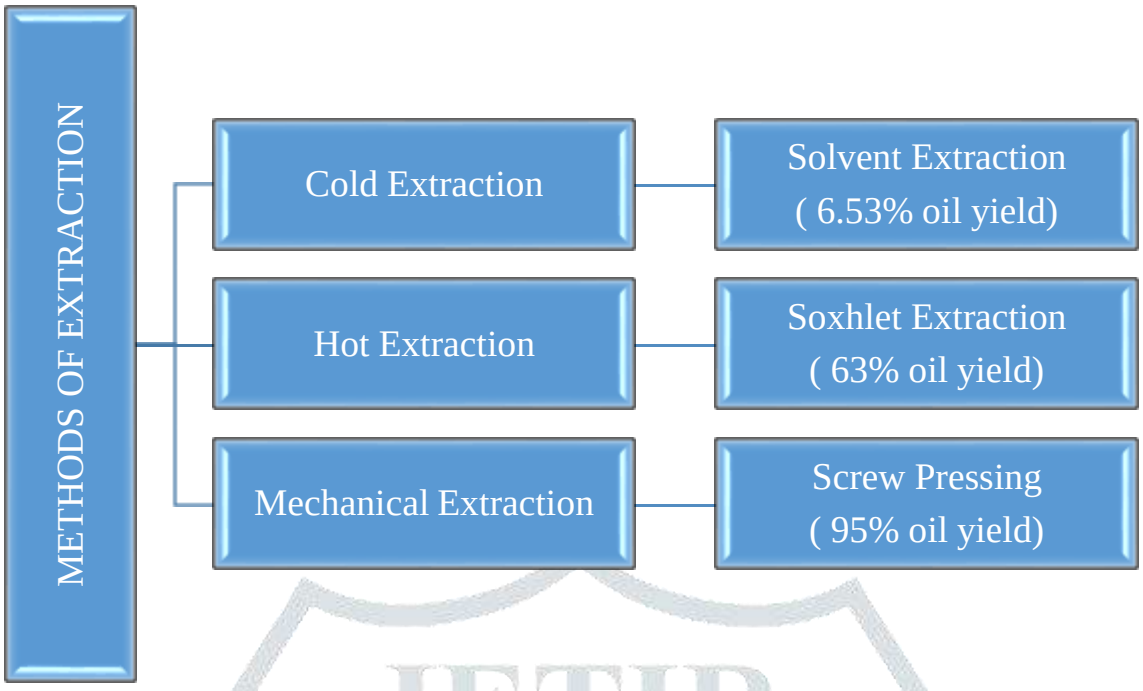

Figure 3: Methods of extraction of Chia seeds

Based on the method of extraction the fatty acid content of the seed oil is evaluated (Ahmed Saad Noshe,2017).

Before use, the seeds must be cleaned thoroughly and stored at a cool and dry place under hermetically sealed conditions (Vanesa Y. Ixtaina,2011). The seeds must be stored at a temperature of about 20˚C with a relative humidity of 65% for about 48hrs, before undergoing the extraction process. Later, further extraction operations must be carried out (Grzegorz Dabrowski,2018). The oil can be extracted by any suitable method of extraction, that is, either solvent, soxhlet or pressing method.

i. SOLVENT EXTRACTION

It is a cold extraction method and in this method, the heat is excluded and the solvent is used in different ratios to have comparative results at different ratios. Initially, the seeds are ground and are being crushed. Then these seeds being crushed are soaked in a solvent(hexane) in the ratio 1:3 in a glass beaker for about a minute. This is the cold extraction method od chia seeds. later the solvent is

extracted out by the evaporation process using a Rotary evaporator held at 35˚C of temperature and the extracted oil is collected in the tubes of the extractor (Ahmed Saad Noshe,2017). Evaporation is carried out under the stream of nitrogen gas and the oil extracted is determined by the weight (dry basis) content (Vanesa Y. Ixtaina,2011). That is the weight of the tubes that determines the oil content being extracted out.

ii. SOXHLET EXTRACTION

Soxhlet extraction is a hot extraction method. Under this process, the oil is extracted by the use of Soxhlet apparatus and hexane is used as a solvent for the extraction of the oil. Firstly, the seeds are ground in a mill and then these groundd seeds are transformed into the thimble containing the solvent (hexane). It is important to note down the weight of thimble, beaker and together with the weight of the thimble and the beaker. The weight must be recorded both before and after the extraction process (Ahmed Saad Noshe,2017).

iii. PRESSING METHOD

Screw press extraction method is a solenoid extraction process. Initially, we need to adjust the moisture percentage of the seeds in to prevent the choking of the equipment and also to have an effect in the oil yield recovery. The maximum oil extract is obtained when the moisture percentage of the seeds is about 10% (Vanesa Y. Ixtaina,2011). The 100g of the seeds are extracted at normal room temperature from the top of the equipment by its opening (small) and thus the extract (oil) is obtained into a container under the opening (Ahmed Saad Noshe,2017).

In the screw pressing technique, moisture plays a major role as it increases the plasticity of the seeds. Hence, attaining a desirable percentage of moisture content is important. At this desirable moisture, the extraction can be carried out effectively. Also, the speed of the screw press must be of about 20rpm and the press is first to run without any feed into it for about 15min. to have a desirable

temperature produced in it. Later the press is run with seeds into it. The oil so extracted is to be stored at a temperature of 48˚C and the oil content is measured by the dry basis (weight method) (Vanesa Y. Ixtaina,2011).

VI. MUCILAGE EXTRACTION

The extraction of the mucilage can be carried out by various methods. The extraction is dependent on various factors, amongst these temperatures is the most important and effective method. It affects the gelling property of the seeds. also, the mucilage concentration is affected by the pH content (Ruaa Naif Abdullah,2017).

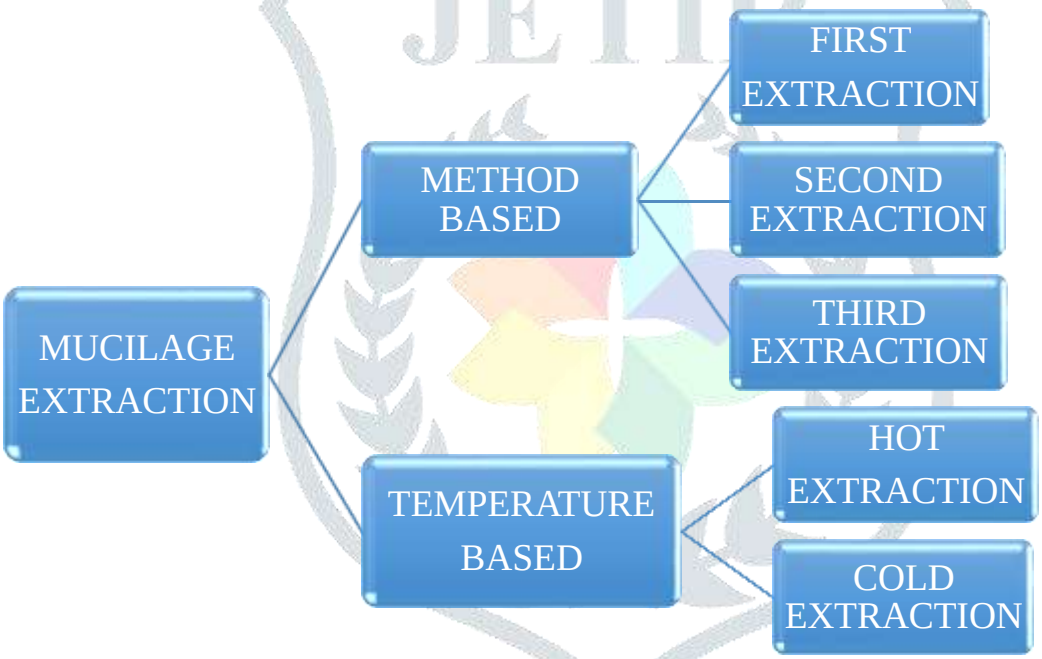

Figure 4: Methods of Mucilage Extraction

i. FIRST EXTRACTION PROCESS

The extraction is carried out by obtaining a ratio of 1:20 of seeds to distilled water respectively. This ratio is collected in a beaker and is stirred continuously on a magnetic stirrer for about 30min. at 45˚C. Then it is placed aside and allowed to cool and then centrifugation is carried out by centrifuging the mixture at 11.60xg (Acceleration) for about 30min. Then the extract (leachate) is separated effectively (Ruaa Naif Abdullah,2017).

ii. SECOND EXTRACTION PROCESS

The seed to distilled water ration (1:30) was dried and then modify the pH by required amounts of HCl or NaOH, in the mixture. Then again the stirring of the mixture is carried out by magnetic stirrer for 2hr. on a hot plate at about 50˚C. Later the mixture is dried in the oven for 10hr. at 60˚C and the dry mass (mucilage) was obtained and then sieved and weighed (Ruaa Naif Abdullah,2017).

iii. THIRD EXTRACTION PROCESS

For this method, the seed to distilled water ratio (1:20) is ground and dried. Again this mixture is stirred at 40-80˚C on the magnetic stirrer for about 10min. Later the leachate is separated after the centrifugation of the cooled mixture. Then it is oven dried for 4-5hr. to record the weight changes at 60˚C of drying (Ruaa Naif Abdullah.2017).

iv. HOT EXTRACTION

It is a method which recovers the maximum yield. The ration of 1:40 of The seed to distilled water is taken, by adjusting the pH of the mixture at 8 and aintaining the temperature conditions. The mixture is then hydrated and stirred magnetically for about 2hr. This suspension is then exposed to a drier for 48hr. at 50˚C. then the mucilage so obtained is sieved and weighed (Lucas Silveria Tavares,2017).

v. COLD EXTRACTION

The extraction process is initiated by a varying ratio (1:10, 1:20, 1:30 &1:40) of the mixture (seed: distilled water) by maintaining temperature and pH conditions. As in hot extraction, here too the mixture is hydrated and stirred. Then the mucilage is extracted by pressing, that is, by the use of ultra-freezer and the freeze-drying technique at -86˚C. Thus the mucilage (dried) is obtained and then is weighed (Lucas Silveria Taveres,2017).

vi. EFFECT OF TEMPERATURE ON MUCIALGE EXTRACTION

The extraction of the mucilage was held at different temperature ranges, but this temperature variation didn't vary much concerning for the extract yield. Though the temperature has not many varying results and it is found more suitable and effective to conduct the extraction at normal room temperature because the seeds had already undergone the centrifugation process at high temperature. The temperature range of 25˚C is found most suitable for the extraction. The separation is easy at This temperature range (Ruaa Naif Abdullah,2017).

vii. EFFECT OF pH ON MUCILAGE EXTRACTION

The acidic and basic pH conditions greatly affect the mucilage extraction. The extraction is suitably held at a pH range of 8, where the extraction provides the desirable results. The pH majorly affects the viscous property of the extract of mucilage and with the changing pH, that is, too acidic or too basic conditions, the viscosity of the mucilage will vary. It is found that separation of the extract is easier by centrifugation at pH 8 (Ruaa Naif Abdullah,2017).

VII. PHARMACOLOGICAL PROPERTIES

Table 3: Pharmacological properties of Chia seeds

PROPERTY	***ROLE AND IMPORTANCE***	***REFERENCE***	***% added***	***In which form added***
Anti-Cancer Property	Salvia hispanica is a rich source of Polyunsaturated fatty acids (PUFA) plays a major role in alleviating cancer in humans. These	(Ramzi Abdul Rashel Abdulkhaleq Gazen,2016)	20:4, n-6	Arachidonic Acid

	PUFAs have a synergistic effect on human health concerning for cancer.			
Anti-Obesity Property	The seed oil has a major effect against obesity. Also, the mixture of seed powder and water is very effective against obesity. It inhibits resistance to insulin and burns body fat.	(John Parker,2018)	12% protein concen-tration	Oxidized form
Anti-Oxidant Property	The chia seeds have a higher antioxidant property than the other cereal grains. The phenolic components present in the seed are rich in antioxidants.	(Mariana Grancieri,2018)	35g/day	Chia seed flour
Anti-Cholestrolenic Property	Chia seeds and its dietary fibres help in reducing the cholesterol level in the body. They block the key enzymes of cholesterol producers into the body.	(Mariana Grancieri,2018)	25g/day	Milled chia seeds

Anti-Inflammatory Property	It prevents the disorders of inflammation which causes swelling and pain. It reduces the inflammation property, thus eliminating the vital loss of the body's function.	(Ramzi Abdul Rashel Abdulkhaleq Gazen,2016)	-	Inflammatory Mediators (linoleic) derived from arachidonic acid
Anti-hypertensive Property	Hypertension is known to be a high blood pressure problem which leads to CVDs risks. Chia seeds reduce the effect of hypertension in adults by exerting its antioxidant and anti-inflammatory effect and activity.	(John Parker,2018)	5%, 10%, 15%, 20%	Seed oil olei fraction
Anti-Diabetic Property	The seeds help the body to fight type-2 diabetes by improving the glucose levels in the body and insulation production by the body.	(Ramzi Abdul Rashel Abdulkhaleq Gazen,2016)	37g/day	In the form of suppliements

VIII. CONCLUSION

Chia seed is a very effective food ingredient which is a complete source of nutrients to proteins, carbohydrates, fatty acids and dietary fibres. Due to their excellent nutritional attributes, the chia seeds and its oil is gaining much importance in the food sector. The seeds have both traditional use and commercial use. The nutritive value of the seeds varies with its varying variety. The extraction of the mucilage of the chia is best at low- temperature ranges. The mucilage content of the seeds is present in the three outer layers, from these three layers it is extracted by various methods like first extraction, second extraction, third extraction, hot extraction and cold extraction. The results from all the extraction methods are being compared later. The extraction is performed at optimum temperature and pH conditions. The mucilage content of the chia seeds has excellent functional properties. The seed oil is obtained by the solvent and soxhlet extraction and also screw pressing method. The solvent extraction gives maximum yield as compared to the screw pressing method. The seed oil is rich in a high amount of polyunsaturated fatty acids. The seeds have excellent characteristics of functional and pharmacological properties. The seeds are rich in antioxidant, anti-diabetic and anti-inflammatory properties.

IX. REFERENCE

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- (Munoz, 2013, p. 40)
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- (Tavares, 2017, p. 10)
- (Ahmed Saad Noshe1, 2017, p. 7)
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- (Maira Rubi Segura-Campos, 2014, p. 6)
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