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ORIGINAL ARTICLE

Evaluation of Phytochemical Contents and their Antioxidant Properties of Sunflower (*Helianthus Annuus* L.) Seeds Collected from Noakhali, Bangladesh

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Abstract

Background: Despite the fact that hydrogen peroxide (H_2O_2) is proactive, it turns to the severely reactive species and harmful hydroxyl radical (HO*) to the cell, which is then enable to interfere with pairs of nucleotides in DNA as well as, the similar way, trigger breakage of the strand due to genotoxicity (carcinogenesis, mutagenesis) and produce reactive oxygen species (ROS). Overproduction of these ROS in the cell leads to various types of chronic degenerative disorders like different types of diabetes, neurodegenerative disease, several types of cancer and so one. Phytochemicals as well as antioxidants perform a crucial task in the prevention of ROS production, which is undergo oxidative stress. However, the potential antioxidant capacity of a molecule is determined by the H_2O_2 scavenging rate. The hydrogen peroxide (H_2O_2) scavenging assay was incorporated utilizing the replacement titration assay and a UV-VIS spectrophotometer. **Objective:** The focus of this investigation was to determine the antioxidant potency of sunflower seeds (*Helianthus annuus* L.) extracts by using H_2O_2 and DPPH scavenging assays, as well as polyphenol, flavonoid, and tannin contents. **Methodology:** We have also studied the water and ethanolic exudates of various sorts of sunflower from the Noakhali region of Bangladesh to check their polyphenol, flavonoid, and tannin residues. The antioxidant potency was also assessed applying the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical reducing capacity. **Results:** The ethanolic extract of sunflower included the maximum number of polyphenols (8.61%), flavonoids (30.67%) as well as obtained in max yields (10.15%). The solution of ethanolic exudate had upmost antioxidant characteristics in both H_2O_2 and DPPH radical-scavenging activity than those in the aqueous extract. The scavenging rate of DPPH and H_2O_2 was brought about in a dose-dependent way. **Conclusion:** Overall, the sunflowers examined in this present study are reliable origin of natural antioxidants that confer unique defensive mechanism adverse to free radical toxicity, are accessible sources of natural antioxidants, also can be used as a potent diet supplement or in the medicinal value against diseases. [*Journal of Current and Advance Medical Research, January 2024;11(1):8-16*]

Keywords: Sunflower seed extract; phytochemical content; free radical scavenging

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Introduction

The sunflower (*Helianthus annuus* L) is recognized as one of the primary oilseeds crops globally, ranking second only to soybeans in terms of overall oil production[1]. The process involves pressing the sunflower seeds to extract the oil. Sunflower oil is a very popular and healthy oil in the world. It is believed a luxurious oil due to its clear appearance, mild flavors, minimal saturated fat, and stability at high temperatures[2]. Most vegetable oils contain phenolic compounds, which are considered stabilizers of the oxidative balance in biological organisms due to their polyunsaturated fatty acid contents. However, the higher the concentration of naturally antioxidant edible oils, the lower the likelihood of developing chronic illness. As a result, sunflower oil has various food applications for dietary and health benefits[3]. Tocopherol, an antioxidant present in sunflower and pumpkin oil, serves to prevent oxidation by functioning as a scavenger for free radicals. LDL modification is not possible for antioxidants. The unprocessed vegetable oil of sunflower has a special form of antioxidant that characterizes the beneficial effects of food in oils[4].

Plant species have been used and consumed for their strong antioxidant properties. The extracts of herbs and natural products are considered the best origin of age-defying and antioxidant qualities. An antioxidant is a protective agent that can hinder or delay the oxidation of cellular molecules and prevent the commencement of an oxidative chain reaction[5]. Lately, there has been a notable concentration of research effort on phenolic acids. About twenty-five phenolic acids have been isolated and identified from various plant species[6]. ROS indicates to a broad spectrum of molecules; furthermore, free radicals originate from molecular oxygen[7]. Superoxide radicals ($O_2^{\bullet-}$), peroxide radicals (*OH), hydrogen peroxide (H_2O_2), singlet oxygen (O_2), and hydroxyls are the major types of ROS that play a conspicuous role in phagocytosis, intracellular signaling transmission, cell proliferation, the synthesis of biological compounds, and antimicrobial activity[8]. ROS may form from internal and external sources. Internal causes can be inflammation, cancer, depression, infestation, and senescence. On the other hand, external free radicals can enter the cell as well as disintegrate because of exposure to heavy transition metals like mercury (Hg), lead (Pb), and iron (Fe), as well as drugs (cyclosporin and gentamicin), smoking, drinking alcohol, different types of radiation, ingestion of food with pesticides. The catalase enzyme converts hydrogen peroxide to water and oxygen, whereas it may also convert hydroxyl radicals, these are too much potent in the appearance of transition metals like iron (Fe). According to Fenton's reaction, iron (Fe^{2+}) donates one electron to hydrogen peroxide to convert hydroxyl radicals[9]. As a result, ROS are produced within our body[10]. Most of the ROS are produced by mitochondria as products of the electron transport chain[11]. Furthermore, ROS can modify not only DNA but also attack the membrane proteins, carbohydrates, lipids, and proteins of a tissue[12]. In biological organisms, the rates of production and mitigation of ROS are in balance, known as oxidative balance. The imbalance between production and mitigation degrades this balance and develops ROS formation, called oxidative stress, which indicates a severe imbalance in the formation and antioxidant defense systems as a result of tissue damage[13]. Oxidation processes can regulate the genesis of harmful free radicals. However, when the body's antioxidant defenses are inadequate, this imbalance can lead to increased vulnerability to diseases and faster senescence[14]. Either human intrinsic enzymes need to be enhanced for removing the damaged molecule in tissue and hindering disease development or ingestion of antioxidant-rich foods.

Several types of enzymes like Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the main organic molecule present in the cell that are responsible for protecting it from the organization of oxidative damage[15]. These enzymes prevent oxidative stress by eradicating free radicals, deterrent lipid peroxidation, also protecting disease formation via a wide range of pathways[16]. Hydrogen peroxide is found in fresh water, rainwater, sea water, different types of beverages like instant coffee, different form of tea (green, black), and also in blood plasma at concentrations greater than 100μM[17]. Synthetic antioxidants and food additives contain high amounts of preservatives[18]. The key synthetic enzymes are butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), also tertiary butyl hydroquinone (TBHQ) which are produce toxic molecule to cell as well as act sometimes as carcinogens[19]. So, detection and study of noble compounds with potential natural antioxidant sources is the key to ensuring sound health[18]. Sunflower seeds are enriched with a myriad of unsaturated fats, phosphorus, iron, furthermore essential diets like vitamin E, the B-complex vitamins, selenium (Se), copper (Cu), zinc (Zn), folate, and phytochemicals, making them a convenient source for meals, tiffin, and snacks[20-21]. Selenium is a trace element necessary for DNA

repair and synthesis in damaged cells, as well as for deterring the amplification of cancerous cells as well as inducing their apoptosis. It also acts as a cofactor for one of the most important enzymes, named glutathione, which is responsible for eradicating toxic substances from our bodies. If glutathione concentrations are insufficient in cell, toxic molecules aren't eradicated, leading to cellular DNA damage and promoting cancer[22].

Sunflower seeds enriched with vitamin K which reduce the risk of colon cancer as well as it used also the treatment the cancer of colon and esophagus[23-24]. Grandiflorolic, kaurenoic, and trachylobanoic acids are isolated from sunflower seeds which hinder the inflammatory mediators like lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. At a threshold level which is nontoxic in form, these molecules declined in a dose dependent manner, NO, PGE2, and TNF-α generation in addition to generation of NOS-2 along with COX-2[25].

One of the components of a sunflower seeds is a magnesium which reduce the acute migraine headache, heart attack risk together with stroke. Besides, it is also necessary for osteogenesis plus energy production. On the other hand, magnesium counter balances the calcium, by blocking the entrance of calcium in cell compartment, by keeping the nerve, blood vessel and muscle relaxed. Imbalance between magnesium and calcium ion causes over activated nerve cell, hypertension, muscle spam, migraines tension and soreness. Sunflower seeds are excellent source of Mg^{2+} to solve this problem. Properties like vitamin E, low saturated fat, high unsaturated fat (linoleic & oleic acid) are found in sunflower seeds[26].

Top most countries producing sunflower in the world are Ukraine, Russia, Argentina and China according to FAO and USDA statistical database, 2022[27]. According to ministry of agriculture, Bangladesh has an annual requirement of 2.4million tones cooking oil, with approximately 88% being imported. Bangladesh bank fiscal year data 2021-22 shows that the country imported edible oil worth Tk2.53billion[28]. The average production of sunflower per hectare is about 3.2t/ha[29].

However, there is insufficient knowledge as well as scientific dossier on the phytochemical contents, antioxidant properties, and deeds of sunflower yield in Bangladesh, especially in Noakhali. Thus, this article was shed light to know the phytochemical contents and determine the antioxidant capacity of sunflower from district of Noakhali, Bangladesh, using several solvent extraction methods.

Methodology

Chemicals and Reagents: Gallic acid, catechin, and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were bought from Sigma Aldrich (St. Louis, MO, USA). L-ascorbic acid, tannic acid (Loba India), $FeSO_4$ Folin-Ciocalteu's phenol reagent, along with ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) were procured from NM Enterprise (Loba India). The chemicals as well as reagents used in this investigation were of analytical grade.

Sunflower Seed Collection: Sunflower seeds were collected from the Sunflower Garden field of Noakhali Science and Technology University (NSTU) which is situated in the district of Noakhali, Bangladesh in February 2023. After collection, the sunflower seeds were loaded into sterile condition before delivering to the Laboratory of the Department of Biochemistry and Molecular Biology, Noakhali Science and Technology University.

Extract Making and Yield Ascertainment: Sunflower seed samples were unadulterated and dried with air in room condition then the test was eradicated. Following seeds were being pasted into a fine particulate matter in a mortar. The fine powdered dust was utilized to prepare not only ethanolic but also water extracts according to Kang's method[30] along with a few modifications. To wrap up, 20% ethanolic exudates was made by mixing seed powder (20g) to a 70% ethanol solution to prepare a 100mL solution. Likewise, for a 20% aqueous exudates preparation, 20g of sunflower powder was disseminated in aqueous medium to prepare a 100mL solution.

Ethanol as well as water extract solutions were kept in the absence of light to avoid unwanted reactions and were shaken in a shaker for 72 hours at 25ºC temperature. After that, the solutions were refined through Whatman No. 1 filter paper and evaporated the existing aqueous and ethanolic residuals from the samples in a rotary evaporator (HAHNSHIN SNT CO, LTD. KOREA) under moderate pressure (100psi) at ~42°C (ethanolic) and ~54°C (aqueous).

The evaporated extracts were stored and kept at -20°C for following experiment. The percentage yield of the exudates was measured likewise the subsequent formula: % yield = [weight of sample extract/initial weight of sample] ×100. Two distinct sunflower seed extracts were prepared for antioxidant analysis (Table 1).

Phytochemical Analysis

Evaluation of Net Polyphenol Content: The Net polyphenol content (NPC) of the sunflower extracts was evaluated by utilizing spectrometrically in consonance with the Folin-Ciocalteu method also elected by Afroz *et al*[31]. In a nutshell, 0.4mL of the extract (0.25mg/mL) was together with 1.6mL of a 7.5% sodium carbonate mixture. Afterwards, 2mL of 10-time diluted Folin-Ciocalteu reagent was fused, as well as the ultimate reaction mixture was rest for 1hour in the absence of light. The luminous density of the blue-colored suspension was determined at 765 nm utilizing a PD303S spectrophotometer (KOREA). The net polyphenol residuals present was measured as gallic acid equivalent (GAE) (25.00, 50.00, 75.00, 100.00g/mL) and was expressed as g of GAE/100g of sunflower.

Evaluation of the Net Flavonoid Content: The net flavonoid content (NFC) was determined by utilizing an aluminum chloride colorimetric study[32]. Firstly, 1mL of the extract (1mg/mL) was mixed with 0.3mL of 5% sodium nitrite as well as added to the reaction mixture. Then correspondingly 5 minutes, 0.3mL of 10% aluminum chloride was mixed. Consequently, after those 6 minutes, a further 2mL of 1M sodium hydroxide (NaOH) was included, further more addition of 2.4mL of distilled water to make a net volume of 10mL. The strength of color of the flavonoid-aluminum mixture was determined at 510nm. The net flavonoid content was assessed as catechin corresponding (CC) (0, 25.00, 50.00, 75.00, 100.00g/mL) and was expressed as g of CE/100g of sunflower.

Evaluation of the Net Tannin Content: The Net Tannin Content (NTC) presence in the sunflower exudates was assessed by utilizing the Folin-Ciocalteu study[33] along with tannic acid counting as a standard. Summarizing, 0.1mL of the solution including 1mg of the exudates was integrated with 7.5mL of distilled water, together with 0.5mL of Folin-Ciocalteu reagent was included. After that, 1mL of 35% sodium carbonate as well as 0.9mL of distilled water (dH_2O) were mixed. Then the mixture was amalgamate furthermore then kept for 30minutes. The strength formation of blue-colored complex was determined at 725nm. The outcome was showed as g of tannic acid corresponding (TC) per 100g of sunflower.

Antioxidant Capacity: The antioxidant capacity of the sunflower samples was assessed utilizing DPPH radical scavenging capacity.

DPPH Scavenging Capacity: The antioxidant capacities of two sunflower extracts were assessed in accordance with their DPPH free radical eradicating capacities, by the study of Braca *et al*. (2002)[34]. To sum up, 1mL of the extract was amalgamate with 1.2mL of 0.003% DPPH in methanol at different concentrations (2.5–80.0g/mL). The percentile of DPPH inhibition was determined by utilizing the following mathematical statement: % of DPPH inhibition = $[(A_{DPPH} - A_S/A_{DPPH})] \times 100$, (1) here, A_{DPPH} is the optical density of DPPH in the absenteeism of a sample and A_S is the optical density of DPPH in the contiguity with sample as well as standard. DPPH scavenging capacity is showed as the amount of a sample need to decrease DPPH absorbance by 50% (IC_{50}). This value can be graphically determined by plotting the absorbance (the percentage of inhibition of DPPH radicals) against the log concentration of DPPH and determining the gradient of a nonlinear regression.

Hydrogen Peroxide Scavenging Assay: The replacement titration method of Zhang[35] with a slight modification involves several steps. Firstly, 1mL of different concentrations of sample extract or standard is taken in different 250mL conical flasks. Following this, 5mL of 2M sulfuric acid (H_2SO_4) is added to each flask, followed by the addition of 3.5mL of 1.8M potassium iodide (KI). Subsequently, 50µl of 8.8M hydrogen peroxide (H_2O_2) is added to each flask, and then 200µl of 3% ammonium molybdate is introduced. Finally, titration is carried out using 1mM sodium thiosulfate ($Na_2S_2O_3$). This method facilitates ion-complex titration and allows for the determination of the concentration of the target species in the sample extract or standard solution. The percentage of scavenging activity of H_2O_2 was calculated as: Inhibition = $[(A_c\text{-}A_s) / A_c \times 100]$, where A_c is the volume of $Na_2S_2O_3$ solution needed to titrate the control (without extract) along with H_2O_2, and A_s is the volume of $Na_2S_2O_3$ solution utilized in the presence of plant exudates.

Statistical Analysis: H_2O_2 scavenging activity was expressed as the density of the extract (IC_{50}) necessary to scavenge H_2O_2 by 50%. The IC_{50} value can be determined graphically by plotting the data (% of inhibition) against the log of the concentration used using the gradient of the nonlinear regression.

Results

Our findings reveal that the most substantial yield of antioxidant compounds is achieved through the ethanolic extraction of sunflower seeds gathered

from the NSTU sunflower field (10.15%). In contrast, the aqueous extraction of sunflower seeds yields the lowest amount (7.11%).

Table 1: The Yield of Various Exudates (Data are expressed as mean)

Parameters	SEE	SWE
Initial weight (g)	113.88	113.88
Yield (g)	11.55	8.09
Yield (%)	10.15	7.11

Polyphenol Content: As depicted in Table 2, sunflower seeds exhibit a notable abundance of polyphenols. Specifically, the total polyphenol content identified in ethanolic extracts markedly surpasses that of analogous aqueous extracts. The Total Polyphenol Content (TPC) in sunflowers varied from 5.28g GAE/100g of sample to 8.61g GAE/100g of sample in aqueous and ethanolic extracts, respectively. Additionally,

Flavonoid Content: The Net Flavonoid Content (NFC) of sunflower is recorded at 18.58% in aqueous extracts and 30.67% in ethanolic extracts, with the latter demonstrating a higher concentration (Table 2).

Tannin Content: Our research findings (Table 2) indicate that sunflower seeds sourced from the Noakhali region serve as a notable reservoir of tannins. Specifically, ethanolic extracts exhibit a significantly elevated NTC of 36.58%, contrasting with the relatively lower NTC of 30.67% observed in water extracts.

Table 2: Concentrations of Total Polyphenols, Flavonoids and Tannins of the Sunflower Seeds Collected from NSTU Sunflower Field (mean ± SD)

Sunflower Extracts	Polyphenols (g GAE/100g of sample)	Flavonoids (g CE/100g of sample)	Tannin (g TE/100g of sample)
SEE	8.61±0.002	18.58±0.1	30.67±0.007
SWE	5.29±0.01	30.67±0.03	36.58±0.08

Antioxidant Capacity: The antioxidant capacity of sunflower samples was evaluated by utilizing DPPH free radical eradicating capacity and a hydrogen peroxide (H_2O_2) scavenging method.

DPPH Radical Scavenging Activity: In accordance with the IC_{50} values (Table 3), ethanolic solution show a high amount of scavenging rate than that of aqueous, which refers to the solvent plays role on determining the antioxidant activity (Figure I).

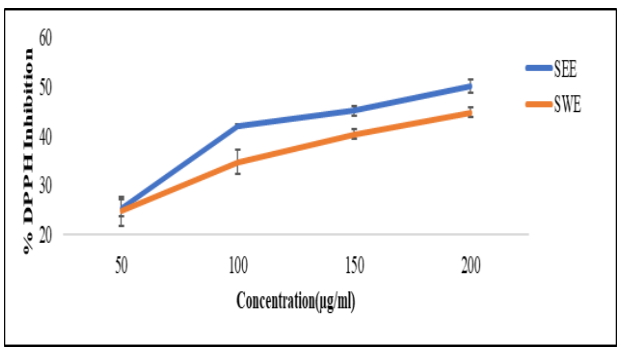

Figure I: DPPH scavenging activities of SWE and SEE at different concentration. Each value represents mean ± SD (n=3)

Table 3: IC_{50} of both DPPH and H_2O_2values of sunflower ethanolic and water extract collected from NSTU sunflower field

Parameters	SEE	SWE
DPPH scavenging activity IC_{50}	3.71	4.61
H_2O_2 scavengingIC_{50} Value	11.10	20.80

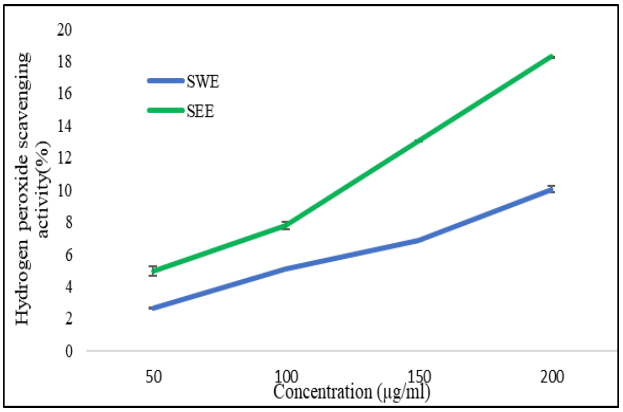

Figure II: H_2O_2 scavenging activities of SEE and SWE at different concentrations; each value represents the mean (n = 3)

The scavenging capacity of hydrogen peroxide: Based on the results, the IC_{50} values (Table 3), ethanolic exudates show a higher amount of scavenging rate than that of aqueous, which indicating that the role of solvents on estimating the antioxidant characteristics (Figure 2) extracts had potent H_2O_2 scavenging activity due to antioxidants and acted as good electron donors for the conversion of H_2O_2 to water. Figure 2 shows the scavenging capacity of SEE and SWE in a dose-dependent manner.

Discussion

This research represents the first recorded investigation into the antioxidant qualities of sunflower seeds obtained from the Noakhali region of Bangladesh. Solvent types and extraction methods have been shown to notably affect the quantity obtained as well as the antioxidant characteristics of the seeds. Plant materials contain numerous antioxidant materials with diverse chemical nature along with polarities, which determine their solubility in various solvents. Ethanol, being a solvent with polarity, is well-suited for extracting phenolic residuals and is deemed secure and dependable for human ingestion[36]. Conversely, polar inorganic solvent water is adept at extracting various bioactive phytochemicals[37].

Phenolics derived from plant sources are notable for their significant levels, attributed to their functional qualities, color, and texture. They play crucial roles not only as scavengers of singlet oxygen but also as free radical scavengers, thereby reducing molecular damage[38]. Primarily, phenolics act as potent antioxidants by stabilizing resonance upon donating hydrogen or electrons, thereby promoting health benefits. Phenolic acids and flavonoids, two forms of phenolics, are recognized as key defenders against reactive oxygen species in maintaining health[39]. Research by Moure *et al*. (2001)[40] highlights those solvents with higher polarity typically yield higher quantities of polyphenolics. However, the considerable variation in Total Phenolic Content (TPC) examined in this investigation may directly correlate with the choice of solvents utilized during the extraction process.

Plants exhibit vibrant colors, and their capacity to scavenge radicals is primarily attributed to flavonoids[41]. Even in small quantities, flavonoids possess antioxidant capabilities that can impede or decelerate the oxidation process of molecules. Non-enzymatic antioxidants such as flavonoids and polyphenols counteract prooxidants and hinder the formation of Reactive Oxygen Species (ROS)[42]. Numerous prior studies have underscored sunflowers as significant natural reservoirs of flavonoids, highlighting their antioxidant properties, ability to scavenge free radicals, potential for preventing coronary heart disease, and anticancer attributes[43].

Tannins represent another noteworthy secondary metabolite derived from plants, recognized for their astringent, antioxidant, and antimicrobial properties[44]. Hydrogen peroxide, while not inherently reactive, possesses a high capability to permeate cellular membranes[45]. In the presence of ferrous ions or superoxide ion radicals within the cell, it can generate hydroxyl radicals[46]. Extracts demonstrate the ability to scavenge hydrogen peroxide and convert it into water, primarily attributed to the presence of phenolics[47].

Reactive oxygen species (ROS) perform a pivotal task in disrupting the oxidative equilibrium within the human body, contributing to various disorders such as several types of diabetes, arthritis, certain type cancer, and atherosclerosis. ROS, including potent forms like hydroxyl radicals, can breach cell membranes and induce oxidative damage in the presence of Fe^{2+} or Cu^{2+}. Although hydrogen peroxide (H_2O_2) may not be highly reactive, its ability to oxidize thiol (-SH) groups found in various enzymes can lead to hazardous conditions within cells. Therefore, the removal of hydrogen peroxide, both endogenously and from external sources, constitutes a primary function of antioxidants. Diseases arise from an imbalance in oxidative equilibrium, emphasizing the importance of managing oxidative stress to prevent or mitigate disease formation.

In this investigation, the antioxidant properties of polyphenols, flavonoids, and tannins were assessed using different biological criteria and compared with established antioxidants such as gallic acid, catechin, and tannic acid. Additionally, to evaluate the capacity of antioxidants to counteract ROS, assays measuring their scavenging activity against H_2O_2 and DPPH were conducted. Results indicated that the samples exhibited increased scavenging capacity in a concentration-dependent manner in both the H_2O_2 and DPPH assays. Phenolics were found to neutralize ROS by donating electrons or hydrogen atoms.

Conclusion

Sunflower seeds sourced from the Noakhali region of Bangladesh hold promise as a rich reservoir of antioxidants due to their elevated levels of

polyphenols, flavonoids, and tannins. Our findings indicate significant values in DPPH free radical scavenging assays together with H_2O_2 scavenging capacities, further highlighting their antioxidant potential. Notably, ethanolic extracts yielded higher antioxidant contents, exhibiting increased Net Phenolic Content (NPC), Net Flavonoid Content (NFC), and Net Tannin Content (NTC), alongside substantial DPPH and H_2O_2 scavenging values. The results of this study might be useful in getting a suitable source of natural antioxidants, as an exogenous dietary source not only for humans but also for fishes, or as a drug source against human diseases. High scavenging value allows them to be used as a convenient source for oxidative stress-associated disorder.

List of Abbreviations

SWE = Sunflower water extract
SEE = Sunflower ethanolic extract
ROS = Reactive exogenous species
TPC = Total polyphenol content
TFC = Total flavonoid content
TTC = Total tannin content
DPPH = 1, 1-diphenyl-2-picrylhydrazyl
H_2O_2 = Hydrogen per oxide
SOD = Superoxide dismutase
CAT = Catalyst
GPx = Glutathione peroxidase
UV = Ultraviolet
HCL = Hydrochloric acid
Fe^{2+} = Iron (Ferrous ion)
Cu^{2+} = Copper (Cupric ion)

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Conflict of Interest
The authors declare 'no conflict of interest.' There is no conflict of interest regarding the publication of this paper.

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Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request. Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
All methods were performed in accordance with the relevant guidelines and regulations. The study lacked ethical approval from the Institutional Review Board. As it did not involve animals, informed consent was deemed unnecessary.

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