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Comparative Analysis of Antioxidant and Hemagglutination Properties of Chia and Basil Seeds

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Authors' contributions

This work was carried out in collaboration between both authors. Author ASH designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SB and ASH managed the analyses of the study. Author SB managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

For many years natural remedies have played a significant part in pharmaceutical biology. Even today, many health organizations depend on the natural products, WHO estimated that more than 80% population depends on the natural remedies. Chia and basil seeds are herbs that contains several beneficial minerals, proteins, vitamins and dietary fibers. They also contain omega 3 and omega 6 fatty acids (healthy) as well as the sufficient amount of polyphenols and antioxidants, like caffeic acids, myricetin and others. Current study consists of comparing the Total Antioxidant Capacity (TAC) of Chai and basil seeds with the help of various assays. Confirmatory tests to verify the presence of phytolectins in both chia and basil seeds have been done by performing hemagglutination tests. These phytolectins are the proteins that binds to the oilgosaccharides and monosaccharides in the reversible manner. Although further studies are required for isolating and characterizing the phytolectins from the seeds.

Keywords: Antioxidant; basil; chia seed; phytolectins; TAC; extracts.

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1. INTRODUCTION

Proper nutrition is an important part of preventing many civilization-related diseases such as diabetes, cardiovascular disease and obesity. Both government and non-governmental organizations issue nutrition guidelines to protect human health, prevent the development of certain diseases, and alleviate symptoms [1]. An increasingly important role in promoting health is due to the biologically active components of food. They have been defined by Biesalski and his colleagues as food ingredients or compounds other than food that are naturally present in raw materials or formed in products in technological processes that can enhance, inhibit or alter the physiological and metabolic functions of the body [2]. The American Dietetic Association supports this definition by further emphasizing the importance of bioactive food safety for health [3]. Bioactive compounds include, for example, polyphenols, carotenoids, plant estrogens, sterols, stanols, vitamins, dietary fiber, fatty acids, probiotics, prebiotics and bioactive peptides. Many studies have been conducted on the on natural food products having several health-related benefits.

1.1 Chia Seeds

Salvia hispanica or generally named as chia, is a yearly herb that bears purple or white hermaphroditic flowers in the summer. It is a short herbaceous plant of around 1m height with small serrated leaves around 4–8 cm wide. The flowers grow in clusters and the plant is cultivated for its seeds. It belongs to the mint family named Lamiaceae. Researchers have discovered how rich the seeds are in polyphenolic antioxidants, constituting about 8.8% of the total chia seed extract on dry matter. Highest amount of polyphenols present are chlorogenic acid, caffeic acid and quercetin [1].

1.2 Basil Seeds

Ocimum basilicum or basil is an aromatic herb belonging to the Lamiaceae family, often used as a culinary ingredient. There are many varieties of basil like sweet basil, Thai basil, lemon basil, holy basil and African blue basil, each with their own distinct aroma and taste. Basil is native to Central Africa and Southeast Asia and is a field crop in India, Burma and parts of the Mediterranean like Italy and Turkey. It is an annual, and sometimes perennial, herb that grows up to 30 cm tall and has small white, pink or purplish flowers in clusters. The leaves are small and serrated [2].

1.3 Free Radicals and Reactive Oxygen Species (ROS)

Free radical is a molecular entity possessing an unpaired electron. ROS are radicals of oxygen formed in the body, physiologically. 1-3% of oxygen in the body, after its utilization, is converted into ROS. They are formed due to various metabolic activities taking place in the cell, like the oxidative phosphorylation in mitochondria, oxidation of unsaturated fatty acids by cytochrome P450 enzyme, phagocytic cells producing ROS during oxidative burst etc. In lower concentrations, free radicals are in fact, necessary for maturation of cellular structures. Phagocytes kill pathogens using free radicals, in a process called oxidative burst [3].

1.4 Antioxidants

The termination of a free radical can happen only if it is either scavenged by an antioxidant or is either neutralized by reacting with other free radical or cell component. Antioxidants are, therefore, compounds that inhibit oxidation. Recently, antioxidants have found widespread applications in food, drug and cosmetic industries both as an active ingredient and to increase the shelf life of the product. They also help prevent various degenerative diseases and cancers by reducing free radicals in the cell [4]. Since most existing antioxidants are synthetically derived, there is a constant hunt for naturally occurring antioxidants that have equal, or more amounts of total antioxidant capacity (TAC).

1.5 DPPH Free Radical Scavenging Assay

The protocol was designed according to the paper published by G. Marinova and V. Batcharov [5], and modified for standardization in accordance to the lab conditions and also with the range of the spectrophotometer. DPPH extra pure was purchased from Sisco Research Laboratories Pvt. Ltd. It is a stable crystalline powder that has free radical molecules. Any presence of antioxidant molecules in a sample reduces the violet-colored DPPH solution to a pale yellow solution of DPPH-H.

2. REVIEW OF LITERATURE

Antioxidants are a need of the hour due to increased incidences in diseases caused by

oxidative damage. They are also used in cosmetics as an anti-ageing ingredient and in food packaging to prevent oxidative damage [6]. Chia and basil seeds have been used for centuries in their native countries for their health benefits. Much work has been done on the antioxidant properties of chia seeds and basil leaves although, TAC of basil seeds has yet to be explored more [7].

Of the extensive literature found on antioxidant activity of chia seeds, it was established that they are potent radical scavengers, have good reducing properties and carry out lipid peroxidation [8]. Although basil seeds have had limited work done on them, studies have established that the seeds have good antioxidant potential.

Through this study comparison of the TAC of chia seeds and basil seeds, which belong to the same family Lamiaceae. And it was also determine if different treatments like soaking and toasting, different soaking time (30 mins, 1 hr, 4 hrs, 8 hrs and 12 hrs) and combining the two seeds in varying concentrations has any effect on its TAC [5,9].

Phytolectins are the largest class of lectins characterized. The potential of chia seeds as agglutinins has not been studied yet. Basil leaves' methanolic extracts, on the other hand, were explored for their hemagglutination activity but the results failed to show agglutination and the authors concluded that phytolectins in basil leaves, were absent [10]. We would like to conduct hemagglutination assay with extracts of chia and basil seeds in phosphate buffered saline and check for the presence of Phytolectins in the seed extracts.

Research Question: what are the antioxidant capacities of chia and basil seeds?

3. DESIGN METHODOLOGY

Samples were prepared by soaking and toasting. After the preparation of the samples assays like DPPH free radical scavenging assay, Phosphate buffered saline (PBS), Blood Sample processing and Haemagglutination assay were performed on these toasted and soaked Chia and Basil seeds. Results are collected on various parameters like Optical density (OD) by DPPH assay, to investigate the presence of phytolectins by performing a simple haemagglutination test with Phosphate Buffered Saline (PBS) extracts of the seeds with 3 blood groups- A+, B+ and O+ and then further these results are analyzed and represented in the form of graphs (Fig. 1).

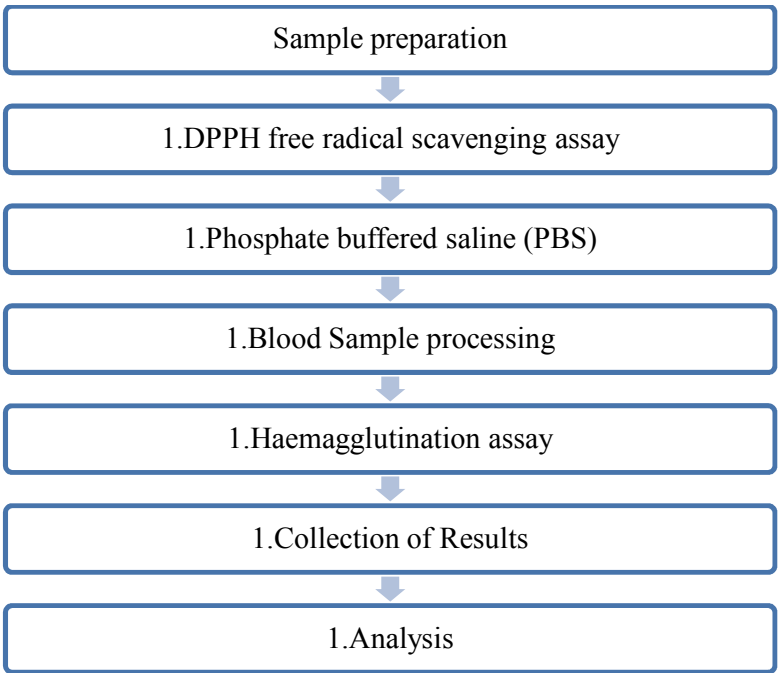

Fig. 1. Flowchart representing the protocol followed throughout the research study to obtain the results

3.1 Sample

For toasted samples, whole seeds were toasted in a skillet for a minute on low flame and then ground to a fine powder and stored in aluminum foil. For soaked samples, the whole seeds were soaked in water in a closed container for appropriate time of 30 minutes, 1 hour, 4 hours, 8 hours and 12 hours. For mixtures, the powdered seeds were mixed in a chia to basil ratios of 30:70 and 70:30 amounting to a total of 1 g. Untreated seeds were ground to a fine powder and were taken as a negative blank.

The samples were weighed 1 g and suspended in 10 ml of methanol for extraction. After keeping it on an orbital shaker for 90 minutes at room temperature, the extract was centrifuged and supernatant was used. Two concentrations of 0.1 g and 0.01 g were prepared in methanol. Fig. 2 (A) is representing the Chia seeds after soaking developing a gelatinous covering and Fig. 2 (B) is representing the Basil seeds after soaking developing a gelatinous covering.

3.2 Instrument

DPPH (2,2- Diphenyl-1-Picrylhydrazyl), methanol and phosphate buffered saline, DPPH free radical scavenging assay for recording the optical density, Phosphate buffered saline (PBS), Blood Sample processing and Haemagglutination assay to investigate the presence of phytolectins. Fig. 3 is representing the extracts of Chia seeds and Basil seeds after completing the centrifugation process.

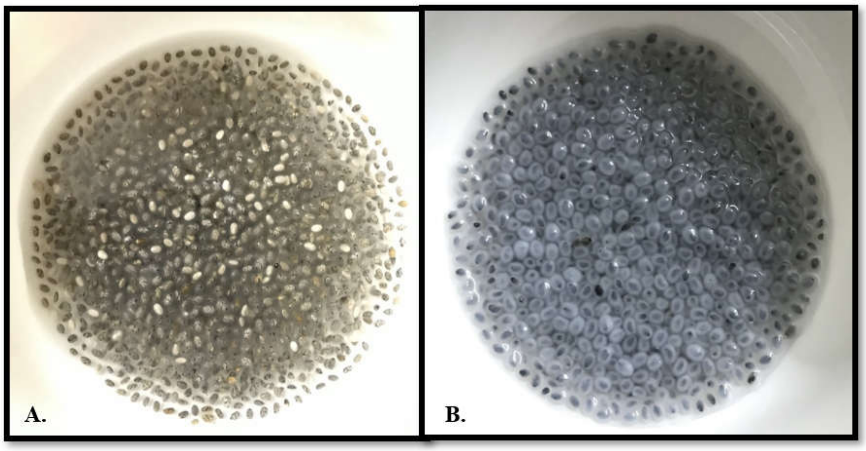

Fig. 2. (A) Chia seeds, after soaking and (B) Basil seeds, after soaking developing a gelatinous covering

Fig. 3. Test Tubes filled with Chia and basil seed extracts in PBS, after centrifugation

Table 1. Physico-chemical (color and texture) evaluation of Chia and Basil seeds soaked in water

Seeds	Colour	Texture	On soaking in water
Chia seeds	Light brown and white	Smooth and slippery	Develops a mucilaginous covering around the seed
Basil seeds	Black	Matte	Develops a mucilaginous covering around the seed

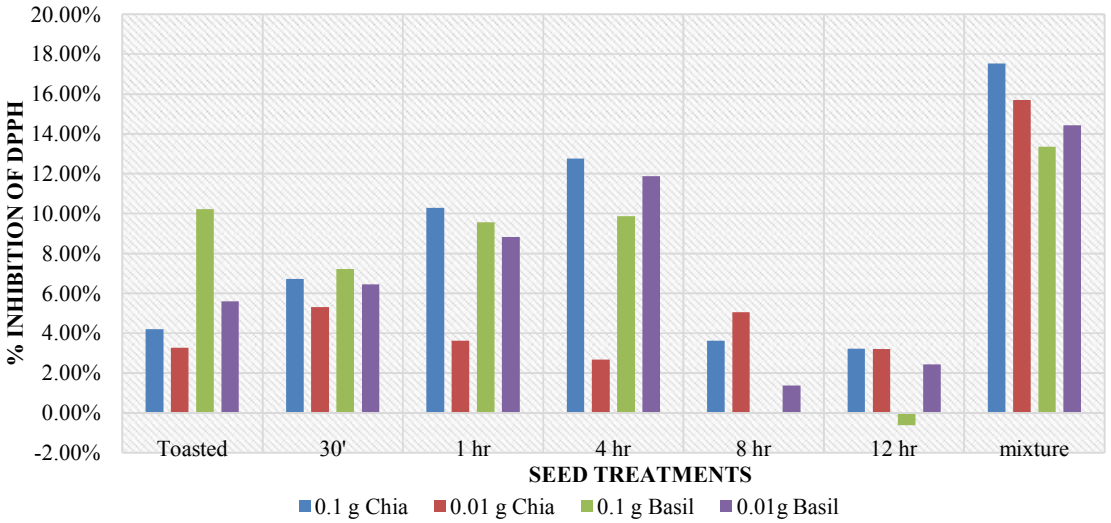

Fig. 4. Difference in percentage inhibition of DPPH, between untreated and treated extracts of chia and basil seeds (Mixture = Chia 30: Basil 70 & Chia 70: basil 30)

3.3 Data Collection

After soaking the seeds of Chia and basil it was reported that there was a significant changes in the physio-chemical properties of both the seeds (Table 1). The results were analysed graphically using charts and graphs to compare the TAC between chia seeds and basil seeds with and without various treatments. Fig. 4 shows the difference between treated samples and untreated samples. Fig. 5 shows a comparison of TAC between untreated chia seeds and basil seeds of two concentrations- 0.1 g and 0.01 g. It is visible that basil seeds have higher TAC than chia seeds. Fig. 6 shows a comparison of TAC of untreated samples of chia and basil seeds with those of toasted samples of the same seeds. From the graph, we can conclude that toasting has a significant effect on improving the TAC of both the seeds, but more so in basil seeds. The TAC has increased by an average of 4.2% in chia in comparison to 10.22% in basil seeds. This could mean that toasting of basil seeds is more efficient in increasing the TAC than in chia seeds. After the blood samples were analyzed for haemagglutination, there was visible agglutination on the slides. The slides were observed under a compound microscope at 40x power. The view showed clumped cells, proving that the RBCs were agglutinated. Fig. 7 (A and B) shows the blood samples added to the seed extracts on a slide, both before and after incubation. In Fig. 8, are the microscopic views of the agglutination observed at 40x, of all three blood groups tested, after 30 minutes of incubation. The slides were a simple wet mound preparation, without the use of any staining dyes.

3.4 Data Analysis

The Fig. 9 shows the effect of soaking period on the 0.1 g concentrations of the seed extracts. The TAC of chia seeds shows a maxima at 240 minutes (4 hours) at 33.03%, after which the

TAC starts to decrease significantly. Basil seeds too, show a peak in TAC at 240 minutes at 33.27%. Fig. 10 shows the effect of soaking period on the 0.01 g concentrations of the seed extracts. The TAC of chia seeds shows a maxima at 30 mins at 22.2% meanwhile, basil seeds show a peak at 240 minutes (4 hours) at 31.66%. From figure 9 and figure 10, it is evident that, the TAC of chia seeds has a maximum at 30 minutes and 240 minutes of soaking in water whereas, TAC of basil seeds peaks at 240 minutes of soaking in water. Fig. 11 shows the variance of TAC in the chia: basil mixture concentrations- 30 chia: 70 basil and 70 chia: 30 basil. This experiment has had the largest variation in TAC of all the treatments that were subjected on to the two seeds.

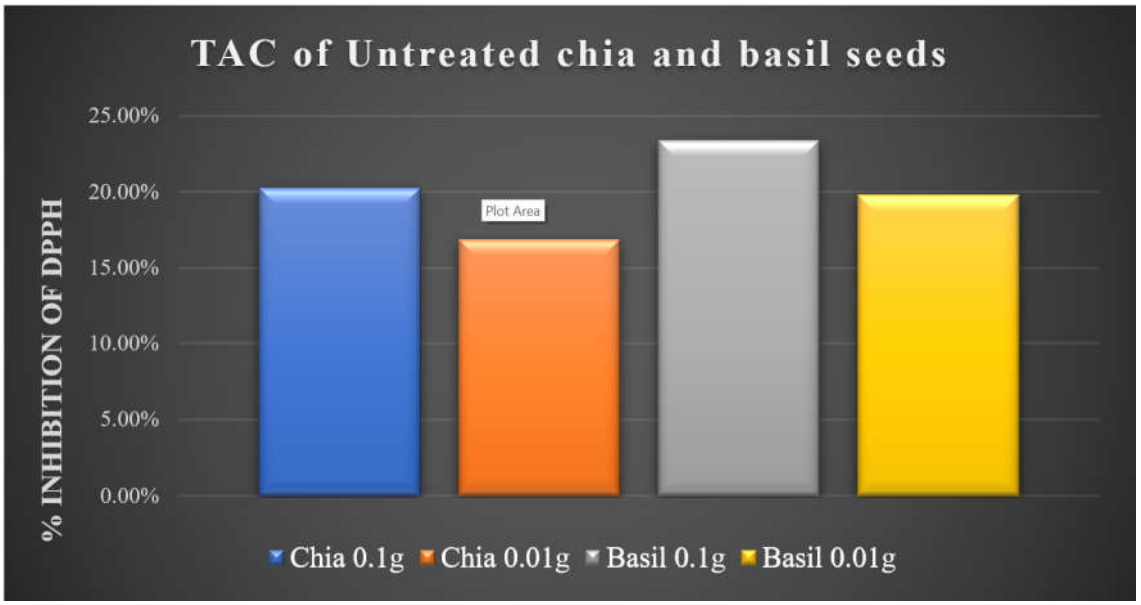

Fig. 5. TAC of chia and basil seeds- untreated samples

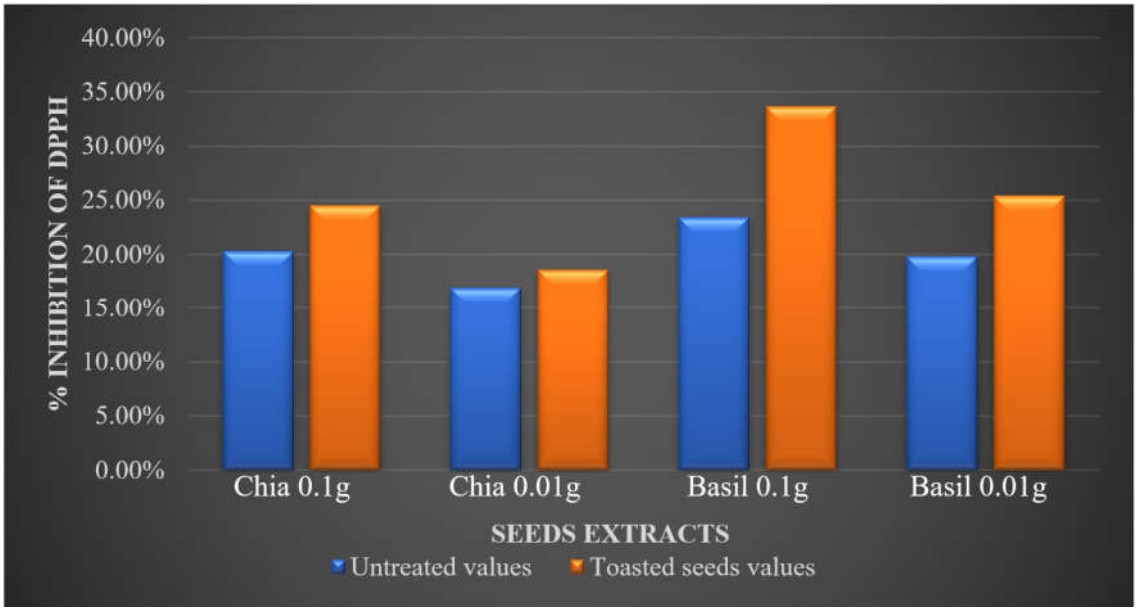

Fig. 6. Comparative analysis of TAC of untreated chia and basil seeds vs toasted chia and basil seeds

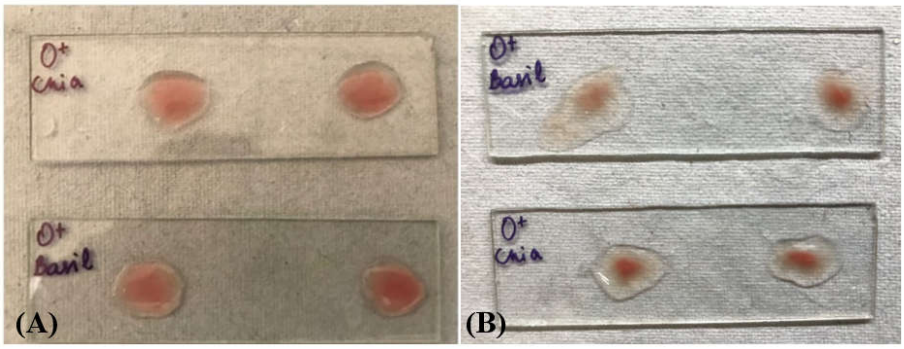

Fig. 7. (A) Before incubation of RBCs with the seed extracts. (B) After incubation of RBCs with seed extracts where, coagulation can be seen

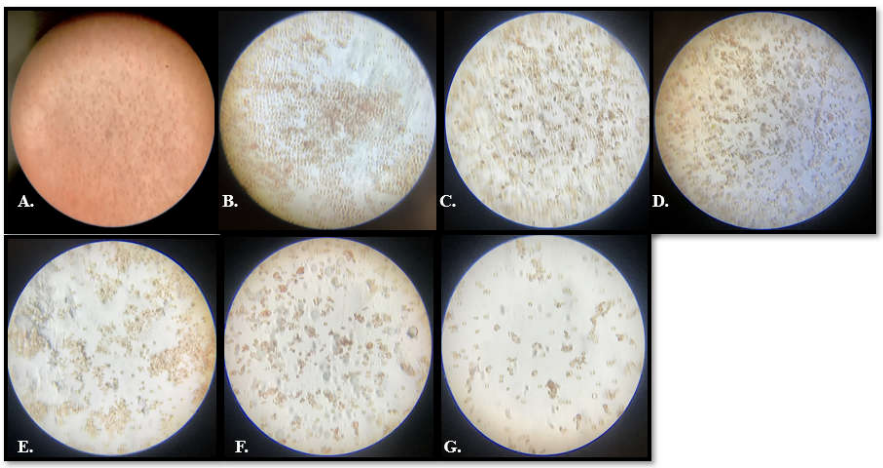

Fig. 8. (A) RBCs without any treatment (positive control). Agglutination observed under 40x in a compound microscope in (B) blood group A+ with chia seed extract (C) blood group A+ with basil seed extract (D) blood group B+ with chia seed extract (E) blood group B+ with basil seed extract (F) blood group O+ with chia seed extract (G) blood group O+ with basil seed extract

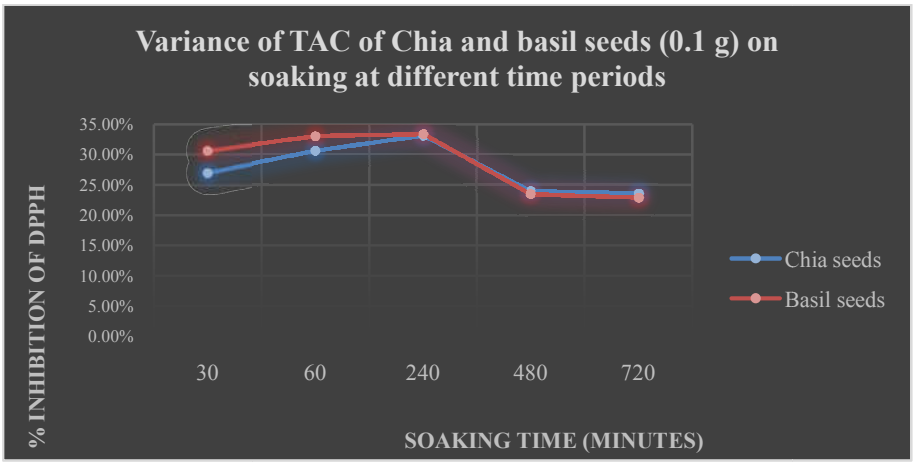

Fig. 9. Comparative analysis of TAC of soaking time of chia and basil seeds of concentrations 0.1 g

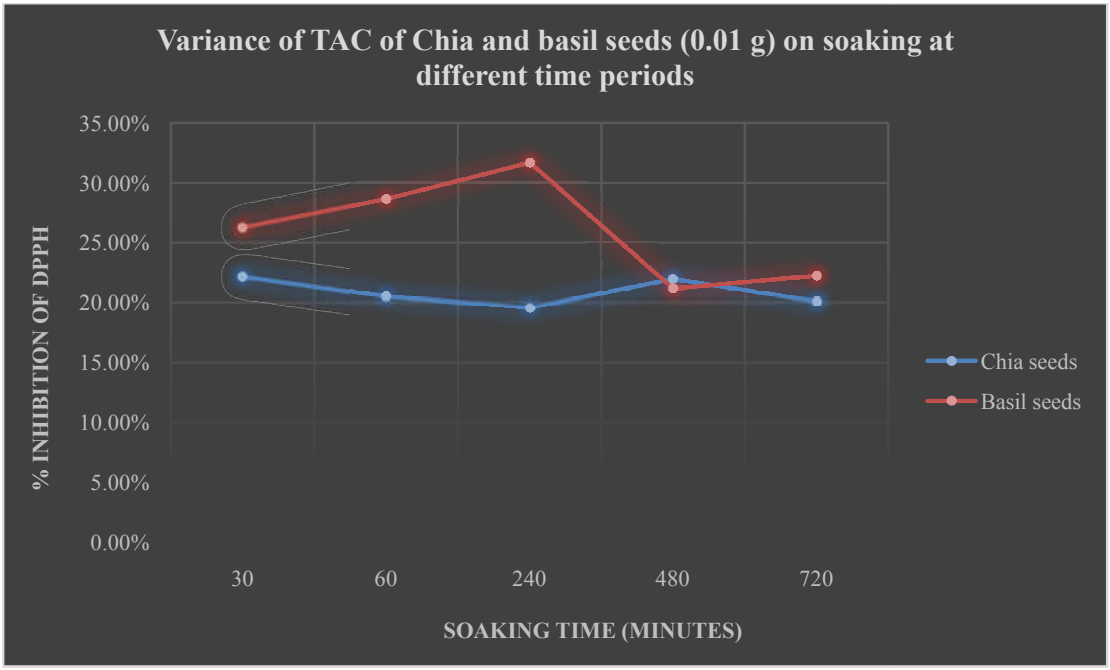

Fig. 10. Comparative analysis of TAC of soaking time of chia and basil seeds of concentration 0.01g

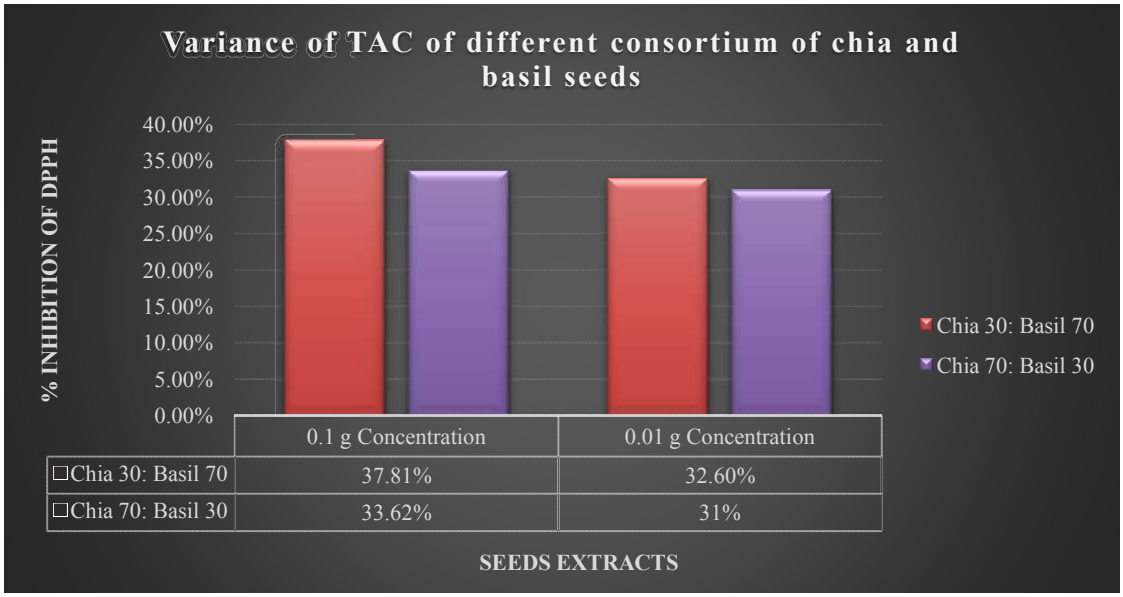

Fig. 11. Variance in the TAC of different consortium of chia and basil seeds in different concentrations

4. RESULTS AND DISCUSSION

Through this study, we were able to determine the variance in Total Antioxidant Capacity (TAC) of the two seeds, when they were subjected to different treatments. While in the untreated samples (not toasted or soaked) of the two seeds, basil seeds showed a significantly higher TAC than chia seeds, by 3.1%. This could mean that basil seeds have more antioxidants compared to chia seeds. Basil seeds are also easier to crush than chia seeds, which are

smoother, making them harder to crush. This reason could have impacted the low TAC values of chia seeds too.

On toasting, the TAC went higher in both the seeds. In chia seeds, the capacity increased by 4.2% in the 0.1 g concentration of the seed extract and 3.28% in the 0.01 g concentration of the seed. Meanwhile, in basil seeds, the concentration went higher than that of chia seeds, at 10.22% for 0.1 g and 5.6% for 0.01 g concentrations. There was a difference of 6.02% and 2.32% in 0.1 g and 0.01 g concentration of the extract. This can be attributed mostly to the activity of heat to lyse the cells and helping the leakage of more cellular contents. Toasting made both the cells rougher, making it easier to grind the cells to a finer powder. These two reasons could be the cause of increased TAC.

The other treatment subjected on to the seeds was soaking. Soaking is the most common way these seeds are consumed. On soaking, these seeds swell and produce a mucilaginous covering which is polysaccharide in nature. The seeds were soaked for 5 different time periods- 30 minutes, 1 hour, 4 hours, 8 hours and 12 hours. It was observed that, the longer they were soaked for, the more water they tended to absorb, making the ground paste more viscous.

As it can be seen in Fig. 9, for chia seeds, the 0.1 g concentration the TAC increased and showed a peak TAC at 240 minutes or 4 hours at 33.03%, after which there is a steady decline in the TAC. In the 0.01 g concentration, however, in Fig. 10 the TAC was highest at 30 minutes at 22.2% after which, the graph was inconsistent. On the other hand, TAC for basil seeds was highest at 240 minutes for both 0.1 g and 0.01 g, at 33.27% and 31.66% respectively as shown in Figs. 9 and 10. This could either be a direct result of soaking for extended period of time, or that it makes the seeds easier to grind and extract the antioxidants from.

The results for consortium study showed the highest TAC of all treatments subjected on the seeds. We tried this to see if the components in the two seeds, when mixed, had any effect on the TAC and the results were positive. The total mixture was 1 g to keep the weight constant. As shown in Fig. 11, the chia: basil ratio of 30:70 showed the highest TAC at 37.81% inhibition of DPPH (17.54% more than untreated samples), compared to chia: basil ratio of 70:30 where % inhibition was 32.6% (13.35% more than untreated samples). The higher concentration of basil in the consortium gave the highest TAC.

Agglutination, in cell biology, means the clumping of cells. It is the basis of many medical diagnostic tests performed, whether it is a simple blood group determination or to detect and treat cancers. Lectins are one of the major agglutinins used in various field of research today. They are proteins that have the characteristics of binding to polysaccharides and polysaccharide-containing proteins with high specificity. They are of great importance in the medical and general biological fields, used for blood typing, bacterial typing, bone marrow transplantation, characterizing polysaccharides and glycoproteins, biomarkers for cancerous cells, molecular architecture of cells, affinity chromatography, affinity electrophoresis, blotting etc. Phytolectins are the lectins derived from plants. They are the largest and best-characterized group compared to animal and bacterial lectins. The second set of experiments were conducted to test for the presence of Phytolectins in the seed extracts of chia and basil. They were performed by looking for hemagglutination.

In a previous study done, the methanolic extracts of *Ocimum basilicum* did not show agglutination, therefore, the choice of solution for extraction of agglutinins from the seeds was selected to be the same as the one in which the RBCs were suspended i.e. PBS. After providing ideal conditions of incubation at 37°C for 30 minutes, there was visible agglutination on the slides. On closer observation under a compound microscope, RBCs could be seen clumped or agglutinated throughout the slides as compared to a positive control, in all the three blood groups tested- A+. B+ and O+. Both basil and chia sees extracted in PBS showed agglutination. This proves the presence of lectins in both chia and basil seeds.

5. CONCLUSION

Through this study, we found that basil seeds, in general, had higher Total Antioxidant Capacity (TAC) than chia seeds. It was also seen that on treatment, TAC of chia and basil seeds increased significantly when compared to untreated seeds. The highest TAC was seen when chia and basil seeds were mixed in fixed proportions, and when the seeds were soaked. Soaking, again, varied TAC depending on how long the seeds were soaked for. We were also able to confirm the presence of Phytolectins in both chia and basil

seeds by performing haemagglutination tests. When the RBCs were made to react with PBS extracts of the two seeds for half an hour, there was both visible agglutination and microscopic clumping of RBCs. Further studies with different methods like OxHLIA and TBARS are required to assess the cause of variance, to perform haemagglutination activity titrimetrically and to isolate and characterize the phytolectins from the seeds.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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