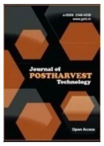

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RESEARCH ARTICLE

Nutraceutical benefits of *Salvia hispanica* and its anti-oxidant and anti-microbial effects

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ABSTRACT

Chia seed is increasing in popularity as a nutritional rich diet and medicinal food. A native of Central and South Mexico, Chia seeds are edible seeds of *Salvia hispanica* which is an annual flowering herbaceous plant belonging to the mint family (Lamiaceae). In recent years, usage of chia seeds has tremendously grown due to their higher nutritional level and medicinal values. Chia was cultivated by Mesopotamian cultures, but then disappeared until the middle of 20th century, when it was rediscovered in 1990s. Chia seeds contain healthy omega -3 fatty acids, dietary fibres, proteins and vitamins and some minerals. Besides these the seeds are an excellent source of antioxidants. Today chia has been analysed in different areas of research. Researchers have been investigating the benefits of chia seeds in medicinal, pharmaceuticals and food industries. Chia oil is today one of the most valuable oils in the market. This study is aimed to characterize the chia seeds (*Salvia hispanica*), get chia seed oil by different extraction methods and analyse their nutritional properties, antioxidant properties and antimicrobial properties. By conducting and analysing the experiment it was found that chia seeds contain a good amount of antioxidant properties, contains phytochemical compounds like alkaloid and flavonoid whereas terpens and tannins were found to be absent. It has also antimicrobial properties in which *E.coli, Klebseilla* are found to be sensitive whereas *Salmonella spp*. was found to be resistant.

Keywords: *Salvia hispanica*, phytochemical, antioxidant, antimicrobial.

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INTRODUCTION

Chia seeds are used as dietary substitute of major food components such as carbohydrates and proteins due to its high fibre content and capacity to reduce cholesterols. This dry indehiscent seed is cultivated worldwide for mass production and recommendation inclusion for dietary plans to benefit human and animal health. With the increasing of Coronary Heart Diseases, an experiment was conducted by feeding chia seeds to rats and the total value of total cholesterols and serum triacylglycerols were found to reduce significantly (Ayerza et al., 2005). It has a high level of omega-3 fatty acid which acts as an antioxidant and a safeguard to protect the seeds from chemical and microbial breakdown. In the fatty acid profile and chemical composition of chia seeds, it was characterised by high percentage of Polyunsaturated Fatty Acids (PUFA) making upto 752 to 623 g/kg of the total fatty acid of the plant during the growth cycle (Peiretti et al., 2009). Chia seeds are small flattened oval shaped measuring on average 2.1mm x 1.3mm x 0.8mm with an average weightage of 1.3mg per seed. They are mottle coloured with

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brown, grey, white, and black patches. The seeds are hygroscopic in nature absorbing up to 12 times their weight in liquid when soaked, they develop a mucilaginous coating that gives them a gel like texture. Dried chia contains 6% water 42% carbohydrates (including high dietary fibre), 16% protein and 31 % fat (Darwish et al., 2018). The seeds are rich in several dietary minerals including calcium, iron, magnesium, manganese, phosphorous, and zinc (all more than 20% DV). The massive nutritional and therapeutic potential of chia seeds is little known, chia offers a great future perspective for feed, food, medical and nutraceutical sectors. Chia seeds can be pressed to extract nutritious oil known as chia seed oil. Chia seeds when placed in water produce a gel, this gel has a good water binding capacity, oil holding capacity, viscosity, and emulsion activity. Chia oil is a versatile ingredient that is used in skin care industries as a potent anti-aging as well as an edible and delicious alternative to oil in kitchens worldwide. It contains alpha-linoleic acid, has the highest source of omega-3. Chia oil improves glucose metabolism but despite the good nutritional value, it is usually used as animal feed, a low value product.

APPLICATION OF CHIA SEEDS AND DERIVED PRODUCTS

Recently, functional foods have gained remarkable considerations worldwide due to health awareness. Contemporarily, chia seeds are used as a healthy oil supplement for humans and animals. Chia seeds along with other food sources such as flax seeds, walnuts etc are found to be effective in reducing obesity, cardiovascular diseases, hypertension, cancer, diabetes mellitus (Dincoglu et al., 2019).

Food industry

Several studies have been performed on the usage of chia seeds in the food industry. In the food industry, chia seeds are used in different shapes: whole, ground, in the form of gel. Chia seeds can be added or mixed into biscuits, pasta, cereals, snacks and cake as supplements. Due to their hydrophilic properties, chia seeds can be used as a substitute for eggs and fat (Kulczynski et al., 2019). Chia oil can replace 25% of the eggs in the cake. Besides, recent studies showed that the mucilage from chia seeds can be used as a functional coating with improved functional properties.

Chia mucilage

Chia mucilage is a rich source of polysaccharides. Due to its binding capacity and viscous texture it can be employed in the food industry as a foam stabilizer, a suspending agent, adhesive or binder. Recent studies showed that chia mucilage can be used as a functional coating with improved functional properties. Mucilage obtained from chia seeds is a novel source of polysaccharide and can potentially generate interesting polymer blends for edible films and coatings (Knez et al., 2019).

Chia gums

Chia seeds are believed to be starting materials in the food industry for their dietary fibre. Gum can be extracted from the dietary fibre fraction by using water as an additive to control viscosity, stability and texture. The chemical composition, molecular structure, and the derivatives properties such as thermal stabilities or gelling ability represents an important factors which determines the appropriateness of polysaccharides in food and pharmaceutical industries. The gum is also stable at high temperatures way up to 224°C (Segura_campos et al., 2014).

Pharmaceutical use

Bilayer emulsion have potential as a deliver system of omega-3 fatty acid from chia oil which represents a high potential in pharmaceutical and food industry since the emulsion can be used directly or subjected to drying process to obtain powders. Due to relative ease of synthesis and economic feasibility, conventional oil in water emulsion is usually the first choice considered to deliver bioactive lipids. Chia oil can be incorporated into oil in water emulsion as omega fatty acid delivery system in food matrices. It is believed that leaves of *Salvia hispanica* L contain an essential oil that comprises β-caryophyllene, globulol, β-pineneα-humoleno and widdrol. Those compounds are believed to have strong repellent characteristics to a wide spectrum of insects (da Silveira et al., 2021).

Therapeutic value

Therapeutic values of chia seeds have been reported well. Incorporating dietary fibre and α linoleic fatty acids into the diet makes chia a prime contender in regulating bodyweight and possibly other comorbidities associated with diabetes. A study on chia seeds and flex seeds by Vladimir Vuksan demonstrated that supplementing 37g/day of chia seeds to an isocaloric diet improved major and emerging risk factors in type 2 diabetes, suggesting its cardio protective potential while maintaining weight (Teoh et al., 2018). A subsequent study by the same group demonstrated that chia acutely reduced postprandial glycemia when added to a meal and prolonged satiety (Vuksan et al., 2017). Other studies were carried out to investigate the therapeutic effects which demonstrate chia seeds as a potential source of several bio-active peptides, essential for the damaged tissue and general wellbeing (Grancieri et al., 2019). Furthermore investigations in chia seeds as an anti-inflammatory agents, antiplatelet, anti-cancerogenic , laxative, hypotensive, cardiac tonic, cardiovascular protector, treatment of anemia, treatment of dermatitis, analgesic, antidepressant, anti-anxiety, vision and immune improver and EPA and DHA improver in blood were carried out (Gazem et al., 2016).

MATERIALS AND METHODS

Collection of chia seeds

Chia seeds from convenient stores with healthy standard practices were collected. Impurities and damaged seeds were separated by hand picking method. The chia seeds were cleaned and further crushed into chia seeds powder which was stored in air tight containers in normal room temperature (Rubavathi et al., 2020).

Preparation of extract from chia seeds using soxhlet apparatus

Chia seed powder of about 15g was weighed. A thimble was made using whatman filter paper and the grinded chia seed was packed inside the thimble. It was then fixed inside the Soxhlet apparatus. 250 ml of hexane, 250ml of petroleum ether, 250ml of ethyl acetate was taken as solvent and were run at 50°C for about 5-6 hours (5cycles), it was then soaked overnight and another 5 cycles were run for 5-6 hours the next day at 50°C. For oil extraction from the solvent, the solvent was first filtered using whatman filter paper, the filtered solvent was then subjected to hot magnetic plate at 45°C for 5-6 hours, 90% of the solvent evaporated and the rest 10% was evaporated at room temperature.

Phytochemical analysis

Phytochemical analysis was done to detect the presence of flavonoids, alkaloid, and tannins in the extracted oil.

Detection of flavonoids

Sulfuric acid reagent: Add drops of concentrated sulfuric acid to the extracted oil, as the appearance of red color indicates positive detection (Noshe et al., 2017).

Detection of alkaloids

Mayer's test: About 1ml of extracted oil were treated with Mayer's reagent. Formation of creamy white precipitate indicates positive indication.

Detection of tannins

About 1ml of extracted oil was treated with few droplets of Fecl3 reagent. Formation of bluish green color indicates positive indication.

Determination of antioxidant efficacy

DPPH assay free radical scavenging activity using 1,1-diphenyl-2 picryl hydrazyl (DPPH) radical scavenging activity was used for evaluating the radical scavenging activity according to the established protocol (Rubavathi et al., 2016). Extracts solution of 50, 100, 150, 200 and 250µg/ml were prepared. Each test tube contained one ml of the sample and 2ml freshly prepared DPPH solution of 40µg/ml in methanol. Absorbance of the mixture was recorded at 517nm after 30 minutes. Ascorbic acid was used as a positive control. The SA was calculated as follows

Scavenging Activity %= (Absorbance of control-Absorbance of sample/Absorbance of control) X100.

Antimicrobial activity of chia oil

Escherichia coli, Salmonella and *Klebseilla* were used as model bacteria to determine the antibacterial activity of chia seed oil. All bacteria were cultivated in appropriate medium at 37°C and passaged in the same medium overnight prior to the assay. A 100 µL of bacterial culture was spread-plated onto Mueller-Hinton Agar (MHA; Merck, Darmstadt, Germany) with sterile cotton swabs. The MHA volume transferred to sterile petri dishes (90 mm diameter) was fixed to 20 mL to obtain reliable results. When cultures were absorbed to the media completely, antibacterial activity assays were performed. Following incubation, inhibition zone diameters were measured using a digital caliper. Three replicates were conducted against each bacteria for each extract.

Agar Disc-diffusion assay: Sterile filter paper discs with a diameter of 6 mm were placed on the MHA and followed by impregnation of either extracts or crude oils (10 µL) to the discs with a micropipette. All plates were incubated at 37°C for 24 hours (Tuncil et al., 2019).

RESULTS

Collected chia seeds after cleaned and powdered were subjected to Soxhlet Apparatus for extraction process using 250ml each of hexane, petroleum ether and ethyl acetate as solvents. The extracts were obtained and subjected to hot magnetic plate in order to evaporate the solvent present. Pure oil was procured after this process. Using Hexane solvent yielded the maximum amount of oil while petroleum ether and ethyl acetate yield were almost negligible. Phytochemical analysis by different methods detected the presence of alkaloid and flavonoid but absence of tannins (Table 1). Antioxidant assay of different concentration of chia seeds oil in terms of percentage of scavenging activity compared to the control group of ascorbic acid is shown in Table 2 and 3. Antimicrobial activity of *Salvia hispanica* was carried out against three pathogenic microbes *E. coli*, *Salmonella* spp and *Klebseilla*. It was found that *E.coli* and *Klebseilla* were sensitive and formation of "zone of inhibition" was detected with a zone diameter of 1mm and 0.5 mm, respectively (Table 4). *Salmonella* however showed resistance and no "zone of inhibition" was formed.

Table 1: Phytochemical analysis of chia seed oil

Phyto constituents	Hexane
Alkaloid	+
Flavonoid	+
Tannins	-

Table 2: Scavenging activity of chia seed oil against ascorbic acid.

Concentration (µg/ml)	Scavenging activity of the sample (%)	Scavenging activity of the ascorbic acid (%)
50	96.6	67.6
100	88	57.5
150	84.2	46.9
200	76	35.3
250	73.3	24.9

Table 3: Antioxidant analysis of chia seed oil.

Concentration (µg/ml)	Concentration Of methanol	Concentration of DPPH	Scavenging Activity (%)
50	900	2ml	96.6
100	850	2ml	88
150	800	2ml	84.2
200	750	2ml	76
250	700	2ml	73.3

Table 4: Anti-microbial test of chia seed oil against pathogenic microbes by disc diffusion method.

Indicator strain	Zone of inhibition	Zone diameter
E. coli	Sensitive	1 mm
Salmonella spp	Resistant	----
Klebseilla spp	Sensitive	0.5 mm

DISCUSSION

Extraction by employing hexane solvent method proved to be the most effective way of procuring chia seed oil from white chia seeds. In an experiment of extraction of chia seed oil by Marcela L. Martinez, it showed that the decrease of seed moisture resulted in an increase of oil yield and the highest values of oil yield were obtained with the lowest values of seed moisture. Reducing the restriction and pressing speed increased the compression exerted on the seeds and the dwell time of the material inside of the press, with a consequent increased of the oil extracted. This combination produced the highest values of oil yield (Martinez et al., 2012).

Over the years, there have been many ways of extraction of oil which yielded different results due to the difference of the geographical region and climatic conditions in which the seeds have been cultivated. Extraction methods also play an important role in procuring high quality of chia seeds oil (Ali et al., 2012). Seed compression method where cold pressing technique and storage at low temperature (4°C) in dark using amber glass bottles without head space (Ixtaina et al., 2012) and Komet screw press at 25-30°C using electrical resistance heating yield better preservation of antioxidant contents than solvent extraction (Capitani et al., 2012). However, this method can only obtain partial recovery of oil yield (Ixtaina et al., 2011). Employing solvent method such as use of Soxhlet apparatus with n-Hexane as the solvent which is less preferable than other methods has its own advantages and disadvantages. It favours the functional characteristics of the oil such as water holding and absorption capacity, organic molecule absorption, and emulsifying stability. On the other hand it causes slight loss of antioxidant content (Capitani et al., 2012) as well as health and environment safety issues of using hexane (Ixtaina et al., 2011). Use of supercritical fluids where carbon dioxide at optimum pressure P=408 and 80°C is more preferable than other methods. It yields better purity and higher Alpha Linoleic Acid/ Linoleic Acid content in the oil (Uribe et al., 2011; Ixtaina et al., 2010). Due to its high nutritional contents the extraction process needs to be efficient for better usage. Use of method such as cold-pressed oils using photochemiluminescence assay can be the solution to achieving high yield (Maria et al.,2016). Chia seed oil promises to be the future of healthy dietary substance due to its multiple nutraceutical characteristics. A lot of research work in this field might produce significant health benefits in the future.

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