Physicochemical Properties and in Vitro Anti-Inflammatory Effects of Indian Chia (Salvia Hispanica L.) Seed Oil

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Abstract: *Salvia hispanica L. also known as chia or salba has been used as a fodder/food in Central America and was one of the chief dietary components of the ancient culture. Owing to the fatty acid profile and nutrition value chia is being reintroduced as a food crop across different countries. Many health beneficial effects of chia seed oil have been reported in vitro and in vivo owing to higher content of polyunsaturated fatty acid particularly alpha-linolenic acid (ALA). High ALA content makes chia a perfect candidate for 'super food' as it is associated with lower incidence of cardiovascular diseases and neuropathies. These metabolic syndromes are mediated by inflammatory pathways. Hence, we assessed the anti-inflammatory property of chia seed oil per se and in combination with other vegetable oils so as to replicate the daily use methods. Here we report the physicochemical and in vitro anti-inflammatory effects of Indian chia seed oil and compared with the previously reported physicochemical parameters of chia from other countries.*

Keywords: *Alpha-linolenic acid, Anti-inflammatory, chia seed oil, physicochemical properties, combination* studies.

I. Introduction

Salvia hispanica L., a biannual oil seed crop (Family: Lamiaceae) also known as chia or salba has been used as a fodder and food in Central and South America and was one of the chief dietary components of the old Aztecs and Mayan culture [1]. Nowadays chia is a regular food component among Mexicans and populations in Central American countries. The usage of chia is in the form of its seed powder and seed oil. Chia is a small herb which grows up to 1 mt tall possessing oppositely arranged leaves with small (3-4 mm) purple flowers and bear seeds of 1mm in size. Chia is adapted to arid and semiarid climates requiring less water, and the genotypes vary across the geographical areas.

The search of functional foods with overall health beneficial effects has gained interest in recent years. Epidemiological and experimental evidences demonstrate a strong correlation between regular dietary consumption of polyunsaturated fatty acids (PUFA) and lower incidence of cardiovascular diseases, diabetes, and other metabolic syndromes [2,3]. Moreover, dietary fortification of α-linolenic acid (ALA) and n-3 long-chain PUFA was observed to exhibit cardio-protective effects in laboratory animals. This lead to clinical intervention studies for assessing the beneficial health effects of dietary ALA and other major PUFAs. The overall chemical composition of chia seeds show regional variation and has high percentage of antioxidants [4]. The seed contains substantial amount of proteins of high biological value and dietary fiber constitutes major part of the total weight of the seed. [5]. Chia seeds and other species of Salvia genus yield 25%-35% oil, which contain high concentrations of PUFA that is 68% of α-linolenic acid (ALA) [6, 7] which is more than flax seed oil (57% ALA) [1].

Reports from different parts of the world suggest that chia seeds and its oil collected from different geographical areas slightly differ in their physicochemical properties. The study of locally available chia seeds may demonstrate to be an incomparable functional food with its distinctive and finer composition. Therefore it is essential to conduct the composition analysis as the seeds may show regional variation depending on the soil texture and climatic conditions.

Numerous reports indicate that inflammatory pathways predominates the pathophysiology of major metabolic syndromes like diabetes and cardiovascular diseases (CVD). It has been noted that obesity and co-morbidities such as diabetes and CVD are resulted from chronic, low-grade inflammation impacting multiple organ systems [8]. The compromised inflammatory state is usually attributed to higher levels of pro-inflammatory signaling from adipocytes [9]. Dietary intervention studies have indicated that n-3 PUFA rich diet alleviates the metabolic syndromes through attenuating the inflammatory status of the system [10, 11]. Hence, it was interesting to study the anti-inflammatory properties of Indian chia seeds in an in vitro condition. Even though chia is not used as a major food component, owing to the high content of ALA, chia is predicted to be an excellent dietary adjuvant. Hence, in this study we fortified chia oil with the other major edible vegetable oils like sunflower oil, palmolein oil, olive oil and soybean oil for the anti-inflammatory assays. It is predicted that

fortification with the other edible oils may replicate the changes in the beneficial effects of chia seed oil when it is used in association with the regular diet [12]. Initially proximate parameters of the Indian chia seeds were assessed followed by physicochemical properties of the chia seed oil. In addition, the anti-inflammatory properties of chia seed oil and edible oils either alone or in combinations were assessed employing in vitro studies.

II. Materials And Methods

2.1. Materials

Seeds of chia (Salvia hispanica L.) were procured locally from Heggadadevana (HD) Kote, Mysuru, Karnataka, India. Authenticity was confirmed by the Department of Studies in Botany, Manasagangotri, Mysore. The chia seeds were cleaned by removing all the impurities and damaged seeds and then stored at 4 ℃ before utilizing for the experiments. The sound seeds were crushed and the flour was used for the analysis.
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Chemicals And Reagents

All solvents and chemicals used in the studies were of analytical grade and purchased from SRL, Mumbai, India.

2.2. Proximate Analysis

The moisture content was determined by drying 2 ± 0.1 g of sample (Chia seeds) at 105 ± 1 ℃ for 40 h and the total ash was determined by incineration of the samples (2 ± 0.1 g) at 550 ℃ for 48h [13]. The crude fibre content was determined by drying 2 ± 0.1 g of the sample [14]. Total lipids of chia seeds was determined using Soxhlet extractor [15]. Crude protein in the oil seeds was determined by Kjeldahl protein units and the protein was calculated as nitrogen (%) $\times$ 6.25 [16]. Total carbohydrate content was calculated as a remainder percentage of sums of all the other components. All proximate determinations were done in triplicates.

For the analysis of fatty acid profile in the chia Seed oil, the fatty acid methyl esters (FAMEs) were prepared from the extracted lipids by the esterification reaction according to the method described by Christi. Gas chromatography was performed using GC-2010 (Gas Chromatography, Shimadzu, Japan) [17, 18]. Individual fatty acids were expressed as mass of fatty acid in 100 g of oil.

2.3. Physicochemical Properties Of Chia Seed Oil

Physicochemical properties of chia seeds were assessed according to a modified version of Indian Standard 548-1 (1964) [19]. For this purpose, oil was extracted from the chia seed powder employing Soxhlet apparatus with n-hexane. The oil was stored at room temperature and used directly for the physicochemical assessment. Refractive index of chia seed oil was estimated using the refractometer abbe with tungsten lamp at 25℃ and specific gravity was determined using a specific gravity bottle at 30℃. Acid value was measured in terms of KOH required for neutralizing free fatty acids. Iodine value was estimated using an indirect titration method by using Wijs reagent and sodium thiosulfate. Saponification value and peroxide value were estimated using potassium hydroxide and sodium thiosulfate respectively.

2.4 In-vitro anti-inflammatory studies
Sample Preparation

The vegetable oils employed in combination studies along with chia seed oil (rich in n-3, linolenic acid) for the present assay differed in their fatty acid composition, hence selected to verify their impact on anti inflammatory properties. More over the olive oil and soybean oil are common cooking oil in western countries and palmolein is regularly used in Indian cooking. The oils used are sunflower oil (standard), olive oil (rich in monounsaturated fatty acid), palmolein oil (rich in saturated fatty acid) and soybean oil (rich in n-6, linoleic acid). The chia oil was tested either alone or in combination with other oils mixed in the ratio 1:3, 1:1 and 3:1 and total samples tested were fourteen in number.

The mixtures of oil samples of different concentrations S1-S14 (10-40 µl/ml) were prepared by using 10% DMSO and following samples were used for the experiments: Sample 1-[Sunflower oil (SFO)], Sample 2-[Chia seed oil (CSO)], Sample 3-[Olive oil (OlO)], Sample 4-[Palmolein Oil (PO)], Sample 5-[Soyabean oil (SO)], Sample 6-[CSO:OlO (75:25)], Sample 7-[CSO:OlO (50:50)], Sample 8-[CSO:OlO (25:75)], Sample 9-[CSO:PO (75:25)], Sample 10-[CSO:PO (50:50)], Sample 11-[CSO:PO (25:75)], Sample 12-[CSO:SO (75:25)], Sample 13-[CSO:SO (50:50)] and Sample 14-[CSO:SO (25:75)].

2.4.1. Human Red Blood Cell (HRBC) Membrane Stabilization Assay

The HRBC membrane stabilization assay was carried out using human red blood cells [20]. A healthy donor was selected who had not taken NSAIDS for the previous two weeks. Guidelines of Indian Council of Medical Research (ICMR)-Govt. of India (vide O. M. No 19015/53/1997-IH Pt) were strictly followed while

collecting blood. Equal volumes of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and blood sample were mixed, centrifuged at 2500rpm. The obtained packed cells were washed with Physiological saline and a suspension of 10% cells was made by using saline. Diclofenac (100μg/ml) was used as positive control. The different concentrations of oil sample 1-14 (10-40 μl/ml) were prepared by using 10% DMSO. To each concentration (10-40 μl), phosphate buffer (1ml), hypo saline (2ml) and 0.5 ml of packed cells were added. Then the test tubes were incubated for 20 min at 37 ˚C and centrifuged at 3000 rpm (15min). The supernatant containing hemoglobin was estimated at 560 nm spectrophotometrically.

The percentage of HRBC protection was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{(\text{control} - \text{test sample})}{(\text{control})} \times 100$$

2.4.2. Protein Anti-Denaturation Activity

The protein anti-denaturation assay was carried out by monitoring the degradation of bovine serum albumin [21]. The reaction mixture contains 1 ml of 0.2% of bovine serum albumin in Tris-buffer saline (pH 6.8), and different concentration of 1ml of oil samples S1-S14 (10-40μl) was added as indicated in the preparation of the samples. 50 μl methanol in 1ml of 10% DMSO served as control and Diclofenac (100 μg/ml) served as positive control. All the tubes were kept in a water bath at 72 ˚C for 5 min and cooled to room temperature. The tubes were centrifuged at 3000rpm for 10 min. The absorbance of the supernatant was determined by spectrophotometer at 660 nm.

The percentage of denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{(\text{control} - \text{test sample})}{(\text{control})} \times 100$$

2.6. Statistics
The results were expressed as Mean ± Standard deviation and all experiments were compared with control and performed in triplicates. Statistical calculation were performed employing Microsoft excel.

III.   Results And Discussion

3.1. Chemical Composition Of Chia Seeds
The results of proximate analysis of Indian chia seeds are presented in table 1. The oil content of chia seed was found to be 28.13% which is lower than the reports of the previous studies where the yield of oil was 33% [22]; 35.13% [23] and 32% [6]. The variation in the oil content from different varieties of chia belonging to different geographical areas is attributed to the edaphic factors like rainfall and water retention capacity of the soil. The protein and ash contents were 20.76% and 5.5% respectively which corroborated with the previous reports [24]. However the protein content was lower when compared to the study of Gujatto et.al [6]. Chia is a good source of dietary fibers and the present sample contained 18.51% of fiber. Significantly higher fiber content in chia has been reported [25]. The present Indian variety of chia possessed 16.88% of total carbohydrates which is markedly high compared to previous studies [26]. The present findings strongly support the concept that the composition of chia seeds cannot be generalized as there is a great variation in the content of seeds grown in India and America and are found to be influenced by the type of species, climate and soil conditions.

3.2. Physicochemical Characterization Of Chia Seed Oil
In the present study, the yield of the chia seed oil was comparable to the study done by Ayerza *et al* [27]. The physical characteristics of the chia seed oil has been presented in Table 2. The relative density of chia seed oil was 0.9246 which was similar to the previous reports [28, 23]. It has been suggested that relative density of oil is directly proportional to the composition of unsaturated fatty acids [29]. Considerable higher relative index of Indian chia seed oil shows that it has high percentage of unsaturated fatty acids, which agrees with the previous results [30]. In our study, the refraction index value of Indian chia seed oil was 1.481 at 25°C and it matched with the refraction indices of chia seed oils obtained from of Mexican, Argentine and Guatemalan variety and the value was similar in all the three studies and it was1.476. The refraction index of any oil is dependent on the temperature of the analysis and content of unsaturated fatty acids and it is established that high analysis temperatures showed lower refraction index values, whereas higher percentage of unsaturated fatty acids in oil increased the refraction index values [29].

Chemical indices of oil depend on the extraction procedure and the storage conditions of the oil. A significant marker of extent of rancidity of oil is measured as acid value which denotes to the free acid content. Acid value of a particular batch of the oil depends on the length of storage of the oil. In the present study the Indian chia seed oil showed acidity index of 1.282 mg KOH/g, which represents 0.71% of oleic acid as free fatty acids in the oil. The results showed that the Indian chia oil tested in this study was stable and are not altered by chemical or enzymatic hydrolysis, hence the acid value obtained was lower compared to chia oil from other

geographical areas (Mexican-2.053; Argentinean-2.05 and Guatemalan-1.64mg KOH/g oil) [5]. Further, the peroxide value, an additional index of rancidity, of the Indian chia seed oil was found to be 2.86 meq O_2/kg oil, which was comparable with other varieties.

The saponification value of the Indian chia oil in the present study was found to be 201.1mg KOH/g and was comparable with chia oils of other areas (Argentinean and Guatemalan-193 mg KOH/g; Mexican-222.7mg KOH/g [5] ; Segura-Campos et al. (2014) [23]. Other edible oils such as safflower (186 - 198 mg KOH/g oil), sunflower (188 -194 mg KOH/g oil), soyabean (189 - 195 mg KOH/g oil) and virgin olive oil (184 -196 mg KOH/g oil, [28, 31] also showed almost similar values. Saponification value is inversely proportional to the chain length of fatty acids [32]. Therefore it indicates that Indian chia oil is constituted by fatty acids of comparable chain length as that of sunflower, safflower, soy and virgin olive oil. The iodine index of oil refers to the total number of double bonds in the fatty acid chains. In this study, Iodine value of Indian chia seed oil was found to be 205.9g I/100g oil which is comparable to chia belonging to various geographical areas [23, 5]. The Iodine values of olive oil, sunflower, soybean oil and safflower oil was found to be 75-95; 118.1;139.2 and 136-148 g I/100 g oil respectively clearly suggesting lower percentage of unsaturated fatty acids in these oils [28, 31]. The iodine value obtained in the current investigation shows higher content of polyunsaturated fatty acids (PUFA) in the Indian chia oil.

3.3. Fatty acid composition of chia seeds oils

The fatty acid profile of the Indian chia seed oil was assessed using gas liquid chromatography (GLC) and the results are presented in table (3). GLC showed that the ω-3 (α-Linolenic, C18:3) fatty acids in chia seed oil was three fold more than ω-6 (Linoleic, C18:2) fatty acids and the percentage was found to be 64.0% and 18.1% respectively. The present results showed a higher content of ALA and palmitic acids when compared with the previous studies of chia from different regions [33, 34]. The current investigation of fatty acid composition of Indian chia seed oil clearly demonstrated rich content in essential (n-3) fatty acids and is 60% of the total fat. The values obtained from Indian chia seed oil are comparable to components of linseed oil which showed 58.8% of ALA and 14%-16% of linoleic acid [35]. The fatty acid profile significantly depends on the variations of the environment such as climatic changes, availability of nutrients, area of cultivation and soil conditions [36, 37]. Thus the environmental conditions affect the chemical composition of chia seed oil and found that their exists an inverse relationship between altitude of cultivation and the content of saturated fatty acids; at low altitude and high temperatures there was increase in the concentration of saturated fatty acids. In Argentina also temperature largely contributes to the type of fatty acid found in the oil [1] wherein the higher temperatures, during seed development resulted in a decreased PUFA content. Further it was observed that ALA content decreased by 23% from the early stage to the maturity of the seed and increased the content of linoleic acid and lignin [38].

3.4. In vitro anti-inflammatory studies of chia seed oil

Previous reports strongly suggest the beneficial effects of chia on human health owing to its high PUFA content. Interestingly, feeding hens with chia resulted in eggs with highest ω-3 ALA content [39]. In another study, rats fed with chia seed rich diet demonstrated a decrease in LDL and serum triglycerides whereas HDL and ω-3 PUFA levels were elevated [40, 41]. In a similar study, pigs and rabbits fed with chia seeds resulted in an increased PUFA content, flavor and aroma in the meat fats [42]. Currently, major part of dietary PUFA is obtained from marine sources. But the psychological stigma of people about biomagnifications of some heavy metals and pesticides in addition to the smell, hinder them from consuming the fish based supplements. Moreover, a typical organoleptic characteristic such as flavor and smell from marine sources were not found in chia making it a desirable vegetable source for PUFA as ALA and is converted enzymatically to long chain PUFA in vivo [43].

The phenomenon of heat induced protein denaturation is similar to the mechanism involved in delayed type III hypersensitivity and arthritis like diseases. The heat induced haemolytic study HRBC assay in vitro also replicates the in vivo inflammatory mechanism. The heat causes damage to the membrane and leads to the release of serum proteins and fluids into the tissues which increase the membrane permeability that leads to inflammation. Potent anti-inflammatory property is attributed to ALA based on the experimental and epidemiological evidences. In this study, we examined the anti-inflammatory property of chia seed oil per se and in synergy with the other vegetable oils employing in vitro systems viz., HRBC membrane stabilization assay and protein denaturation assay. Our study demonstrated the anti-inflammatory property of chia seed oil alone and in combination with the other vegetable oils. There was increase in the anti inflammatory activity as the dose of oil increased from 10-40 μl/ml. Fortification with the other vegetable oils is a mandatory method of activity assessment in case of not so frequently used products like chia, since this method reveals the complementary effects of these dietary components [12]. The results from HRBC membrane stabilization assay clearly indicates the potent membrane stabilizing property of chia seed oil (63%) and olive oil (82%) when

compared to SFO (54%), PO (69%) and SO (58%). Interestingly, the ability of chia seed oil to inhibit membrane disintegration was lower to that of olive oil. Mixing of chia seed oil with the olive oil slightly increased the effect, however it was not substantial (Fig 1). Further, similar results were observed in the protein anti-denaturation assay. The inhibition of protein denaturation was evident with chia seed oil (65%), however was similar when compared to the other oils like olive oil (65%) when compared to SFO (58%), PO (68%) and SO (58%) respectively. In combination studies chia oil with olive oil or palmolin oil gave good inhibitory effect compared to that of soybean oil (Fig 2). Previous reports suggest that blending of different oils resulted in better storage and improved antioxidant properties in vitro [44, 45]. Moreover, health benefits from functional foods as chia seeds are usually conspicuous on a chronic supplementation. However, further experiments are underway to elucidate the potent health benefits from chia seed oil.

IV.   Figures And Tables

Fig. 1: The anti-inflammatory effects of different oil samples (S1-S14) by HRBC membrane stabilization assay showing percentage of inhibition
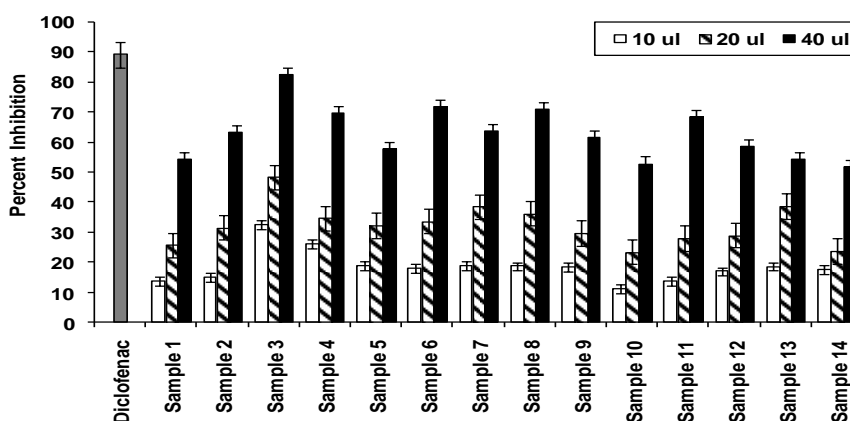

Fig. 2: The anti-inflammatory effects of different oil samples (S1-S14) by anti-denaturation activity showing percentage inhibition
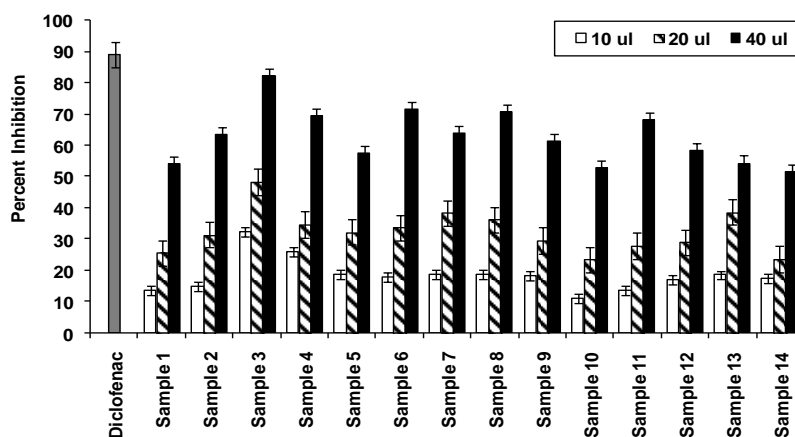

Table 1: Chemical composition of Indian chia seeds (% weight)

SL. No.	Component	Content (% g) Mean ± SD
1	Moisture	8.95 ± 0.06
2	Protein	20.76 ± 0.05
3	Oil	28.1 ± 0.5
4	Crude Fibre	18.51 ± 0.04
5	Ash	5.5 ± 0.5
6	Carbohydrate	16.88 ± 0.63

Table 2.Comparison of physical and chemical properties of chia seed oil from different countries.

Sr. No.	Property	Indian chia oil	Mexican chia oil (Segura-Campos et al., 2014) [18]	Argentinean chia oil (Ixtania et al., 2011) [5]	Guatemalan chia oil (Ixtania et al., 2011) [5]
1	**Specific grafity**	0.9246 ± 0.0002	0.9241 ± 0.003	nr	nr
2	**Refraction index**	1.481 ± 0.001	1.4761 ± 0.00	1.4768	1.4763
3	**Acidity index (mg KOH/g oil)**	1.2816 ± 0.08	2.053 ± 0.03	2.05	1.64
4	**Saponification index (mg KOH/g oil)**	201.133 ± 1.45	222.66 ± 0.29	193.09	193.01
5	**Unsaponifiable matter(g/kg)**	-	0.839 ± 0.10	1.27	nr
6	**Iodine index (g I/100 g oil)**	205.867 ± 1.81	193.45 ± 0.54	210.5	215.0
7	**Peroxide index (meq O2/kg oil)**	2.86 ± 0.0005	17.5 ± 0.07	nr	1.64

Table 3. Fatty acids profile of chia oil.

Fatty acids	Composition (%)			
	Indian chia oil	Mexican chia oil (Segura-Campos et al., 2014) [18]	Ayerza and Coates (1999) [27]	Craig and Sons (2004) [28]
Saturated fatty acids (SFA)				
Myristic C14:0	-	0.04 ± 0.00	0.0	0.1
Pentadecaenoic C15:0	-	0.02 ± 0.00	nr	nr
Palmitic C16:0	7.1± 0.3	7.47 ± 0.09	6.5	6.7
Palmitoleic C16:1 cis-9	0.1± 0.007	0.06 ± 0.00	0.1	0.1
Heptadecaenoic C17:0	-	0.05 ± 0.00	nr	0.2
Heptadecaenoic C17:1 cis-10	-	0.02 ± 0.00	nr	0.1
Stearic C18:0	3.3± 0.25	0.29 ± 0.07	2.9	3.0
Arachidic C20:0	0.3± 0.022	0.15 ± 0.03	0.3	0.3
Behenic C22:0	-	0.06 ± 0.06	nr	0.1
Monounsaturated fatty acids (MUFA)				
Oleic C18:1 cis-9	6.4± 0.2	2.43 ± 0.03	7.2	6.9
Gadoleic C20:1 cis-11	-	0.03 ± 0.01	0.1	0.1
Polyunsaturated fatty acids (PUFA)				
Linoleic C18:2 cis-9, 12	18.1± 1.6	20.40 ± 0.09	20.3	18.8
α-Linolenic C18:3 cis-9, 12, 15	64.0±2.0	68.52 ± 0.02	62.0	58.8
γ-Linolenic C18:3 cis-6, 9, 12	-	0.31 ± 0.02	nr	0.1
Eicosatrienoic C20:3 cis-11, 14, 17	-	0.01 ± 0.00	nr	0.1
Arachidonic C20:4 cis-5, 8, 11, 14	-	0.13 ± 0.10	nr	nr
Docosahexaenoic C22:6 cis-4,7,10,13,16,19	-	0.05 ± 0.029	nr	nr

nr=Not reported

V. Conclusion

Local availability, global climatic adaptability, rapid growth, significant chemical composition and economic price make chia a prime candidate as a health supplement and animal feed for improving the food quality. However, knowledge of chemical characteristics and composition of chia seed oil from various geographical sources should be obtained for the consideration for general animal feeds or human use. Here, we have presented physicochemical and anti-inflammatory properties of Indian local chia seeds and accordingly compared with the previous reports. We have also made efforts to assess the combined effects of chia seed oil with the other vegetable oils. From our findings it is anticipated that chia may be promoted as an excellent dietary adjuvant or fortifier however; further data about the potent biological activities of Indian chia seed oil need to be investigated.

Acknowledgement

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