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# Stability of Phytochemicals in Pumpkin Seeds and Sunflower Seeds Using Ethanol Extraction

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Pumpkin seeds (*Cucurbita moschata*) and sunflower seeds (*Helianthus annuus*) are underutilised as food in Malaysia. Benefits of seeds are often overlooked. This research showed that 95% ethanol extract of pumpkin seeds exhibited greenish colour, while 95% ethanol extract of sunflower seeds extract exhibited yellowish colour. Crude fat analysis (Soxhlet) contained 29.53±0.58% in pumpkin seeds and 27.74±0.31% in sunflower seeds. Crude protein was significantly higher in pumpkin seeds (36.69±1.26%) compared to sunflower seeds (12.03±0.14%). FTIR spectroscopy revealed functional groups such as hydroxyl, nitrile, nitric oxide in 95% ethanol extract for both seeds compared to 50% ethanol extracts and powdered forms. ICP-OES indicated that potassium was abundant (4408.20±0.00 mg/100g) and copper least (3.42±0.00 mg/100g) in pumpkin seeds. Sunflower seeds contained 3665.85±47.46 mg/100g potassium and 6.78±0.03 mg/100g copper. Alkaloid content was significantly higher in 95% ethanol extract of sunflower seeds (5.01±0.10 mg/g) compared to 50% ethanol extract (4.60±0.16 mg/g). Pumpkin seeds, 95% and 50% ethanol extracts contained 3.16±0.10 mg/g and 3.04±0.10 mg/g) of alkaloids, respectively. The 95% ethanol extracts had higher tannin content, with pumpkin seeds containing 0.91±0.008 mg CE/mL for pumpkin seeds and sunflower seeds (0.81±0.003 mg CE/mL), compared to 50% ethanol extracts. This research aligns with sustainability approach and SDG 12 framework.

**Keywords:** *Cucurbita moschata*; *Helianthus annuus*; ethanol extraction; minerals; FTIR; colorimetry

## I. INTRODUCTION

The pumpkin seeds market in Malaysia is valued at about 70 million and is projected to rise to USD 110 million by 2028 (ScopeGrid Analytics, 2025). Pumpkin is widely known for its fruit and seed, which can be eaten raw or processed to make baked goods, soups, purees, jams and pastries (Yang *et al.*, 2022). Singh and Kumar (2023) stated that every part of the pumpkin can be used. The edible part of a pumpkin consists approximately 78.69% flesh, 17.95% peel and 3.63% seeds. According to Mandal and Mishra (2020), pumpkin and sunflowers seeds are a nutrient-dense powerhouse. Super seeds are a plant-based source of important minerals and amino acids in vegetarian and vegan diet such as calcium, zinc, copper and magnesium. Super seeds include pumpkin and sunflower seeds. The seeds have a rich, nutty flavour and chewy texture. Pumpkin seeds are consumed in roasted and salted forms, used in bakery industries and are gaining popularity as a healthier alternative to fried snacks. Pumpkin seeds also have been utilised for extracting edible oil since they contain a lot of minerals and bioactive compounds. They have a natural protein content of 25-37% and an oil content of 37-45%. Pumpkin seed is a good source of fibre and amino acids. Pumpkin seeds were proven to alleviate anaemia due to rich source of nonheme iron, especially among adults (Ray & Singh, 2023).

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Meanwhile, sunflower seeds have become more well-known as they are popular snack by Malaysians. In 2024, the revenue for sunflower seeds in Malaysia is USD 9.2 million (Data Bridge Market Research, 2024). The seeds are gaining increasing importance as an edible oil and protein source, with comparable nutritional quality to soybeans and other oilseed proteins (Tenyang *et al*., 2021). Ray and Singh (2023) stated that sunflower seeds are high in polyunsaturated fatty acids (PUFAs) which can help lower the total cholesterol hence reducing the risk of cardiovascular disease. Rasheed *et al*. (2023) found that the combination of seeds (pumpkin and sunflower), contains high levels of trace minerals, antioxidants, omega 3 and omega 6 fatty acids, protein and carbohydrates and these nutrients support normal progesterone in females.

There is a need to utilise natural food sources that contain essential nutrients and bioactive compounds to promote a healthy lifestyle particularly in line with United Nation Sustainable Development Goals (SDGs) of Good Health and Well-Being (SDG3). COVID-19 have impacted daily lives of global population. Cross-sectional survey conducted in year 2020 indicated that 759 Malaysians adopted healthier eating habits (Ahmad *et al*., 2022). Yuan *et al*. (2023) stated that older individuals consume plant-based diets can lower their risk of developing chronic illnesses such as diabetes, heart disease and stroke as well as reduce overall death rates.

Mounes *et al*. (2024) stated that pumpkin seed cake possesses strong antioxidative qualities and increased enzyme activity such as superoxide dismutase, catalase and glutathione peroxidase. According to Oh *et al*. (2024), pumpkin seed extract contributes an antioxidative role in cardiovascular disease, triacylglycerol accumulation in the body and dysfunction in energy generation by reducing oxidative stress brought on by menopause and aging. In cookie production, the combination of mocaf flour and pumpkin flour (77.5%:22.5%) respectively, show greatest antioxidant activity by using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Anastasia Wheni Indrianingsih *et al*., 2024). According to Gao *et al*. (2022), pumpkin seed meal is effective in creating a sturdy, flexible and edible texture with antioxidant properties. Pumpkin seeds are not only used in bakery products but can also be used in meat as preservatives. Improved sensory qualities was noted when 2% crude pumpkin seed extract added into minced meat (Gadekar *et al*., 2025). According to Abbas *et al*. (2024), sunflower sprouts have higher antioxidant activity than sunflower seeds due to the increased levels of total phenolic, melatonin and total isoflavones during the sprouting process.

Various health risks in Malaysia such as non-communicable disease as well as malnutrition could be addressed by implementing the plant seeds in daily diet (Aziz *et al*., 2023; Patel *et al*., 2024; Cheenam & Leena, 2016). Although pumpkin seeds and sunflower seeds are underutilised as food ingredients and functional food in Malaysia, they could offer solutions for these problems. However, there is very little research using these seeds in terms of supporting public health in Malaysia. This study contributes to promoting the consumption of underutilised seeds in Malaysia with their nutritional composition and potential therapeutic effects. These seeds can be further developed into functional food products or commercialisation of pharmaceutical products that can act as immunity booster for public health. This paper aims to determine the physical characteristics of pumpkin and sunflower seeds, their nutritional composition such as fat, protein, minerals analysed using ICP-OES and chemical characteristics such as alkaloids and tannins as well as functional groups using FTIR method. The efficiency of solvent extraction was studied using 95% and 50% ethanol as some studies stated mixture of alcohol and aqueous could improve the efficiency of extraction.

## II. MATERIALS AND METHOD

### *A. Materials*

Pumpkins (6 kg) were purchased from a local supermarket located at Seksyen 7, Shah Alam, Selangor, Malaysia. The fruits were peeled and the seeds were detached from its flesh. As for sunflower seeds, 1 kg of sunflower seeds were purchased from Jalan Timur Laut, Georgetown, Pulau Pinang, Malaysia.

### *B. Sample Preparation and Extraction*

Firstly, the seeds were cleaned under running tap water to avoid surface contamination and rinsed. Then, the pumpkin and sunflower seeds were oven-dried at 60 °C for approximately 4 hours. The dried pumpkin and sunflower seeds were ground into fine powder using an electric grinder (Khind, model BL1012, Malaysia) for 10 minutes and kept in an airtight plastic container for further analysis (Danbature *et al.*, 2013). Solvent extraction has been carried out using the maceration method described by Gowtham *et al.* (2022). Seed powder (5 g) extracted with 100 mL 50% ethanol and 95% ethanol using a shaker set at 200 rpm, at 30 ℃ for 24 hours. The extracts were filtered using Whatman No.1 filter paper and concentrated using a rotary evaporator at 40 ℃ to obtain ethanolic extracts. The extracts were stored at 4 ℃ for further analysis (Jane Monica *et al.*, 2022).

### *C. Physical Analysis*

#### *1. Colour measurements*

Colour measurement of pumpkin seeds and sunflower seeds followed the method proposed by Rana *et al.* (2020) as mentioned by Razzak *et al.* (2023). The colour of samples was determined by using a colorimeter. The colorimeter was calibrated before measurement was taken. Ground seed samples were placed on a glass plate to test colour values (H, L*, a*, b* and C*). Same procedures were repeated using the solvent-extracted samples. Colour in terms of lightness or darkness was represented by the L* value. Black is denoted by L*=0 and white by L*=100. On the other hand, redness or greenness was indicated by its a* value, where a* > 0 denotes redness and a* < 0 denotes greenness. Yellowness or blueness was represented by its b* value, where b* > 0 denotes yellowness and b* < 0 denotes blueness. Each measurement was done three times and average of the result for each sample was calculated.

### *D. Chemical Analysis*

#### *1. Determination of fat*

The method to estimate the fat content in the seeds followed the method described by Danbature *et al.* (2013) with a slight modification. 2 g of pumpkin seeds and sunflower seeds samples, ($W_1$) were placed on a filter paper and put into the Soxhlet extractor for extraction into a pre-weighed round-bottom flask with low-boiling petroleum ether (40 °C - 60 °C) using a Soxhlet extractor for 8 hours. The flask and its content were placed in a steam bath at 95 °C until no steam is observed. Finally, the flask was heated at 105 °C in an oven for 1 hour and cooled in a desiccator and weighed. The process of heating and cooling was repeated until a constant weight was obtained.

$$Fat\ content\ (\%) = \frac{Weight\ of\ fat\ materials\ (g)}{Weight\ of\ samples\ (g)} \times 100\%$$

$$Weight\ of\ fat\ materials = Weight\ of\ (fat + flask) - Weight\ of\ flask$$

#### *2. Determination of protein*

The determination of protein in the seeds followed the method by Magomya *et al.* (2014). 0.6-0.8 g of the powdered sample was digested in a Kjeldahl digestion flask by boiling it with 20 mL of concentrated $H_2SO_4$ and 2 pills of Kjeldahl digestion tablet (selenium catalyst) until the mixture becomes clear. The samples were allowed to cool. Before the distillation, the pH meter was calibrated. In the distillation unit, 20 mL of 2% boric acid was added into the flask. The tube was placed securely. 50 mL of deionised water was added to the digestion tube and 80 mL of 2% of NaOH added to the digestion tube by pressing the NaOH button. The blank (sulfuric acid + catalyst) was placed in the distillation unit and titrated with 0.1 M HCl followed by the samples. The volume of HCl used for sample and blank was recorded. The amount of protein has been calculated as follows:

$$Total\ nitrogen\ (g) per\ 100\ g\ of\ food = \frac{(Titre - Blank) \times 1.4 \times 100}{1000 \times sample\ weigh}$$

Crude protein (g/100 g) = Total nitrogen × conversion factor for food sample analysed
Conversion factor:
Pumpkin = 6.25 (Danbature *et al.*, 2013)
Sunflower = 5.30 (Li *et al.*, 2024)

#### *3. Determination of functional groups by using FTIR*

The determination of functional groups in pumpkin seeds and sunflower seeds was carried out using Fourier-Transformation Infrared spectroscopy (FTIR) (Perkin Elmer, Spectrum One) following the method described by Gowtham *et al.* (2022) using the 50% ethanol and 100% ethanol seed extracts. Solid samples were crushed into a thin pallet using an agate mortar, followed by milling with potassium bromide (KBr). The samples were analysed between 4000-450 cm$^{-1}$.

*4. Determination of minerals by using ICP-OES*

The dried sample was placed in a muffle furnace at 550 °C for 3 hours. After that, the ash samples were taken from the furnace and allowed to cool in desiccators provided with a desiccant agent. The acid digestion method was used to determine elemental constituents. The ash samples were digested with 20 mL of 65% of $HNO_3$ for 24 hours at room temperature (25 °C) followed by boiling at 100 °C until the red fumes disappeared. After that, 2 mL of 35% hydrogen peroxide, $H_2O_2$ was added. After cooling, the samples were filtered using ashless filter paper until a clear solution is obtained. The solution was subsequently transferred to a 100 mL plastic volumetric flask and diluted with deionised water by taking 4.61 mL of the stock solution. The elements analysed using an ICP-OES were Na, K, Ca, Mg, Fe, Zn and Cu according to Shajan *et al.* (2024) and Liu *et al.* (2013) for pumpkin seeds and sunflower seeds respectively. The multi-element standard (Sigma Aldrich, St Louise, MO, USA) was prepared by taking 10 mL of each element and mixing it in 100 mL of deionised water.

## E. Phytochemical Composition

*1. Determination of alkaloids content*

This was done by the alkaline precipitation gravimetric method according to Enujiugha *et al.* (2014). A volume of 0.2 mL extracts was dispersed into 50 mL of 10% glacial acetic acid solution in 95% ethanol. 10 drops of 30% ammonium hydroxide solution were added into it, and it was allowed to stay for 4 minutes. It was then placed on the hot plate to concentrate the solution at 50 °C. 10 drops of 30% ammonium hydroxide were added again and filtered using a weighted filter paper, $W_0$. The weight of filter paper after filtration was recorded, $W_1$. The filter paper with the residue was dried in the oven at 80 °C for 10 minutes and then it was allowed to cool in the desiccator for 10 minutes. The weight of filter paper after drying was recorded, $W_2$. The alkaloid content was calculated and expressed as a concentration (g/mL) of the volume of sample analysed.

$$Alkaloid\ (mg/g) = \frac{Weight\ of\ wet\ residue\ (W_3) - Weight\ of\ dried\ residue\ (W_4)}{Volume\ of\ sample\ (mL)} \times 100\%$$

$$W_3 = (Weight\ of\ filter\ paper\ +\ wet\ residue, W_1) - Weight\ of\ filter\ paper, W_0$$

$$W_4 = (Weight\ of\ filter\ paper\ +\ dried\ residue, W_1) - Weight\ of\ filter\ paper, W_0$$

*2. Determination of tannin content*

Tannin content was measured using the method described by Akullo *et al.* (2023) with slight modifications. Tannins were measured using a modified vanillin-hydrochloric acid method. Catechin hydrate was used to make standards at concentrations of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL ($R^2$= 0.9650). 1 mL of each sample extracts and standards were placed in test tubes. To the blank, 5 mL of 4% HCl in methanol without the reagent (vanillin) was added. Sample and standards were treated for 20 minutes with 5 mL of vanillin-HCl reagent (made by mixing the equal volumes of 4% HCl in methanol and 1% vanillin in methanol). The absorbance of the blank, standard and sample were measured at 500 nm and tannin content was estimated as concentration catechin equivalent (CE) using a standard calibration curve derived from catechin absorbance at various concentrations.

## F. Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 29.0 version was used for the analysis of data. The p-value of less than 0.05 was considered as significant. An independent t-test was applied to compare pumpkin seeds and sunflower seeds without the use of two different concentrations of seeds extract as well as to compare the concentration of extract within the same seeds. In order to analyse the differences in colour characteristics, one-way Analysis of Variance (ANOVA) was applied followed by the Duncan post-hoc test (Shoeb, 2016).

# III. RESULT AND DISCUSSION

## A. Physical Analysis

*1. Extraction method efficiency*

The yield of each solvent extraction is shown in Table 1. Pumpkin seeds that were extracted with 95% ethanol resulted higher yield (45.69%) compared to 50% ethanol extract (23.88%). Similarly, sunflower seeds extracted with 95% ethanol was significantly higher (40.33%) compared to 50% ethanol extract (20.12%). Conventional solvent extraction method uses organic solvent but varying many factors such as temperature, stirring, time, pH and heat. However, this method utilises large amount of solvent and time consuming. Several studies have reported differences in yield according to extraction method. Pumpkin seed extracted using chloroform and methanol (2:1) resulted in 440-524 g/kg fresh weight (Kim *et al.*, 2012). Pumpkin seeds extracted using microwave assisted extraction using hexane (1:10) for 5-30 minutes (200 W) resulted in 51-62.5% (Hernandez-Santos *et al.*, 2016). The extraction yields of pumpkin seed oil were 48.99% for UAE (50 °C, 100 W) and 30.28% for pressurised ethanol extraction (50 °C, 6 mL/min, 50 bar) (Amarante *et al.*, 2025). UAE offers extraction of desired constituents by varying ultrasonic power, time, solvent to solid ratio, temperature and sample size. These variations may influence the depth of cavitation and disruption of pumpkin seed cell walls (Amarante *et al.*, 2025). Meanwhile, pumpkin seeds extracted using microwave assisted extraction using ethanol water (100-150 °C, 20 min, 2.45 GHz) resulted phenolic compounds 35-84 mg GAE/g dry weight (Stupar *et al.*, 2021). Microwave assisted extraction for pumpkin seed oil using hexane (5-30 min, 200 W, 1:10 g/mL) yielded 51-62.5% (Hernandez-Santos *et al.*, 2016). Microwave energy combined with effective magnetic stirring allows swift extraction to below 30 minutes while minimising solvent usage. Meanwhile, microwave roasted sunflower seeds resulted in higher phenolic compounds (895.6 mg/100 g) compared to boiling method (598.5 mg/100 g) (Kiczorowska *et al.*, 2019). Various extraction techniques are employed to plant seeds; however, extraction efficiency depends on many factors including analyte to be extracted and instrumental parameters.

Table 1. Extraction yield for pumpkin seeds and sunflower seeds

| Samples | Extract | Yield (%) |
|---|---|---|
| Pumpkin seeds | 50% ethanol | 23.88[a] |
| | 95% ethanol | 45.69[a] |
| Sunflower seeds | 50% ethanol | 20.12[b] |
| | 95% ethanol | 40.33[a] |

Note: Values are the means ± standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$.

*2. Colour measurement*

As indicated in Table 2, the colour for both seeds was expressed as L*, a* and b* values. Based on the data, the powdered pumpkin seeds had significantly higher lightness (L* value) compared to the fresh pumpkin seeds as well as extracts of 50% and 95% ethanol respectively. This indicated that the powder and fresh seeds were lighter, while the pumpkin seeds extracts were darker as shown in Figure 1. Both pumpkin seeds and sunflower seeds with 95% ethanol extract had the lowest value of a*, indicating a greenish colour. Pumpkin seed extract had a higher -a* value compared to sunflower seeds. As for the sunflower seeds, powdered seeds had the highest b* value followed by 95% of ethanol extract, 50% ethanol extract and fresh seeds. Sunflower seed extracts had a higher value of b*, indicating that they are towards the yellow colour. The 95% of ethanol extract is effectively extracting the colour and pigments (Dan *et al.*, 2009). Pigments from coloured plants can be useful for many applications in food, pharmaceutical and textile industries. Artificial colours in the food business have increased as they are more stable and have versatile colours (Nor-Aleesya *et al.*, 2023). However, despite current studies into synthetic chemicals, natural pigments have emerged in response to recent findings about the toxicity and adverse consequences of several synthetic dyes (Novais *et al.*, 2022). Food industries have begun replacing artificial colours with natural colourants because they have both pigmentation and health benefits. Plant pigments considered to be valuable as colourants (Ebrahimi *et al.*, 2023). Sunflower seeds might be associated with yellow pigments as it exhibits a higher b* value in their extract compared to pumpkin seeds. Xanthophylls and carotenes contributes to the yellow pigment in sunflower seeds (Kiczorowska *et al.*, 2019). Chloroplasts mainly responsible to contribute for greenish colour in pumpkin seeds (Boujemaa *et al.*, 2020). (Cvitković *et al.* (2021) found that the highest concentration of chlorophyll a and chlorophyll b were found in ethanol extracts.

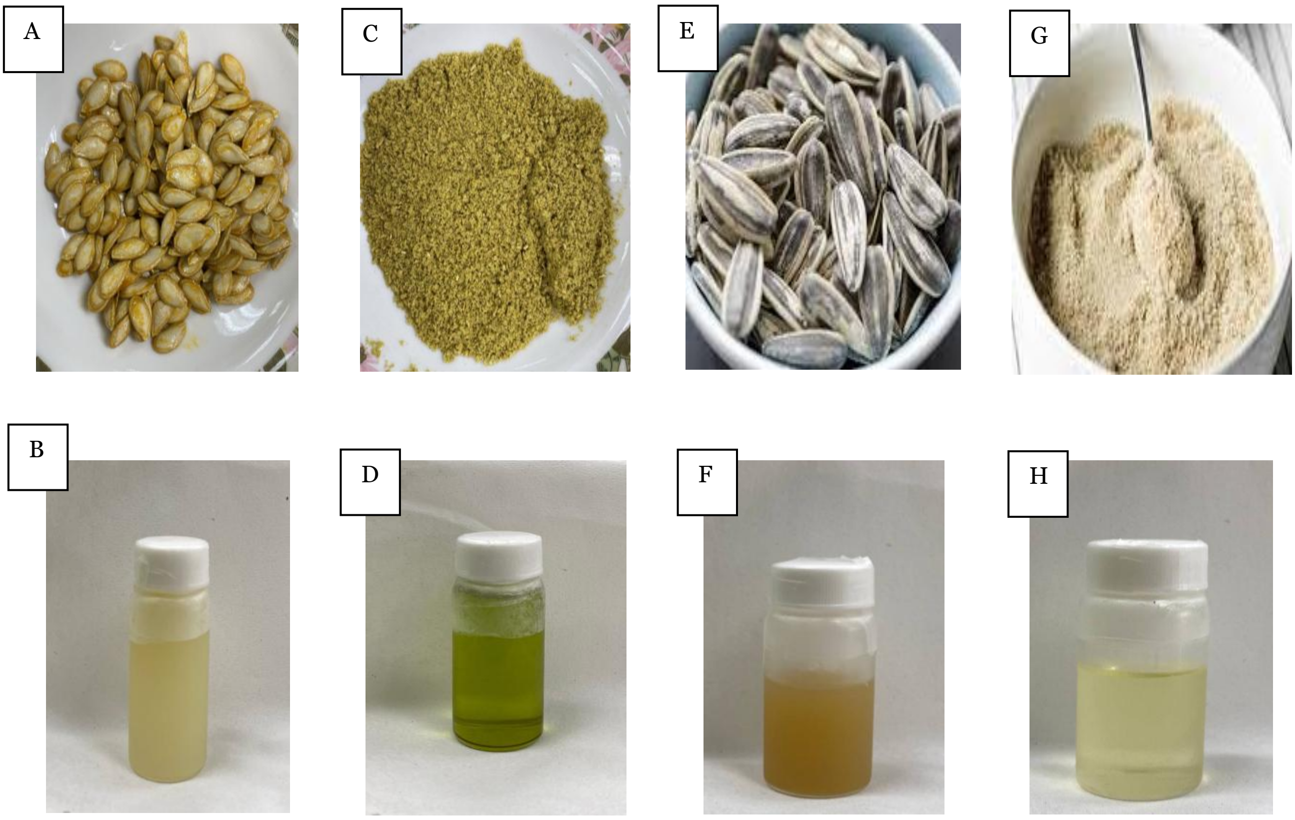


Figure 1. Images indicating (a) pumpkin seeds, (b) pumpkin seeds 50% ethanol extract, (c) pumpkin seeds powder, (d) pumpkin seeds 95% ethanol extract, (e) sunflower seeds, (f) sunflower seeds 50% ethanol extract, (g) sunflower seeds powder and (h) sunflower seeds 95% ethanol extract.

Table 2. Colour measurements for pumpkin seeds and sunflower seeds

| **Samples** | | **L*** | **a*** | **b*** |
|---|---|---|---|---|
| Pumpkin seeds | Fresh seeds | $65.46\pm0.36^{bA}$ | $3.37\pm0.35^{aA}$ | $17.82\pm0.13^{bA}$ |
| | Powdered seeds | $69.17\pm0.40^{aA}$ | $-0.92\pm0.03^{bB}$ | $26.46\pm0.31^{aA}$ |
| | 50% ethanol extract | $39.62\pm0.14^{cA}$ | $-1.90\pm0.16^{cB}$ | $1.94\pm0.17^{dB}$ |
| | 95% ethanol extract | $30.15\pm0.36^{dB}$ | $-3.48\pm0.15^{dA}$ | $8.66\pm0.24^{cB}$ |
| Sunflower seeds | Fresh seeds | $56.75\pm0.50^{bA}$ | $1.52\pm0.35^{bB}$ | $6.80\pm0.51^{cB}$ |
| | Powdered seeds | $69.10\pm0.10^{aA}$ | $2.92\pm0.03^{aA}$ | $15.40\pm0.14^{aB}$ |
| | 50% ethanol extract | $30.76\pm0.57^{dB}$ | $0.43\pm0.07^{cA}$ | $4.80\pm0.12^{dA}$ |
| | 95% ethanol extract | $42.38\pm0.78^{cA}$ | $-3.63\pm0.14^{dA}$ | $14.08\pm0.48^{bA}$ |

Note: Values are the means ± standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$. Capital letter indicates the effect of seeds at the specific conditions on the colour (A-B). Small letters indicate the effect of seed conditions on the colour (a-d).

### *B. Chemical Analysis*

#### *1. Determination of fat*

The results of fat analysis of pumpkin seeds and sunflower seeds are presented in Table 3. The crude fat content was determined using Soxhlet extraction method for both plant seeds. The fat content analysis revealed that pumpkin seeds had a significantly higher amount of crude fat (29.53%) compared to sunflower seeds (27.74%). Different species of pumpkin seed oils contains high levels of unsaturated fatty acids (Boujemaa *et al*., 2020). Infrared radiation resulted in higher fat content compared to boiled and microwave roasted sunflower seed (Kiczorowska *et al*., 2019). Pumpkin seeds are rich in bioactive compounds including unsaturated fatty acid, promoting its health benefits towards cardiovascular disease. The confectionary sunflower seeds are widely applied in bakery products. Sunflower seed extract shows a positive effect on reducing body fat in adults with obesity. Kim *et al*. (2024) mentioned that supplementation with sunflower seeds extract not only resulted in a significant reduction in body weight gain and adipose tissue, it also inhibited adipogenesis and promoted lipolysis and thermogenesis. This result differs with studies from Polyzos *et al*. (2024) that found 42.74 g/100g dw fat in pumpkin seeds. Meanwhile, Petraru *et al*. (2021) reported that sunflower seed contained 65.42% fat, 32.50% fat in kernel and 8.81% in its hull. Their nutritional values are influenced by various factors such as genotype, soil characteristics, agricultural practices, climatic conditions and processing conditions.

Table 3. Crude fat content of pumpkin seeds and sunflower seeds

| Samples | Crude fat content (%) |
|---|---|
| Pumpkin seeds | $29.53 \pm 0.58^{a}$ |
| Sunflower seeds | $27.74 \pm 0.31^{b}$ |

Note: Values are the means ± standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$.

#### *2. Determination of protein content*

The protein content of protein seeds and sunflower seeds are presented in Table 4. The Kjeldahl method was used to calculate the crude protein content of pumpkin seeds and sunflower seeds using a conversion factor 6.25 for pumpkin seeds and 5.30 for sunflower seeds according to the Food Regulations 1985, Regulations 18B. Although the amount of protein in plant seeds is not comparable to the other source of protein such as fish, eggs, poultry or meat, research has shown that they have a high protein content among fruits and vegetables.

From Table 3, pumpkin seeds had a significantly higher crude protein content compared to sunflower seeds. The crude protein in pumpkin seeds was 36.69%. Findings from Sinkovič *et al.* (2021) reported the protein content in pumpkin seeds ranged from 38.3% to 39.5% among different pumpkin cultivars. The difference due to geographic location, cultivars could also contribute to differences in protein content. Sunflower seeds had a protein content of 12.03%. Protein content in raw sunflower seed was higher (244.5 g/kg) compared to boiling, infrared and microwave roasted sunflower seeds (Kiczorowska *et al*., 2019). Previously, Petraru *et al.* (2021) found 33.85% protein content in sunflower seeds, 23.73% in kernel and 7.82% in its hull. Barsby *et al.* (2021) reported that sunflower seeds contained 21% of protein using the Qubit Fluorometric Protein Assay Kit and used aqueous extract of sunflower seeds. Nzotta and Onabanjo (2021) found 29.83% protein in pumpkin seed. Nicoleta *et al.* (2024) stated that the pumpkin by-products, especially pumpkin seeds, are rich in protein. This bioactive compound exhibits diverse biological activities such as antihypertensive, antioxidant, antibacterial and immunomodulatory effects. Protein as a primary metabolite of pumpkin seeds play a crucial role in disease prevention and improvement in human health (Patel *et al*., 2023). A study from Sá *et al.* (2021) revealed that pumpkin seeds contain numerous essential amino acids such as histidine, isoleucine, leucine, lysine, threonine, valine, methionine as well as cysteine. Each amino acid is the building block for a human body that makes up the enzymes which initiates many chemical reactions for usual body homeostasis. Proteins originating from plant-based food contains lower total and LDL cholesterol compared to diet with red meat (Mounes *et al*., 2024).

One of the health benefits of pumpkin seeds is its antidiabetic property. The hypoglycaemic effects of protein isolate from various pumpkin seeds varieties within the Cucurbitaceae family including *Cucurbita moschata* cultivar have been reported (Syed, 2019). In addition, Batool *et al.* (2022) stated that pumpkin seeds protein isolate had the potential to reduce the level of aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), and lactate dehydrogenase (LD), indicating its potential benefits in managing liver dysregulation, as well as enhancing antioxidant activities. Furthermore, protein-rich food also contains tryptophan that acts as a precursor of serotonin and melatonin. Arora *et al.* (2023) stated that pumpkin seeds are a natural source of tryptophan. Although serotonin cannot cross the blood-brain barrier, the synthesis of serotonin in the central nervous system can be influenced by a diet rich in tryptophan. This kind of diet may serve as a protective factor against depression and is positively associated with improving social cognition (Storz, 2021).

Although the protein content of sunflower seeds is comparatively lower than pumpkin seeds, sunflower seeds is widely utilised in food products especially bakery products such as bread, pancakes, cookies as well as brownies. According to de Oliveira Filho and Egea (2021), sunflower protein isolates are free from toxic substances and possess lower levels of antinutritional factors compared to other protein-rich sources. Thus, utilising agro-industrial byproducts for protein production represents a sustainable approach that enhances the commercial value of these byproducts. The advantages include producing valuable chemicals at cheaper cost, biomass reuse, bioproducts, wastewater treatment and reduced agricultural waste management cost (Benelia *et al.*, 2025; Wan-Mohtar *et al.*, 2023).

Table 4. Crude protein content of pumpkin seeds and sunflower seeds

| **Samples** | **Crude protein (%)** |
|---|---|
| Pumpkin seeds | $36.69 \pm 1.26^a$ |
| Sunflower seeds | $12.03 \pm 0.14^b$ |

Note: Values are the means ± standard deviation of two replicate analyses. Means values with different superscripts are significantly different at $p < 0.05$.

*3. Determination of functional group using FTIR*

FTIR results demonstrate how the ethanol extraction enhances the functional groups linked to the bioactive compound indicating that processed extracts can have even more therapeutic potential. This highlights the value of including sunflower seeds and pumpkin seeds in a balanced diet due to their potential to prevent disease and enhance general health in addition to their nutritional advantages. The functional groups in the powder and ethanol extracts of pumpkin seeds and sunflower seeds were characterised based on absorption using an infrared spectrum and the results are displayed in Figure 2 and Figure 3. The results of the FTIR spectrum assisted in identifying the active components listed in Table 5 and Table 6. From the results obtained, 95% of ethanol extract had the most functional group and the highest transmittance for both seeds compared to the powder and 50% of ethanol extract samples. Rajhard *et al.* (2021) stated that polar solvent and mixture of alcohol and water can extract polar compounds. Hence, since 95% of ethanol extract is less polar, it was able to extract less polar and nonpolar compounds, such as hydrocarbons.

In pumpkin seeds, the prominent peak at 1745.86 $cm^{-1}$, indicated the presence of aromatic rings, pointing to the possibility of polyphenols such as flavonoids and tannins being present. Tannins, which are well-known for their cardioprotective, anti-inflammatory and antioxidant benefits have been associated with a lower chance of developing chronic illnesses like diabetes, cancer and cardiovascular disease (Cosme *et al.*, 2025). The presence of hydrocarbon-based molecules is further supported by the C-H stretching (3000-2840 $cm^{-1}$), which validates the existence of lipid structures that support metabolic health and energy storage. Bikmurzin *et al.* (2022) stated that the lipid region is linked to C-H stretching modes around 3000-2840 $cm^{-1}$. The presence of alcohols and phenols is shown by the broad O-H stretching (3200-3550 $cm^{-1}$), which supports pumpkin seeds' antioxidant capacity. Phenols with one or more aromatic rings and hydroxyl groups serve as antioxidants, disease inhibiting agents as well as colourants (Peng *et al.*, 2021). Both the N-O stretching (1500-1550 $cm^{-1}$) and the C-N stretching (1266-1342 $cm^{-1}$) indicate the presence of proteins and nitrogen-containing substances. Bioactive peptides and proteins (BAPPs) are functional molecules with distinct amino acid sequences that have a role in a wide range of biological activities such as immunomodulation, biocatalysis, signalling pathway activation and inhibition and the control of cellular purpose and behaviour. As a result, they exhibit an excellent potential as treatment agents for a number of illnesses including diabetes, tissue damage, cancer, infection and chronic pain (Hao *et al.*, 2024).

In sunflower seeds, the existence of phenolic and flavonoid compounds is shown by the prominent peak at 1745.26 cm$^{-1}$, which corresponds to C-H stretching in aromatic compounds. Flavonoids which are present in natural dyes, cosmetics and skin care products as well as anti-wrinkle agents have important medical uses such as anticancer, antibacterial, antiviral, antimalarial, antioxidant, neuroprotective, antitumor anti proliferative (Ullah *et al.*, 2020). The C-H stretching (2840-3000 cm$^{-1}$) further confirmed the existence of lipid molecules, indicating the presence of important fatty acids such as linoleic acid. Linoleic acid lowers total and low-density lipoprotein cholesterol when compared with saturated fatty acids and carbohydrates (Jackson *et al.*, 2024). The O-H stretching (3200-3550 cm$^{-1}$) indicated a high concentration of phenolic substances. Numerous scientific studies have proposed that substances produced from plants have biological effects such as preventing disease linked to oxidative stress as these actions have been associated with the polyphenol compounds found in plants (Wongsa *et al.*, 2022). The presence of proteins and polyphenols in the seeds is confirmed by the C=C stretching (1626-1662 cm$^{-1}$) and C-N stretching (1266-1342 cm$^{-1}$) which aids in muscle repair, enzyme activity as well as anti-inflammatory properties. Furthermore, distinct functional groups like nitrile (C≡N at 2222-2260 cm$^{-1}$) and isothiocyanate (N=C=S at 1990-2140 cm$^{-1}$) are seen in sunflower seeds, indicating the presence of sulphur-containing bioactive compounds with detoxifying characteristics and cancer-protective properties.

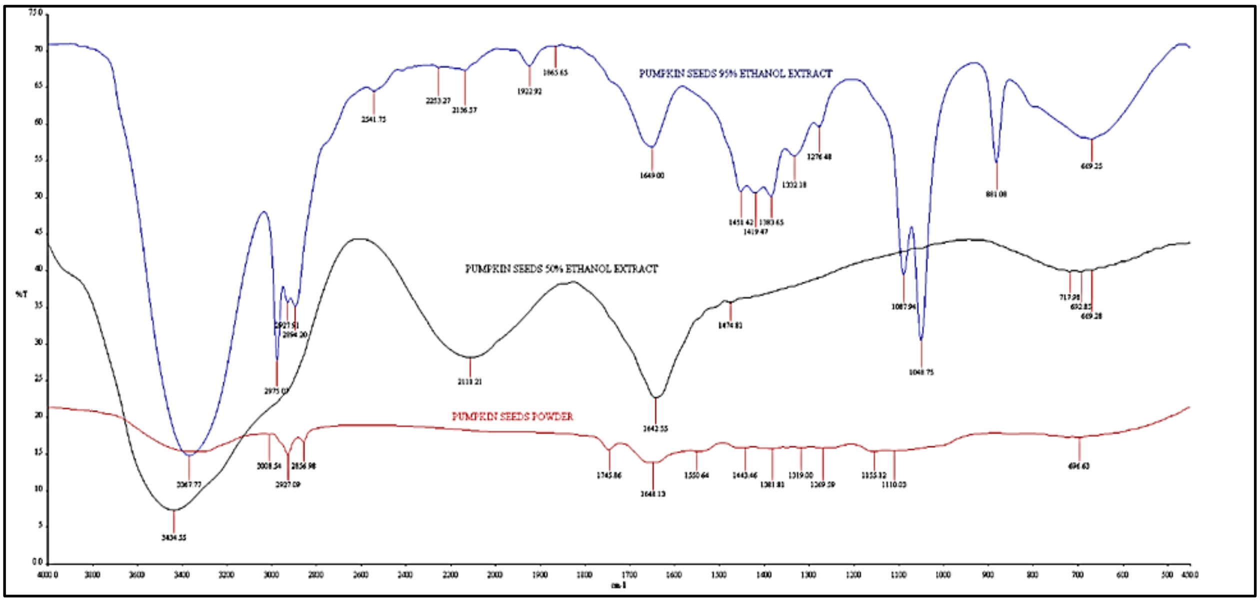


Figure 2. FTIR spectrum of pumpkin seeds.
Red line: Powdered seeds, Blue line: 50% ethanol extract, Black line: 95% ethanol extract

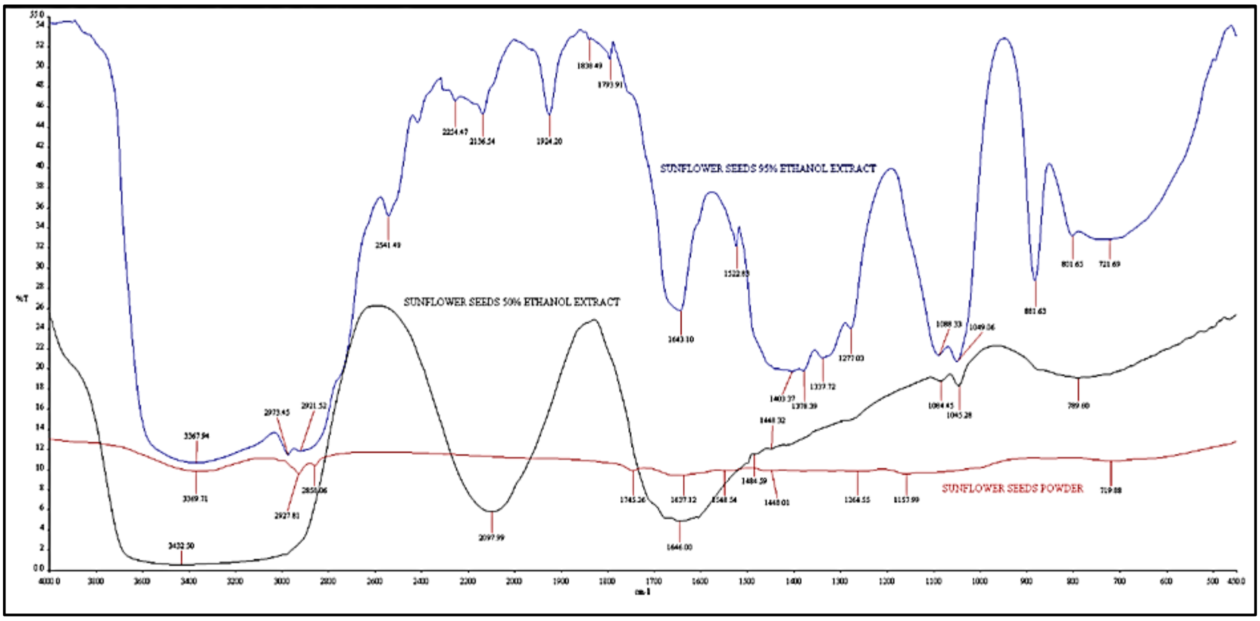


Figure 3. FTIR spectrum of sunflower seeds.
Red line: Powdered seeds, Blue line: 50% ethanol extract, Black line: 95% ethanol extract

Table 5. Peak value, functional group and class for FTIR spectra of powdered and extracts of pumpkin seeds

| Wavenumber (cm $^{-1}$) | Pumpkin seeds | | | Functional group | Class |
|---|---|---|---|---|---|
| | Powdered seeds | 50% ethanol extract | 95% ethanol extract | | |
| 3300-3400 | | | 3367.77 | N-H | Aliphatic 1' amine |
| 3200-3550 | | 3434.55 | | O-H | Alcohol |
| 2840-3000 | | | 2975.07 | C-H | Alkane |
| | | | 2927.91 | C-H | Alkane |
| | | | 2894.20 | C-H | Alkane |
| 2700-3200 | 3008.54 | | | O-H | Alcohol |
| 2500-3300 | 2927.09 | | 2541.75 | O-H | Carboxylic acid |
| | 2856.98 | | | | |
| 2190-2260 | | | 2253.27 | C≡C | Alkyne |
| | | | 2136.57 | C≡C | Alkyne |
| 2100-2140 | | 2111.21 | | C≡C | Alkyne |
| 1900-2000 | | | 1922.92 | C=C=C | Allene |
| 1650-2000 | 1745.86 | | 1865.65 | C-H | Aromatic compound |
| 1648-1658 | 1648.13 | | 1649.00 | C=C | Alkene |
| 1626-1662 | | 1642.55 | | C=C | Alkene |
| 1500-1550 | 1550.64 | | | N-O | Nitro compounds |
| 1450 | 1443.46 | 1474.81 | 1451.42 | C-H | Alkane |
| 1380-1390 | 1381.81 | | | C-H | Phenol |
| 1330-1420 | | | 1419.47 | O-H | Alcohol |
| 1310-1390 | 1319.00 | | 1383.65 | O-H | Phenol |
| | | | 1332.18 | | |
| 1266-1342 | 1269.59 | | 1276.48 | C-N | Aromatic amine |
| 1087-1124 | | | 1087.94 | C-O | 2' alcohol |

| | | | | | |
|---|---|---|---|---|---|
| 1030-1070 | | | 1048.75 | S=O | Sulfoxide |
| 1020-1250 | 1155.12<br>1110.03 | | | C-N | Amine |
| 860-900 | | | 881.08 | C-H | 1,2,4-trisubstituted,<br>1,3-disubstituted |
| 680-720 | 696.63 | 717.98<br>692.85 | | | Benzene derivative |
| 665-730 | | 669.28 | 669.25 | C=C | Alkene |

Table 6. Peak value, functional group and class for FTIR spectra of powdered and extracts of sunflower seeds

| **Wavenumber (cm $^{-1}$)** | **Sunflower seeds** | | | **Functional group** | **Class** |
|---|---|---|---|---|---|
| | **Powdered seeds** | **50% ethanol extract** | **95% ethanol extract** | | |
| 3200-3550 | 3369.71 | 3432.50 | 3367.94 | O-H | Alcohol |
| 2840-3000 | 2927.81<br>2858.06 | | 2973.45 | C-H | Alkane |
| 2700-3200 | | | 2921.52 | O-H | Alcohol |
| 2500-3300 | | | 2541.49 | O-H | Carboxylic acid |
| 2222-2260 | | | 2254.47 | C≡N | Nitrile |
| 2100-2140 | | | 2136.54 | C≡C | Alkyne |
| 1990-2140 | | 2097.99 | | N=C=S | Isothiocyanate |
| 1900-2000 | | | 1924.20 | C=C=C | Allene |
| 1650-2000 | 1745.26 | | 1838.49<br>1793.91 | C-H | Aromatic compound |
| 1626-1662 | 1637.12 | 1646.00 | | C=C | Alkene |
| 1580-1650 | | | 1643.10 | C=C | Alkene |
| 1500-1550 | 1548.54 | | 1522.88 | N-O | Nitro compound |
| 1450 | 1448.01 | 1484.59<br>1448.32 | | C-H | Alkane |
| 1395-1440 | | | 1403.37 | O-H | Carboxylic acid |
| 1330-1420 | | | 1378.39<br>1337.72 | O-H | Alcohol |
| 1266-342 | | | 1277.03 | C-N | Aromatic amine |
| 1250-1310 | 1264.55 | | | C-O | Aromatic ester |
| 1020-1250 | 1157.99 | 1084.45<br>1045.28 | 1088.33<br>1049.06 | C-N | Amine |
| 860-900 | | | 881.63 | C-H | 1,2,4-trisubstituted,<br>1,3-disubstituted |
| 790-840 | | | 801.65 | C=C | Alkene |
| 760-800 | | 789.60 | | C-H | 1,2,3-trisubstituted |

| | | | | |
|---|---|---|---|---|
| 680-720 | 719.88 | | | Benzene derivative |
| 665-730 | | 721.69 | C=C | Alkene |

*4. Determination of mineral using ICP-OES*

The minerals composition of pumpkin and sunflower seeds is presented in Table 7. Minerals are inorganic nutrients that are vital for sustaining life and supporting various physicochemical processes within the body. Minerals can be classified into two categories: macro elements and mineral elements. Macro elements are essential nutrients required by the body in relatively large quantities compared to other minerals as they play a critical role in maintaining overall health and physiological functions (Siddiqui *et al.*, 2014). In pumpkin seeds, potassium exhibited the highest concentration followed by magnesium, calcium and sodium. The amount of concentration of minerals obtained contradicts findings from Elinge *et al.* (2012), however they still found that potassium has the highest concentration in the pumpkin seeds. The slight difference in the mineral content might be due to the pumpkin varieties chosen as well as soil characteristics and climate conditions in certain countries. On the other hand, sunflower seeds contain potassium as the highest amount of mineral, followed by magnesium, calcium and sodium respectively. Petraru *et al.* (2021) in their study found a slightly different amount of mineral content with this study.

From the result obtained, potassium is the most abundant mineral in both pumpkin and sunflower seeds. Hypokalaemia is one of the consequences of electrolyte imbalance due to low potassium levels in the blood and supplemental potassium has been recommended for treating hypokalaemia (Alfano *et al.*, 2021). Aside from potassium, both plant seeds are also rich in magnesium. The magnesium content of the samples was found 2112.99 mg/100g and 1052 mg/100g for pumpkin seeds and sunflower seeds respectively. Storz (2021) stated that magnesium-containing food could enhance sleep quality, prolonged sleep duration. The iron and zinc content in pumpkin seeds were significantly higher than in sunflower seeds. Pumpkin seeds can be processed into pumpkin seed flour through the drying process, this nutrient-rich flour has potential applications as a supplement in food products, notably in bakery items. Hussain (2023) found that biscuits formulated with pumpkin seeds flour, which is rich in iron and zinc could serve as an effective means of enhancing immunity in children. Zinc plays a crucial role for the immune system, contributing to both antiviral and antibacterial properties (Younes, 2024).

Table 7. Mineral composition of pumpkin seeds and sunflower seeds

| **Concentration (mg/100g)** | **Pumpkin seeds** | **Sunflower seeds** |
|---|---|---|
| Calcium, Ca | $518.99 \pm 0.31^{b}$ | $594.12 \pm 0.60^{a}$ |
| Sodium, Na | $114.02 \pm 0.31^{a}$ | $77.99 \pm 2.57^{b}$ |
| Potassium, K | $4408.20 \pm 0.00^{a}$ | $3665.85 \pm 47.46^{b}$ |
| Magnesium, Mg | $2112.99 \pm 2.14^{a}$ | $1052.92 \pm 6.43^{b}$ |
| Iron, Fe | $46.53 \pm 0.30^{a}$ | $21.02 \pm 0.03^{b}$ |
| Copper, Cu | $3.42 \pm 0.00^{b}$ | $6.78 \pm 0.03^{a}$ |
| Zinc, Zn | $41.77 \pm 0.09^{a}$ | $22.32 \pm 0.21^{b}$ |

Note: Values are the means ± standard deviation of two replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$

## *C. Phytochemical Analysis*

*1. Determination of alkaloid content*

The alkaloid contents of sunflower seeds were significantly higher compared to pumpkin seeds (Table 8). In addition, the alkaloid content of sunflower seeds treated with 95% ethanol is significantly higher compared to 50% ethanol. A study from Krisridwany *et al.* (2023) reported that the alkaloid content in pumpkin seeds to be 1.77% using the UV-Vis Spectrophotometer method. A study by Prakash *et al.* (2024) reported that alkaloids were abundant in sunflower seeds through phytochemical screening using the Mayer's test, Wagner's test and Hager's test. Agubosi *et al.* (2022) further reported that sunflower seeds contain 3.86% alkaloids. These differences might be due to the different extraction methods and solvent used. Yan *et al.* (2021) mentioned that alkaloids exhibit antibacterial activity through multiple mechanisms such as suppressing bacterial nucleic acid and protein synthesis, altering cell membrane permeability, causing damage in cell membrane and cell wall, disrupting bacterial metabolism. The presence of small amount of alkaloids in edible plant parts is indicative for their potential to exhibit various activities such as antimalarial properties, anticancer activity and antimicrobial effects (Aziz *et al.*, 2021). Alkaloids also shown to portray the ability to target multiple therapeutic pathways and exhibit strong antiviral activity (Majnooni *et al.*, 2021).

Table 8. Alkaloid content in pumpkin seeds and sunflower seeds in ethanolic extract

| Sample | Solvent | Alkaloid concentration (mg/g extract) |
|---|---|---|
| Pumpkin seeds | 50% ethanol | $3.04 \pm 0.10^{aB}$ |
| | 95% ethanol | $3.16 \pm 0.10^{aB}$ |
| Sunflower seeds | 50% ethanol | $4.60 \pm 0.16^{bA}$ |
| | 95% ethanol | $5.01 \pm 0.10^{aA}$ |

Note: Values are the means ± standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$. Capital letters indicate the effect of seeds at the specific concentration on the alkaloid content (A-B). Small letter indicates the effect of ethanol concentration on the alkaloid content (a-b)

*2. Determination of tannin content*

The results for the tannin contents of both extracts of the two plant seeds are presented in Table 9. Tannin content in pumpkin seed extract ranged from 0.16 to 0.91 mg CE/mL extract, while sunflower seeds extract ranged from 0.21 to 0.81 mg/mL (Table 8). It is worthwhile to note that the tannin content of pumpkin seed and sunflower seed extracts using 95% ethanol were significantly higher compared to 50% ethanol. The tannin content in the pumpkin seeds contradicts with the finding from Nzotta and Onabanjo (2021) that reported 0.01%. However, for sunflower seeds, the total tannin content is supported by the study from Muttagi and Joshi (2020) that reported 30.91 mg/100g tannic acid for sunflower seed variety Confectionery-1 from Bengaluru, India. The differences in results might be due to the different extraction method and the standard used to measure the tannin. Nzotta and Onabanjo (2021) used a mixture of acetone and glacial acetic acid (80:20) to extract the tannin and used tannic acid to estimate the total tannin content, while this study used ethanol at 50% and 95% concentration to extract the tannin compound and catechin hydrate as a standard used. Tannins are categorised into two main types; hydrolysable tannins and condensed tannins or also known as catechin tannins or proanthocyanidins (Smeriglio *et al.*, 2016).

Table 9. Tannin content in pumpkin seeds and sunflower seeds in ethanolic extract

| Sample | Solvent | Tannin concentration (mg CE/mL extract) |
|---|---|---|
| Pumpkin seeds | 50% ethanol | $0.16 \pm 0.002^{bB}$ |
| | 95% ethanol | $0.91 \pm 0.008^{aA}$ |
| Sunflower seeds | 50% ethanol | $0.21 \pm 0.002^{bA}$ |
| | 95% ethanol | $0.81 \pm 0.003^{aB}$ |

Note: Values are the means ± standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$. Capital letters indicate the effect of seeds at the specific concentration on the tannins content (A-B). Small letter indicates the effect of ethanol concentration on the tannins content (a-b).

## IV. CONCLUSION

The colour, nutritional compositions such as fat, protein, minerals analysed using ICP-OES and chemical characteristics such as alkaloids and tannins as well as functional groups using FTIR method of pumpkin seeds and sunflower seeds extracted with 50% and 95% ethanol were determined. From the analysis conducted, the colour of 95% ethanol pumpkin seed extract was greenish and yellowish colour. Pumpkin seeds had significantly higher amount of fat, protein and potassium compared to sunflower seeds. Analysis of the functional group using FTIR revealed that the 95% ethanol extract exhibited the most spectra of the functional group compared to 50% ethanol. Potassium, magnesium, sodium, iron and zinc was found significantly higher in pumpkin seeds compared to sunflower seeds. In terms of alkaloids, 95% ethanol extract of sunflower seeds had the highest alkaloid content compared to the 50% ethanol extract. For tannin analysis, the 95% ethanol extract pumpkin seeds had significantly the highest condensed tannin content compared to 50% ethanol extract.

## V. ACKNOWLEDGEMENTS

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