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Article

Antioxidant Properties of *Sesamum indicum* Seeds using Different Solvents

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Abstract: Sesamum indicum is considered an underutilized oil-bearing seed in the semi-arid regions of Ghana. Nonetheless, it is a promising source of food with both nutritional and therapeutic benefits. The aim of the present study was to evaluate the antioxidant properties S. indicum seeds using different extraction solvents. The seeds were obtained from the local farmers and prepared for analysis. The bioactive compounds present in the seeds were extracted using hexane, ethyl acetate, ethanol and water and their yields quantified. Total phenolic content (TPC), Condensed tannin content (TTC) and Total antioxidant capacity (TAC) , and DPPH radical scaveging assay were analyzed using standard methods. Antinutrients such as saponins, alkaloids, phytates and oxalates were also analyzed from the powdered seeds. Two chemometric methods; hierarchical cluster analysis (HCA) and Pearson correlation were employed to evaluate the interdependence of the various parameters to result in their antioxidant properties. The re-sults revealed that the solvents utilized had a significant impact on the extraction yield, phyto-chemical component concentration, and antioxidant activities. Hexane extracts of S. indicum seeds significantly exhibited the highest antioxidant activity ($p < 0.05$). It was marked with the highest TAC value of 232.6 ± 6.267 mg/g AAE and a strong DPPH scavenging activity with an IC50 of 52.81 ± 2.30 µg/mL. Correlations ($p < 0.05$) was established between TPC,CTC, TAC and DPPH radical scavenging activity) of the extracts. Antinutrients such as; phytate, oxalate, saponins and alkaloids were found to be 7.691 ± 0.8576, 1.501 ± 0.1375, 21.33 ± 4.619 and 317.33 ± 30.29 mg/g respectively. Data obtained suggest that S. indicum possess rich bioactive compounds that can be used in neutraceuticals and food products.

Keywords: dietary plants; medicinal plants; extraction; solvent; phytoconstituents; antioxidants; anti-nutrients; radical scavenging

1. Introduction

Plant secondary metabolites are non-nutrient bioactive compounds with anti-oxidantive properties which may reduce the risk of many health disorders are associated with free radicals generated during body metabolic processes [1]. Several environmental activities contribute significantly to the release of chemicals, radiation, which lead to the contamination of water and food sources. The presence of these contaminants if found in the human body has the ability to induce the expression of some abnormal proteins which

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may lead to oxidative damage [2, 3]. The oxidative damage leads to the production of free radicals, which result in the risk of tissue damage which is usually evident in a wide variety of disease conditions in the human body.

These antioxidant compounds protect tissues from oxidative damage which are caused by free radicals. Polyphenolic compounds exhibit antioxidant activity as a result of their redox properties. This allows the phenolic compounds to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [5]. Most edible plants show high medicinal value based on the composition of their phytochemical constituents. Some of the reported essential phytochemical compounds include phenolics, tannins, flavonoids, alkaloids and glycosides [6].

The daily intake of food containing significant antioxidant compounds has been associated with the promotion of good health. For example, in the prevention of various diseases related to oxidative/nitrosative stress, including cardiovascular diseases, neurological disorders and cancer [7, 8]. The presence of antioxidant compounds in plants may occur in all parts of the plant. They may be found in the seeds, roots, leaves and fruits [9]. Oil-bearing seeds, for example, soybean, flaxseeds, sunflower, sesame and pumpkin has gained a lot of consumer interest as a result of the many reports claiming the existence of diverse nutritional compounds, minerals, antioxidants and phytochemicals of essential health benefits.

Sesamum indicum (Sesame) is a valuable oil-bearing seed crop found in the savannah areas of Ghana. The seeds are consumed by the indigenous people as food for energy. It is regarded as an exotic crop because it is not grown in the nation in large-scale commercial agriculture. According to studies conducted, *S. indicum* has high nutritional value and exhibit significant medicinal properties [10]. Roasted white sesame seeds, offered as a health-promoting condiment and packaged in sachets, have been introduced as a result of Ghana's growing embrace of traditional medicine. Although Ghanaian farmers typically only dedicate a small fraction of their farmlands to newly imported exotic crop, it is projected that commercial sesame production will rise in Ghana [11].

This study seeks to evaluate the extraction yield, bioactive compounds and the antioxidant properties of extracts obtained from *S. indicum* seeds using different solvents. The nature of a particular (polar, mid-polar and non-polar in an extraction process has significant effect on the percentage yield and kinds of bioactive compounds that may be extracted. Polar solvents such as water, methanol and ethanol are among the common solvents usually employed for the extraction of phenolic compounds whilst hexane, chloroform, and petroleum ether are employed to extract oils and fats as well other non-polar compounds [12]. The nature of the extraction solvent and conditions of extraction has significant effect on the yield and compounds obtained in the extraction [13]. Therefore the current study considered water, ethanol, ethyl acetate and hexane as solvents for the extraction of the phytoconstituents of present in Sesamum indicum oilseeds and their antioxidant properties analysed. Chemometric techniques, including unsupervised hierarchical cluster analysis (HCA) and Pearson bivariate analysis, were applied to evaluate and develop a classification model to assess all extracts to ascertain the correlations between the bioctive compounds and the antioxidant activities.

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2. Materials and Methods

2.1. Sample Collection and Preparation

The study sample was collected from farmers in the Upper East region of Ghana. They were identified and authenticated by a botanist at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST). The seeds were then cleared of all extraneous matter by sorting and sieving, air-dried, milled into coarse powder and kept in an air-tight container until ready for use.

2.2. Reagents and Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), catechin, gallic acid, ascorbic acid, vanillin, and Folin-Ciocalteau (FC) reagent were purchased from Sigma-Aldrich, UK. All other drugs and reagents used were of analytical grade.

2.3. Extraction of Plant Samples

The powdered plant materials (300 g) each was cold macerated in water (aqueous), ethanol, ethyl acetate and hexane. After 72 hrs of exhaustive extraction, the mixtures were filtered and the filtrate lyophilized. The concentrates were dried by evaporation on a water bath, the percentage yield calculated and the sample kept in a desiccator for subsequent use. The extracts were coded using the common names of the seeds and name of solvent; SA (sesame aqueous), SE (sesame ethanolic), SETAC (sesame ethyl acetate), and SH (sesame hexane)

2.4. Antioxidant Activity Assays

2.4.1. Total Antioxidant Capacity (Phosphomolybdate Assay)

Ammonium molybdate (4 mM), disodium hydrogen phosphate (28 mM) and sulphuric acid (6 mM) were used in the preparation of the reagent solution. The extracts solutions (500 μg/mL: 1 mL) were taken into labelled test tubes and 3mL of the reagent solution was added to each. Ascorbic acid solutions (200-1.5625 μg/mL) were prepared and 3 mL of the reagent solution added to 1 mL of the solutions. The reaction mixtures were incubated at 95 °C for 90 minutes and absorbances measured at 695 nm using the Synergy H[1] Hybrid Multi-Mode Microplate Reader, BioTek Instruments to plot the calibration curve. The total antioxidant capacity of the extracts determined from the linear equation of the calibration curve was expressed as mg of ascorbic acid equivalent (AAE) per g of the extract [13].

2.4.2. DPPH Free Radical Scavenging Assay

The ability of the extracts to scavenge free radicals and ascorbic acid (reference compound) was evaluated using a method reported by Sarpong et al [14]. The extract solutions (1000- 31.25 μg/mL, 1 mL) were added to 3 mL methanol solution of DPPH (20 mg/L) in labelled test tubes. The reaction mixtures were incubated in the dark at room temperature for 30 minutes. The reaction process was repeated for the reference compound, ascorbic acid of different concentrations (200- 1.5625 μg/mL). The absorbance of the

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residual DPPH was determined at 517 nm in the multimode microplate reader. The DPPH free radical scavenging activity was calculated from the following equation:

$$\% \text{ DPPH radical scavenging activity} = \left(1 - \left(\frac{\text{Abs of sample}}{\text{Abs of control}}\right)\right) \times 100\%$$

The percentage of DPPH radical scavenging activity was plotted against the log concentration of the reference compound and extracts. The concentration required to scavenge 50% of DPPH radicals was expressed in IC_{50}'s.

2.5. Quantitative Phytochemical Screening

2.5.1. Assessment of Total Phenolic Content (Folin-Ciocalteau Assay)

The reference compound - gallic acid solutions (200- 1.5625 µg/mL) were prepared and 0.5 mL were measured into test tubes and mixed with 2.5 mL Folin-Ciocalteau (FC) reagent (10%) and neutralized with 2 mL aqueous Na_2CO_3 (75 mg/mL). The extracts (0.5 mL) were also taken through the same procedure as the reference compound. The reaction mixtures were incubated at 50 °C for 10 minutes and absorbances were measured at 760 nm in the multimode microplate reader. The total phenolic content determined from the equation of the line from the calibration curve was expressed as gallic acid equivalents (mg GAE/ g extract) [15].

2.5.2. Analysis of Condensed Tannins Content

Condensed tannins content was evaluated colorimetrically using the vanillin-HCl assay reported by Frempong *et al.,* 2021 [16] with some modifications. The extracts (0.5 mL) were placed into test tubes wrapped in aluminium foil due to the light-sensitive nature of the experiment. Freshly prepared vanillin methanol reagent (3 mL; 4%, w/v) and 1.5 mL concentrated HCl was added to the extracts and mixed thoroughly. The reaction mixtures were kept in the dark at room temperature for 15 minutes and absorbances read at 500 nm. Catechin solutions (200 -1.5625 µg/mL) was prepared and taken through the same procedure and absorbances were measured at the same wavelength. The condensed tannin content of the plant extracts was determined from the calibration curve and expressed as mg/g CE (Catechin Equivalents).

2.6. Quantification of Antinutrients

2.6.1. Saponins

The amount of saponins was determined using the method described by Unofin *et al.,* 2017 [17] with some modifications. 1.25 g of the powdered plant sample was added to 25 mL of 20% ethanol and heated with continuous stirring on a water bath at 55 °C for 4 hrs. It was filtered and residue re-extracted with another 25 mL of 20% ethanol. The filtrates were combined and reduced to 40 ml on a water bath at 90 °C. The concentrate was introduced into a separating funnel and shaken vigorously with 20ml diethyl ether. The ether layer was discarded and the aqueous layer was retained. 60 ml of n-butanol was added to the retained aqueous layer in the separating funnel and shaken vigorously. The butanol layer was retained and shaken twice with 10 ml of 5% aq. NaCl. The remaining solution was collected, evaporated in a water bath and dried to a constant weight in an oven at 40 °C. The saponin content was calculated using the following equation;

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$$\text{Saponin content} = \left(\frac{\text{weight of residue (mg)}}{\text{weight of original sample (g)}}\right)$$

2.6.2. Alkaloids

The powdered plant material (1.25 g) was mixed with 50 ml of 10% acetic acid in ethanol, covered and left to stand for 4 hrs. It was filtered and the filtrate was concentrated on a water bath to about 15 ml. Concentrated ammonium hydroxide was added in drops until precipitation was complete. The solution was left to settle and washed with dilute ammonium hydroxide and filtered. The residue was collected, dried to a constant weight [17]. The alkaloid content was calculated using;

$$\text{Alkaloid content} = \left(\frac{\text{weight of residue (mg)}}{\text{weight of original sample (g)}}\right)$$

2.6.3. Oxalates

Oxalates in the powdered plant sample were quantified using a method described by Badu *et al.,* 2020 [10]. Exactly 1 g of the powdered plant sample was weighed into a beaker and sulphuric acid (75 mL; 1.5 N) was added. The mixture was stirred continuously for one hour using a magnetic stirrer and filtered. The filtrate (25 mL) was titrated hot against 0.1 M $KMnO_4$ till a pink colour which persisted for about 30 seconds was formed at the endpoint. The oxalate content of the sample was calculated as = (titre value × 0.9004) mg/g

2.6.4. Phytates

The phytate content of the powdered plant sample was evaluated using a method described by Badu *et al*., 2020 [10] with some modifications. 2 g of the sample was weighed into a conical flask and HCl (50 mL; 2% v/v) was added. The mixture was continuously stirred for three hours and filtered. The filtrate (25 mL) was measured into a conical flask, 50 mL of distilled water was added to the filtrate and 5 mL of 0.3% ammonium thiocyanate indicator was added. The solution mixture was titrated against 0.00195 g/mL iron(iii) chloride solution till a brownish-yellow colour which persisted for about 5 minutes was observed. The phytate content was calculated using the following equation;

$$\text{Phytate content (mg/g)} = \frac{8.24(titre\ value)}{weight\ of\ sample\ taken}$$

2.7. *Statistical Evaluations*

The results are shown as means ± standard deviation (SD) values. The statistical analysis was done by One-way ANOVA using Minitab®19.2020.1 (copyright© 2012 Minitab Inc., Philadelphia, PA, USA) and GraphPad Prism version 6. The differences between treatments and the standard data were considered significant at $p < 0.05$ using Tukey's HSD test. A chemometric approach which was the unsupervised hierarchical cluster analysis (HCA) was performed using Minitab®19.2020.1 (copyright© 2012 Minitab Inc., Philadelphia, PA, USA). The HCA method was utilized to assess the relationships between the *S. indicum* extracts. The squared Euclidean distance and Ward's linkage method was used to generate the dendrogram for the plant extract's samples determined each+ cluster. Pearson correlation was also undertaken using Minitab®19.2020.1 (copyright©

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2012 Minitab Inc., Philadelphia, PA, USA) to evaluate the possible relation between TPC, CTC, TAC and antioxidant activity of the studied extracts

3. Results

3.1. Extraction of Compounds

From the extraction process, the percentage yield of extracts obtained were estimated, and the results are shown in Table 1. The results revealed a significant difference ($p < 0.05$) in extraction yield depending on the nature of the solvent. Hexane had the highest percentage extraction yield (49.34 ± 1.474, $p < 0.05$) and water had the least extraction yield (11.9 ± 0.721, $p < 0.05$).

3.2. Total phenolic Content, Condensed Tannin Content and Antioxidant Activity

Effect of different solvents types phytochemical components and their associated antioxidant properties were analysed. As illustrated in Table 1, the aqueous extract exhibited the highest TPC (17.12 ± 0.041 mg GAE/ g of dried extract , $p < 0.05$) and CTC (64.27 ± 4.711 mg CE/ g of dried extract, $p < 0.05$). Ethanol and hexane had similar total phenolic content (14.83± 0.123 and 14.66 ± 1.474 mg GAE/ g of dried extract respectively, $p < 0.05$) ; CTC: 37.07 ± 1.588 mg CE/ g of dried extract, $p < 0.05$). Ethyl acetate had the lowest TPC content. The ethanol extract gave the highest total antioxidant capacity (232.6 ± 6.267 mg AAE/ g of dried extract, $p< 0.05$) followed by the hexane extract (210.9 ± 26.86 mg AAE/ g of dried extract, $p< 0.05$).

The antioxidant activities of various *S. indicum* extracts was measured using the DPPH radical scavenging activity are shown in Table 2 and Figure 1. The different extracts had varying free radical scavenging capabilities ($p <0.05$). Among the tested extracts, hexane extract was the most potent with IC_{50} of 52.81 ± 2.30 µg/mL.

Table 1. Total phenol content (TPC), Condensed tannin content (CTC) and Total antioxidant capacity (TAC) of the plant extracts

Sample	% Yield	TPC mg GAE/ g of dried extract	CTC mg CE/ g of dried extract	TAC mg AAE/ g of dried extract)	DPPH IC_{50} (µg/mL)
SA	11.9 ± 0.721^{c}	17.12 ± 0.041^{a}	64.27 ± 4.711^{a}	35.44 ± 0.926^{c}	290.9 ± 8.00^{a}
SE	13.3 ± 0.99^{c}	14.83 ± 0.123^{b}	37.07 ± 1.588^{b}	232.6 ± 6.267^{a}	61.49 ± 1.99^{c}
SETAC	24.5 ± 0.714^{b}	6.442 ± 0.714^{c}	20.75 ± 12.46^{bc}	188.0 ± 7.494^{b}	254.90 ± 4.01^{b}
SH	49.34 ± 1.474^{a}	14.66 ± 1.474^{b}	12.59 ± 4.711^{c}	210.9 ± 26.86^{ab}	52.81 ± 2.30^{c}
ASCORBIC ACID	ND	ND	ND	ND	20.71 ± 1.315^{ab}

Values are represented as mean ± standard deviation (n = 3); Statistical significance: Means in columns that do not share the same superscript letter are significantly different. ($p < 0.05$). (mg GAE)/g = milligram gallic acid equivalent per gram of dried extract; mg CE)/g = milligram of catechin equivalent per gram of dried extract; (mg AAE)/g = milligram of ascorbic acid equivalent per gram of dried extract; n.d= not determined

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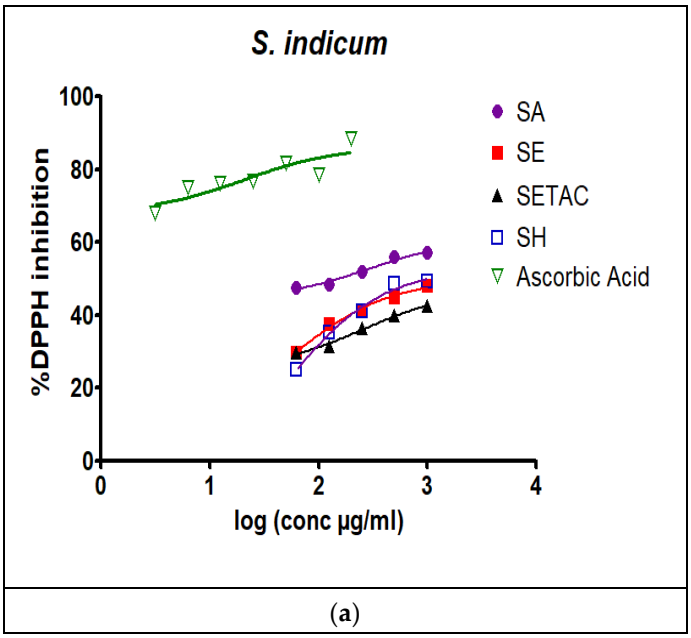

(a)

Figure 1: DPPH free radical scavenging activity of extracts and ascorbic acid

3.5. Chemometric Analysis

A comparative analysis (Pearson Corrrelation) was conducted to explore the relationship amongst the variables measured. Results of the analysis are shown in Figure 2 and Table 2. A strong negative linear correlation coefficient between TAC and DPPH(-0.784). TPC was weakly correlated with TAC (-0.387) and DPPH (-0.253) which implied that the total phenols was not a major contributing factor to the high total antioxidant capacity and free radical scavenging property which could result from other components found in the seeds of the plant. A cluster analysis was also employed to provide information about the associations and patterns that exist in our data. In this study, there was no assumption about the likely relationships within our data. A cluster analysis of the solvent extracts of *S. indicum* seeds was performed using data on the % yield, TPC, CTC, TAC and DPPH.

The dendrogram obtained from the unsupervised hierarchical cluster analysis (Figure 3) displayed a certain correlation among the solvent extracts of the plant sample. The analysis of the results showed that the samples created a non-homogenous data set. Three separate clusters (C1-C3) were generated. Both SH and SE were found in one cluster which is characterized by high antioxidant capacity and strong radical scavenging ability. SA belonged to the second cluster, characterized by high TPC level and low antioxidant capacity and low radical scavenging ability. SETAC was found in the third cluster with low phenolic content and weak antioxidant activity.

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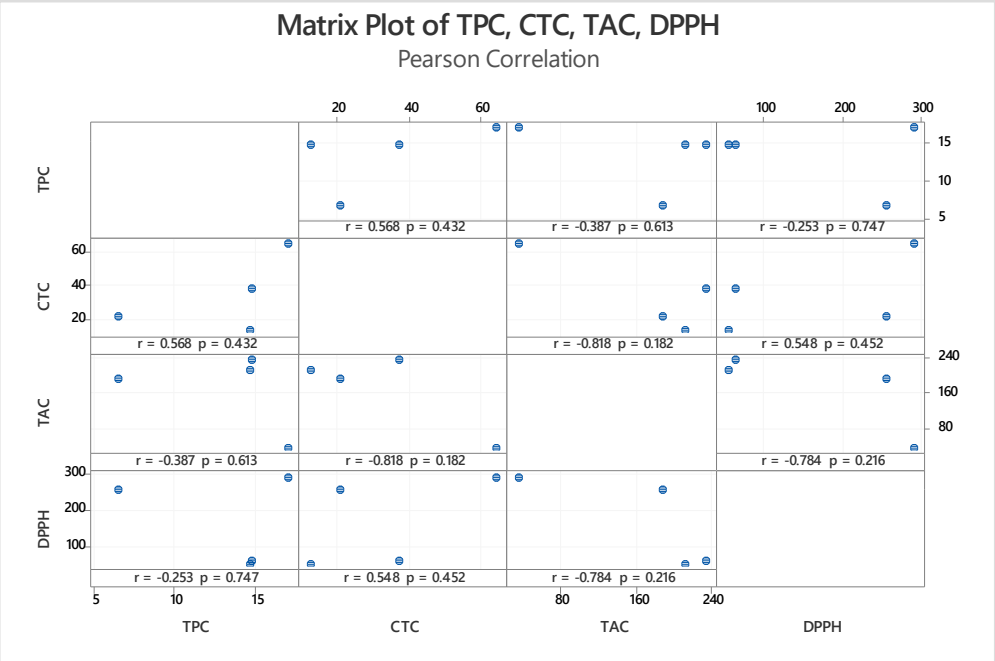

Figure 2: Bivariate Pearson Correlation Matrix Plot of TPC, CTC, TAC and DPPH

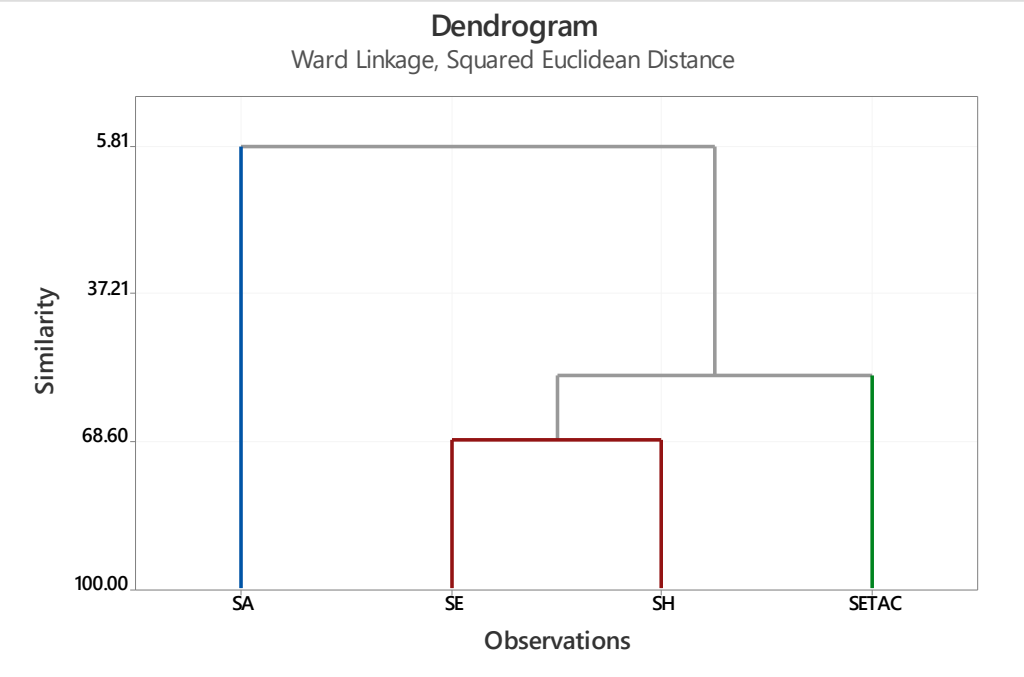

Figure 3: The dendrogram obtained by hierarchical cluster analysis using Ward's method and squared Euclidean distance metric. For data of TPC, CTC, TAC, DPPH and percentage yield of extraction of the extracts. SiA, '*Sesamum indicum* aqueous extract'; SE, *Sesamum indicum* ethanolic extract ; SETAC, ''*Sesamum indicum* ethyl acetate extract'; SH, ''*Sesamum indicum* hexane extract'

Table 2. Bivariate Pearson correlation coefficients (R) between total phenolic, condensed tannin contents, total antioxidant capacity and DPPH radical scavenging activity of the tested extracts of *S. indicum*

	TPC	CTC	TAC
CTC	0.568		

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TAC	-0.387	-0.818	
DPPH	-0.253	0.548	-0.784

3.4. Antinutritional content of powdered seeds.

The result of the antinutritional content for total saponin content, oxalates, total alkaloids and phytate in *S. indicium* is shown in Table 3. A higher alkaloid content of 317.33 ± 30.29 mg/g ($p< 0.05$) was observed whereas the oxalate content in the powdered seeds was however found in low levels (1.501 ± 0.1375 mg/g, $p < 0.05$).

Table 3. Antinutritional content of *S. indicum*

Antinutrients	*S. indicum* (mg/g of powdered sample
Saponins	21.33 ± 4.619[b]
Alkaloids	317.33 ± 30.29[a]
Oxalates	1.501 ± 0.1375[b]
Phytates	7.691 ± 0.8576[b]

All data were recorded as mean and their standard deviation. $p < 0.05$. Means in the same column that do not share the same letter are significantly different.

4. Discussion

Estimation of percentage extract may be used to evaluate the presence of phytochemical compounds present in the oilseed using a particular solvent. According to literature reports, the extraction method, time, temperature, and the nature of solvent greatly influences the quality, yield and efficiency of the extraction [18]. Using solvents of different polarities, results from this study showed that *S. indicum* contained high amount of non-polar compounds as shown in the high percentage yield recorded by the hexane extract. The aqueous extract represent the polar compounds exhibited the highest phenolic content with the ethyl acetate extract showing the least. Polyphenolic compounds are characterized by the presence of an aromatic ring and a hydroxyl group in their molecular structure. The compounds are reported to be soluble in methanol and hot water [19]. This may account for the high total phenolic content obtained in the aqueous extract for the seeds. The results in Table 1 showed that the aqueous extract of *S. indicum* exhibited the highest condensed tannin content, followed by the ethanol extract with the hexane extract showing the least. Condensed tannins consist of flavonoid units (essentially flavan-3-ols and flavan-3,4-diols) which have suffered varying degrees of condensation, and are associated with carbohydrates and traces of amino and imino-acids [20]. These compounds are easily extracted using solvents such as ethanol, ethanol in water mixture due to their high polarity [21]. For TAC, however, SA gave the lowest total antioxidant capacity. Whilst SH gave the highest yield. This results signify that there may be other components in the plant other that the phenolics which are contributing factors to the total antioxidant capacity and the radical scavenging activities [22]

From the graph in Figure 1, a trend of concentration dependent DPPH scavenging activity was exhibited by all the extracts as well as the reference compound. The lower the

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IC_{50}, the higher the antioxidant activity. The ascorbic acid as expected exhibited the highest DPPH scavenging activity. Among the extracts, SH had highest antioxidant activity with the least IC_{50} while the SA showed the least activity. This trend was also noticed for the TAC of the extracts. According to Jadid *et al.,* 2017 [23], extracts with IC_{50} values ranging from 10 -50 μg/mL have a strong antioxidant activity, 50 – 100 μg/mL: intermediate and > 100 μg/mL have weak antioxidant activity. *S. indicum* has been found to be rich in phytosterols and tocopherols [24]. Researchers have credited the antioxidant of these phytosterols to the formation of an allylic radical and its isomerization to other relatively stable free radicals [25]. Based on this, the hexane extracts of *S. indicum* can be classified as having strong antioxidant activity. Hexane solvents have also been reported to extract terpenoid-derived compounds known to play vital role in boosting human health. Terpenoid-derived compounds are also known for providing provitamin A or β-carotene, a compound *S. indicum* also have been reported to have in rich quantity [26]. Provitamin A has a high antioxidant potential [27]. This also could be the contributing factor to *S. indicum* exhibiting a strong antioxidant activity. Furthermore, a high TPC content does not automatically imply a high level of bioactivity. A researcher reported fruits from Mexican *Opuntia robusta* with high TPC having low antioxidant activity [28]. The biological or antioxidant potency of an extract may be as a result of synergistic effects of several secondary metabolites the extract possesses [29]. Aruwa et al, 2019 in their research showed the TPC of extracts of Southern African *Opuntia ficus-indica* fruit pulp and peels generally decreased in the order ethanol > methanol > hexane > water. The hexane extracts however showed the lowest EC_{50} of DPPH values (15.59–80.22 mg/mL) and had the highest antioxidant activity [30] which was consistent with our results

The dendrogram obtained from the unsupervised hierarchical cluster analysis displayed in figure 3 generated three separate clusters (C1-C3). One cluster was generated with similarity more than 60% and two clusters each having a single observation (SA with similarity of 5.81 and SETAC with a similarity of 54.71 which were considered as outliers. Cluster 1 consists of SA with a similarity of 5.81 and is distinguished by having the highest TPC yet lowest TAC (35.44 ± 0.926 mg AAE/ g of dried extract), and the lowest antioxidant activity among the extracts. SE and SH, grouped as Cluster 2 are also characterised by their high TAC and very high antioxidant activities with lower IC_{50} values compared to the other extracts. The hexane extract in this cluster had the high percentage yield of extraction. Hexane is commonly used to extract steroids, oils and carotenoids. Hexane can also be used for defatting plant materials. This could be the result of a high amount of non-polar components in the sample which was extracted by hexane.

Cluster 3 had a single observation, i.e., SETAC which was characterised by having the lowest TPC, low antioxidant capacities which may provide explanation for its comparatively low DPPH radical scavenging activity.

Pearson correlation (Figure 3 and Table 3) was used to establish the relationship between TPC, CTC and TAC in the different plant extracts to inform the contribution of these variables on the extracts to their total antioxidant activity [31]. There was a negative correlation between TAC and DPPH ($r = -0.784$, $p < 0.05$) in the extracts, demonstrating that the total antioxidant capacity influenced the antioxidant activity. TPC also negatively correlated with the TAC ($r = -0.387$, $p < 0.05$). This suggested that the extracts' total

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antioxidant capacity wasn't primarily due to their phenolic content. It was evident from our Pearson's correlation analysis that a high TPC in an extract did not always correspond to a high TAC. Total phenolic content has been used as an indicator of antioxidant properties in plant extracts and reported severally in literature. A strong positive correlation ($p \leq 0.01$) between TPC and TAC was reported by by Parikh and Patel, 2018 in determining the total phenolic content and total antioxidant capacity of common Indian pulses and split pulses [32]. Amponsah et al, 2014 also stated a high positive correlation between the total antioxidant capacity and the phenolic content ($r^2 = 0.9195$). This suggested that the phenolic content of *Artocarpus altilis* may have accounted for 92% of its antioxidant activity, with the remaining secondary metabolites accounted for the remaining 8% [33]. The rise in TPC, CTC could be linked to the increase in antioxidant activities which is evidenced by lower IC_{50} (DPPH). As a result, TPC and CTC are negatively correlated with IC_{50} (DPPH). Anjum and Tripathi, 2015 [34] reported a negative correlation between total phenols and DPPH radical scavenging acrivities of *Hippophae rhamnoides* L. Berries. Another author also reported TPC in black sesame seed extract was significantly negative correlated with IC_{50} ABTS value ($r = -0.828$, $p < 0.01$) [35]. This could signify phenolic compounds being a main a contributor to antioxidant activities using DPPH and ABTS methods. However, this was not observed in our studies as there was no significant correlation between TPC and DPPH radical scavenging activity. The antioxidant activities could possibly be owing to other phytochemicals like carotenoids [36], antioxidant polypeptides [37], and Vitamin C or E or rather than TPC, it could be due to synergistic or antagonistic actions between chemical components such as TPC, terpenoids, and so on. In a crude extract which is heterogenous mixture, the antioxidant capacity of each metabolite cannot be determined or measured since all phytochemical present in the extract that act as antioxidants react concurrently to determine the sample's scavenging ability[38, 39]. In an effective way, it is imperative to use diverse methods that specifically address different mode of actions of the individual compnents [40].

Anti-nutritional factors are chemicals present in dietary or medicinal plants which are either synthesized by the plants themselves or introduced synthetically from the environment (pesticides, fertilizers etc.) [41]. These anti-nutrients though have been reported to have medicinal properties, beyond certain limits inhibit the availability of nutrients to the body and could also be poison to the system [42]. Knowledge on the anti-nutritional content of the food we eat is key to healthy living. For instance, high saponin content of above 10% has been reported by Unuofin *et al.*, 2017 to be hazardous to the body since it inhibits growth, reduces the bioavailability of nutrients and prevents biochemical reactions which facilitates protein breakdown in the body [17]. The seeds of *S. indicum* can be classified as safe since they both contain less than 10% saponins. Saponins are known to exhibit medicinal properties such as anti-inflammatory, antimicrobial, anti-cancer properties which are all linked to antioxidant activity [27]. Alkaloids, a well-known therapeutic compound, at high levels could also be harmful to human health. For example, consumption of tropane-an alkaloid has been reported to lead to rapid heartbeat, paralysis and even death. Alkaloids are also known for cell membrane disruption in the digestive tract at high levels. Mbaebie et al, 2010 reported a total alkaloid content of 6.28 ± 4.19 mg /g of powdered sample in the sesame seeds [43]. Samuel and Genevieve., 2017 presented a

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total alkaloid content of 4.80 mg /100g of powdered sample [44]. Neeta et al, 2015 made a comparative analysis of secondary metabolites present in black and white sesame seeds. The black sesame seeds had a lower alkaloid content (15mg /g of powdered sample) than the white variety (56 mg/g of powdered sample) [45].

In our study, *S. indicum* exhibited a significantly higher alkaloid content compared to results reported in literature. When oxalate is taken into the body, it has the capacity to bind with calcium and magnesium to form insoluble salts which cannot be absorbed into the body further causing kidney stones. Phytates also form insoluble salts with ions of mineral elements such as calcium, magnesium, zinc and iron lowering their bioavailability to the body and causing mineral deficiencies [17, 42, 10]. Olagunju and Ifesan., 2021 stated in their report the raw sesame seeds containing phytic acid content of 31.59 ± 0.95 mg/g and oxalate content of 1.05 ± 0.10 mg/g [46]. They however observed a 69% reduction in the phytic acid and oxalate content of the sesame seeds following a 96-hour fermentation. Fermentation has been identified as one of the most effective processing techniques that reduces phytates [47]. Jimoh et al., 2011 also observed significant differences ($p < 0.05$) in the antinutrients composition between the raw and the processed sesame seeds [48]. A reduction trend was also observed in the various samples with processing time. The data presented by Akusu *et al*, 2020 on phytate and oxalate content of raw and processed sesame seed flour showed a significant ($p < 0.05$) reduction in the level of phytate, and oxalate from the raw to the processed seeds. It was attributed to initial soaking, hydration and cooking of the dehulled seeds which may have caused leaching of some amounts of the antinutrients into the processing water before the actual fermentation process. Also, they stated the diminishing effect of enzymes such as phytase could be a contributing factor in the reduction these anti-nutrients during fermentation. [49]. Phytates and oxalates are considered thermolabile in nature [50] The toxicity of most anti-nutrients in dietary plants can be reduced or removed when they are well-treated thermally before employed for consumption purposes.

From our study, the sesame seeds can be classified as having low phytate and oxalate content since they contain less than 5% oxalates and phytates.

This results signify that there may be other components in the plant other that the phenolics which are contributing factors to the total antioxidant capacity and the radical scavenging activities

5. Conclusions

This study confirms the presence of different bioactive compounds in extracts of *S. indicum* seeds. The different extracts of the seed showed varying levels of antioxidant activities, and this was found to be dependent on the solvent for extraction. Also, the study indicated that hexane extracts (fats and oils) exhibited high total antioxidant capacity and strong DPPH scavenging activity. The low antinutrient content found in both seeds makes them safe for consumption and can be incorporated into the diet.

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