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Research Article

Phytochemical Quality and Antioxidant Effects of *Solanum retroflexum* Dun. Leaf Extracts on Oxidation Markers in a Sunflower Oil–Based Salad Dressing Emulsion

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Synthetic compounds that are commonly employed to hinder lipid oxidation in high-fat foods have been linked to numerous detrimental health effects. The use of plant extracts that exhibit antioxidant activity is preferred and deemed natural. The current study is aimed to evaluate the phytochemical quality (total flavonoid content (TFC) and total phenolic content (TPC)) and antioxidant activity (FRAP and ABTS) of aqueous reconstituted *Solanum retroflexum* methanolic leaf extracts. Thereafter, the phenolic profile was analyzed with high-performance liquid chromatography (HPLC), followed by the determination of the antioxidative effects of the *S. retroflexum* leaf extracts in salad dressing. The TFC and TPC of the *S. retroflexum* leaf extracts were 575.35 mg quercetin equivalents/g (dw) and 130.00 mg gallic acid equivalents/g (dw), respectively. The antioxidant activity was 1054.39 μM Trolox equivalents/g (dw) (ABTS) and 176.77 μM Fe equivalents/g (dw) (FRAP). Phenolic compounds identified with HPLC included protocatechuate, ellagic acid, and 4-hydroxycinnamic acid. All the extracts of *S. retroflexum* retarded hydrolysis of fat in salad dressing under accelerated Schaal oven test conditions, that is, as effective as butylated hydroxy anisole. In addition, *S. retroflexum* leaf extracts, when present at 300 mg/mL, slightly delayed peroxide formation in salad dressing perhaps owing to their high polar plant phenolic concentration. The outcomes of this research point out that *S. retroflexum* leaf extracts show potential as natural sources of antioxidants in high-fat foods like salad dressing emulsions. Further studies must determine how incorporation of the leaf extracts influences the sensory quality of the salad dressing.

Keywords: antioxidant activity; plant phenolics; salad dressing; *Solanum retroflexum* Dun.

1. Introduction

Approximately 1.3 billion tons of food, representing around one-third of the annual global food output, is squandered or misused [1]. South Africa with equally high levels of food waste is consequently encountering food security issues due to both water scarcity and food losses [2, 3]. Food losses are mostly attributed to inefficient farming practices, premature harvest, insufficient storage facilities, inadequate food safety practices, and inadequate infrastructure [4]. These postharvest spoilage or losses can be prevented using several methods such as chilling, reduction of water activity, restricting nutrients, freezing, acidification, fermentation, applying irradiation, thermal energy, or pressure, and using antimicrobial agents [5].

The primary cause of spoilage in emulsions, including salad dressing, and various other high-fat food products is autoxidation of polyunsaturated lipids and the resulting quality changes, instead of microbial degradation [6]. Autoxidation of polyunsaturated lipids results in the development of rancidity, a process that causes financial loss, while also reducing salad dressings' sensory attributes, shelf-life, functional properties, nutritional value (because of the loss of essential fatty acids), and safety [7]. Synthetic

chemical compounds having antioxidant properties, for instance, butylated hydroxy anisole (BHA), which are commonly employed to inhibit lipid oxidation have been associated with numerous adverse effects, including toxic effects and cancer [7–9]. These compounds neutralize the surplus of free radicals that induce lipid oxidation and increase the risk of chronic illnesses linked to oxidative stress [10, 11]. One way of improving the shelf-life of foods is to preserve them without the use of synthetic preservatives. This satisfies consumer needs for natural products and can be achieved by incorporating plant extracts exhibiting antioxidant activity into food products, like salad dressings.

Plant phenolics, namely, phenolic acids as well as flavonoids, have been identified in extracts derived from underutilized edible African leafy green vegetables, including *Solanum retroflexum* [12, 13]. *S. retroflexum* can act as sources of natural antioxidants, partly because of their phenolic content that can potentially inhibit or delay oxidation. The polyphenolic antioxidants' known modes of action in preventing oxidation include the scavenging of free radicals through hydrogen atom donation or just single electron transfer (ET), the inactivation of metal catalysts through chelation, the reduction of hydroperoxides into hydroxyl derivatives, and interactions with various other reducing agents [14]. However, despite the growing demand for safe natural antioxidants from underutilized indigenous plants and their potential economic benefits, limited research exists on using *S. retroflexum* extracts as natural antioxidants or preservatives for emulsions like salad dressings, which are frequently needed for delivering polyunsaturated fatty acids (PUFAs) [15, 16]. These PUFAs contribute to synthesizing essential hormones, like prostaglandins, which control physiological parameters like arterial pressure, cholesterol levels, and the genital system [7, 15]. Hence, this study aimed to assess the effectiveness of *S. retroflexum* leaf extracts as antioxidants and their potential application in salad dressings.

2. Experimental (Materials and Methods)

2.1. Materials. Analytical and high-performance liquid chromatography (HPLC) grade chemicals were procured from Sigma-Aldrich (Johannesburg, South Africa) and ACE chemicals (Pty) Ltd (Johannesburg, South Africa), while food-grade ingredients for salad dressing preparation were acquired from Pick n Pay (Johannesburg, South Africa). Raw sunflower oil was kindly supplied 2 weeks prior to salad dressing preparations by Siqalo Foods (Pty) Ltd (Johannesburg, South Africa).

2.2. Methods

2.2.1. Plant Material Collection. S. retroflexum fresh leaves were procured from local growers at Thohoyandou, Limpopo province, South Africa and authenticated by the Department of Plant Biotechnology and Botany, University of Johannesburg. These fresh leaves were transported under cool conditions, thoroughly cleansed and any surplus water was discarded. To preserve them, the foliage underwent freeze-drying using an in-lab freeze dryer (Telstar Lyoquest 55, United States). Thereafter, they were ground into an extremely tiny powder utilizing a mortar and pestle and kept in sealed Eppendorf tubes at room temperature until subsequent utilization.

2.2.2. Methanolic Extract Preparation. S. retroflexum leaf samples were extracted following the procedure reported by Abu-reidah et al. [17], Moyo et al. [18], and Sikwese and Duodu [19] with slight adjustments. Briefly, 20 mL of 80% aqueous methanolic solution was combined with 2 g of dried leafy material. To enhance the extraction yield, the resulting mixture was agitated for 30 min at environmental temperature. The samples thereafter underwent centrifugation at 4°C for 10 min at a velocity of 3000 rpm. Then, the supernatant was concentrated to 2 mL with a Labtech EV 311+ rotary evaporator under vacuum at 35°C. The extract was oven dried over night at 35°C in order to eliminate nonfood-grade methyl alcohol. After reconstituting with phosphate-buffered saline (PBS), the resultant extract was kept at −20°C until its next usage. The extractions were conducted three times.

2.2.3. Quantification of Total Phenolic Content (TPC). The TPC in *S. retroflexum* extracts was estimated with the Folin-Ciocalteu (FC) procedure outlined by Ainsworth and Gillespie [20]. The extract (10 μL) was transferred to Eppendorf tubes and mixed thoroughly using a vortex mixer. FC solution (50 μL) was added to every tube, and sodium carbonate (50 μL) thereafter. Utilizing the Bio-Rad iMark microplate absorbance reader (labs 168-1130), the absorbance of extracts/standards was determined at 750 nanometers and each value was calculated in mg gallic acid equivalents/g dry weight of sample through the use of standard curves.

2.2.4. Quantification of Total Flavonoid Content (TFC). The TFC in *Solanum retroflexum* extracts was calculated with the aluminum trichloride colorimetric procedure proposed by Kalita et al. [21] and Al-Farsi and Lee [22]. Extracts or standard (10 μL) was transferred to each Eppendorf tube and shaken thoroughly. First, sodium nitrite (30 μL), then aluminum trichloride (30 μL), and finally sodium hydroxide (100 μL) were added into every tube. Utilizing the Bio-Rad iMark microplate absorbance reader (labs 168-1130), the absorbance of standards/extracts was determined at 450 nm and each value was quantified in mg quercetin equivalents/g dry weight of sample through the use of standard curves.

2.2.5. Quantification of Antioxidant Activity

2.2.5.1. ABTS (Diammonium 2,2′-Azino-Bis-[3-Ethylbenz thiazoline-6-Sulfonate]). The antioxidative activity of *S. retroflexum* leaf extracts was evaluated by means of the ABTS method proposed by Moyo et al. [18]. The plant extract (10 μL) was transferred to microtiter plate wells, and then, 0.26 mM ABTS free radical cation solution (290 μL) was added. The 96-well microtiter plate was incubated at 37°C in the absence of light for 15 min after being enveloped in the aluminum foil. Extract absorbance was determined at 750 nm utilizing Bio-Rad iMark microplate absorbance

reader (labs 168-1130). Each value was quantified in μmol Trolox equivalents/gram dry weight of sample through the use of standard curves.

2.2.5.2. FRAP (Ferric Ion Reducing Antioxidant Power). S. retroflexum extract antioxidant activity was assessed using the FRAP technique as outlined by Adedapo et al. [23], Ahmed Salatou [24], and Gohari et al. [25] with various alterations. Trolox was used as the standard. The extract (30 μL) was combined with fresh working FRAP solution (900 μL), and100 μL each of the mixture was dispensed into the wells of the microtiter plate and left to incubate in the absence of light for 30 min. Absorbance measurements were conducted at 595 nm using a Bio-Rad iMark microplate absorbance reader (labs 168-1130). Each value was quantified in μmol Fe equivalent/gram dry weight of sample employing the standard curves.

2.2.6. Quantification of the Phenolic Profile of S. retroflexum Leaf Extracts. HPLC was utilized to analyze the phenolic profile following the technique by Molehin et al. [26] with minor changes. Leaf extracts underwent initial filtration through a 0.45-μm syringe filter. Analysis was done with HPLC (Agilent 1200 Infinity) series instrument featuring a diode array detection system (Agilent Technologies, Waldbronn, Baden-Württemberg, Germany) fitted with Agilent Zorbax Eclipse Plus C18 (4.6 mm × 1.5 mm × 3.5 μm) (Agilent, Newport, California, United States) column at 25°C. Solvents, 0.1% HPLC grade methanoic acid in water (A) and 0.1% methanoic acid in HPLC grade methyl cyanide (B), were run at gradient with flow rate of 0.8 mL/min. The solvent gradients were established as follows: 40% solvent B for 2 min, 10% solvent B from 2 to 27 min, 30% solvent B from 27 to 30 min, and 90% solvent B from 30 to 35 min. Afterwards, 92% solvent A was employed to facilitate the re-equilibration of the column. Gallic acid, 2,4-dihydroxybenzoic acid, protocatechuate, gentisic acid sodium, ellagic acid, *trans*-ferulic acid, quercetin, quercetin-3-rutinoside hydrate, 4-hydroxycinnamic acid, chlorogenate, and *trans*-caffeate were used as standards.

2.2.7. Salad Dressing Sample Preparations for Antioxidant Effectiveness Tests. Salad dressing samples were prepared according to Khoza [27] and Nejad et al. [28] method with few alterations. Salad dressings were formulated in batches weighing 1.5 kg. The formulations for the salad dressing samples were outlined in Table 1. The selection of the aforementioned concentrations was based on existing literature [29]. Powdery BHA (150 mg/kg) was used as a reference antioxidant under the legally permissible threshold of 200 mg/kg specified by the Department of Health, South Africa (DOH) (1972). The prepared emulsions were stored at 4°C within Schott Duran glass bottles wrapped with aluminum film to shield them from light. Before preparation, methanol-free plant extracts (1 mg) were recomposed in distilled water (1 mL) because PBS solution is unsafe for human consumption.

2.2.8. Oxidative Stability Study of Salad Dressing Samples When Exposed to Accelerated Storage Conditions. All prepared salad dressings were stored in a forced-draft oven in the absence of light at 65°C. The aforementioned temperature was selected to avoid the phase separation in the emulsion, with every day of storage assumed to equate to 1 month at ambient temperature (21°C) [24, 30, 31]. The oxidative stability of each sample was assessed through determination of peroxide values (POVs) and free fatty acids (FFAs) at 3-day intervals over a 12-day storage duration.

2.2.8.1. Determination of FFA. Salad dressing sample FFA was calculated in triplicate through titration with sodium hydroxide (0.1 M) using AOAC official method 940.28 [30, 32, 33].

FFA (%) were calculated by applying the subsequent formula:

$$\text{Free Fatty acid}\,(\%) = \frac{28.2 \times \text{v} \times \text{N}}{\text{W}}$$

In which, v represents sodium hydroxide's volume in mL utilized during titration; N represents its normality, while W represents the weight of the salad dressing sample weight in g.

2.2.8.2. Determination of POV. Salad dressing sample POVs were obtained in triplicate by titrating with sodium thiosulfate, utilizing 1% starch as indicator [30, 33]. POVs were obtained by applying the subsequent calculation.

$$\text{POV} = [(v - v_0) \times \text{t/M}] \times 103\ \text{milliequivalents of oxygen per kilogram}\,(\text{mEq/kg})$$

In which, v indicates the titrated volume in mL; v_0 represents the blank volume in mL; t represents sodium thiosulfate's molarity in mol/L (moles/L), while M represents salad dressing mass utilized in g [30, 33].

2.2.8.3. Determination of Oxidative Stability Index (OSI) With Rancimat Method. Prior to determining the OSI induction time, an extraction of oil from salad dressing was performed. The oil phases of salad dressings were retrieved utilizing the technique stated by Pavlović et al. [34] and Jacobsen et al. [35], with certain tweaks. To obtain the oil phases, salad dressing samples were subjected to freezing for 24 h at −80°C, defrosted at environmental temperature, and thereafter centrifuged at 4°C for 10 min.

OSI induction times of salad dressing oils were measured in triplicate using the technique recommended by the American Oil Chemists' Society (AOCS) procedure Cd 12b-92 [36]. Rancimat (Metrohm) from Wilmar SA PTY (Ltd) was used. At 110°C, samples (2.5 g ± 0.03) were studied with a continuous air flow (20 L/hr).

TABLE 1: Formulations of salad dressings utilised for the antioxidant efficacy tests.

Ingredients and amounts added	Salad dressing with *Solanum retroflexum* extract (100 mg/mL)	Salad dressing with *Solanum retroflexum* extract (300 mg/mL)	Salad dressing with *Solanum retroflexum* extract (400 mg/mL)	Salad dressing with butylated hydroxy anisole (BHA), (0.15 mg/mL)	Salad dressing without *S. retroflexum* extract (control)
Unrefined sunflower oil	45%	33.83%	33.83%	45%	45%
Distilled white vinegar	23.11%	23.11%	23.11%	23.11%	23.11%
Salt	1%	1%	1%	1%	1%
Antioxidant added	10%	30%	40%	0.015%	0%
Egg albumin	0.18%	0.18%	0.18%	0.18%	0.18%
Xanthan gum	0.05%	0.03%	0.03%	0.05%	0.05%
Water	20.66%	11.85%	1.85%	30.66%	30.66%

2.2.9. Statistical Analysis. Data gathered were examined using one-way analysis of variance (ANOVA) in IBM SPSS Statistics version 25.0. The findings were reported as mean value of triplicates ± standard deviation. Significant differences between the means of the samples were established by employing the Tukey HSD test with a significance level of 95%. A correlation analysis was carried out utilizing Pearson's correlation coefficient at a correlation level of 1% to examine the potential relationships between *S. retroflexum* leaf extracts' antioxidant activities (FRAP and ABTS) with their TFC and TPC.

TABLE 2: Total phenolic content, total flavonoid content, and antioxidant activity of *Solanum retroflexum* leaf extracts.

Analyses	Total phenolic and flavonoid contents and antioxidant activity
TPC	130.00 ± 19.63 mg gallic acid equivalents/g (dw)
TFC	575.35 ± 43.07 mg quercetin equivalents/g (dw)
ABTS	1054.39 ± 6.23 μM Trolox equivalents/g (dw)
FRAP	176.77 ± 8.89 μM Fe equivalents/g (dw)

Note: Values represent the mean ± standard deviation of three experiments done in triplicate. TPC denotes the total phenolic content, TFC refers to the total flavonoid content, and dw stands for dry matter weight.

3. Results and Discussion

3.1. TPC, Flavonoid Content, and Antioxidant Activity of S. retroflexum Leaf Extracts. Prior studies have documented the occurrence of polyphenols in *Solanum* species [37–39]. The TPC determines the amount of phenolic compounds, while the TFC determines the amount of flavonoids, a class of phenolic compounds exhibiting antioxidant capacity [14, 21]. The TPC, TFC, ABTS, and FRAP of *S. retroflexum* extract are displayed in Table 2. In this study, the TPC (130.00 mg gallic acid equiv./g dw) of *S. retroflexum* extract and its TFC (575.35 mg quercetin equiv./g (dw)) were substantially greater than those found by Daji [12] (4.57 mg gallic acid equiv./g (dw) and 3.03 mg quercetin equiv./g (dw), respectively) and that reported for other methanolic leaf extracts from *Solanum* species such as *Solanum nigrum* (97.96 mg gallic acid equiv./g (dw) and 16.42 mg quercetin equiv./g (dw), respectively) and *Solanum tuberosum* (10.25 mg gallic acid equiv./g fresh weight) [40, 41]. The variation in findings may emanate from the relatively lower drying temperature (35°C) used in our extraction method when compared to other studies that dried at 37°C [40] and 40°C–50°C [12]. Low drying temperature results in higher yield of polyphenols in extracts since polyphenols are heat-sensitive and easily oxidized [42]. Also, the difference in plant species, differences in the stage of growth, and the different growing conditions of plants including drought stress, alkalinity, salinity, and UV stress have been reported to affect the flavonoid and phenolic contents [18, 43, 44]. For instance, under drought stress, the TPC increases in leaf extracts in species such as *Achillea* species since stress triggers secondary metabolite synthesis [43]. In addition, younger leaves of plants such as *Pistacia atlantica* have higher phenolic content than old mature leaves due to their greater levels of chemical and mechanical defences as compared to mature leaves [45].

With antioxidant activity of *S. retroflexum*, the ABTS yielded an antioxidant activity of 1054.39 μM Trolox equiv./g, while the FRAP assay yielded 176.77 μM Fe equiv./g (Table 2). It must be noted that both assays evaluate the comparative abilities of antioxidants to neutralize synthetic radicals in relation to conventional antioxidants, specifically ferrous sulphate heptahydrate for FRAP and Trolox for ABTS [46]. Therefore, the observed differences between the two methods were thought to be due to that FRAP is an assay that involves single ET, whereas ABTS decolorization assay combines two modes of ET, namely, hydrogen atom transfer (HAT) and ET [47]. Compared to literature, the antioxidative activity of *S. retroflexum* extract in the current research was greater than those found by Daji [12] of 60.17 μM Trolox Equiv./g (dw) and 17.2 μM Fe equiv./g when determined with ABTS and FRAP, respectively. Similarly, other *Solanum* spp. including *Solanum sisymbriifolium, Solanum incanum, Solanum khasianum, Silphium integrifolium, Solanum aethiopicum*, and *Solanum*

torvum leaf extracts were reported to have a lower antioxidant activity with FRAP values ranging from 0.72 to 8.11 μmol Trolox equiv./g and ABTS values ranging from 2.7 to 12.94 μM Trolox equiv./g, in comparison to the present research [39]. The relatively great antioxidant results of this study could be explained by the reasons given for TPC above. Also, the high antioxidant activity demonstrated by the *S. retroflexum* leaf extract indicates its potential to retard autoxidation of polyunsaturated lipids and provide health-promoting properties.

TPC and TFC of *S. retroflexum* leaf extracts showed a strong positive correlation with the FRAP assay (1.00) as well as with the ABTS assay (1.00), respectively. The aforementioned findings align with a research conducted by Daji [12] and Moyo et al. [48] which similarly reported a strong positive correlation among TFC, TPC, and antioxidant activity assays for *S. retroflexum* and *S. nigrum* extracts, respectively. The correlation coefficients demonstrate that the *in vitro* antioxidant potential of *S. nigrum* and *S. retroflexum* leaf extracts, as measured by FRAP and ABTS tests, might be connected to the occurrence of polyphenols like flavonoids and phenolic acids. Antioxidant capacities of phenolic acids originate from the reactive nature of the hydroxy group (-OH) attached to the aromatic, benzene-like ring, with radical scavenging being their main mechanism of antioxidative action [14, 24]. The significant correlation between FRAP assay and flavonoids, which is an ET assay utilizing iron (II) chelation, could potentially be accounted for by metal-complexing sites present within flavonoids including the ortho-hydroxy groups attached to the B ring, the 3-hydroxy groups linked to the 4-carbonyl group, or the 5-hydroxy groups linked to the 4-carbonyl group [14].

3.2. Phenolic Profile of S. retroflexum Leaf Extracts Determined Using HPLC. The phenolic acids identified in methanolic extract of *S. retroflexum* are presented in Table 3. Additionally, the chromatogram of mixed standards is displayed in Figure 1. Two commonly occurring compounds, hydroxybenzoic acids (protocatechuate and ellagic acid) and one hydroxycinnamic acid (4-hydroxycinnamic acid) were detected in *S. retroflexum* extracts. The compound p-CoQA–p-coumaroylquinic acid, a 4-hydroxycinnamic acid ester, was also detected elsewhere in *S. retroflexum* extracts with UPLC-qTOF-MS [37]. The most prevalent phenolic acid found in the *S. retroflexum* leaf extract was ellagic acid (3649.84 μg/mL), while the least prevalent hydroxybenzoic acid was protocatechuate (58.41 μg/mL). Protocatechuate and ellagic acid possess antimicrobial and antioxidant activities [49, 50]. Notably, the protocatechuate concentration in *S. retroflexum* extract was greater than the one recorded by Huang et al. [38] in *S. nigrum*, which was 10.59 μg/g.

The sole hydroxycinnamic acid identified in the *S. retroflexum* leaf extract here was 4-hydroxycinnamic acid present at 641.44 μg/mL. Prior studies reported that 4-hydroxycinnamic acid has antioxidant and antimicrobial activities by disrupting fungal cell membrane and inhibiting the expression of tumor necrosis factor alpha [51]. Likewise, the concentration of 4-hydroxycinnamic acid in *S. retroflexum* from this study was greater than that published by Huang et al. [38] of 7.85 μg/mL for *S. nigrum* extracts and lower than that found by Degrain et al. [52] of 1115 ± 0.76 μg/mL in *S. retroflexum* extracts. The discrepancies in the aforementioned results might possibly be attributable to the distinct extraction technique, extractant employed and growing conditions. Mokgope [53] states that hydroxycinnamic acids, namely, 4-hydroxycinnamic acid, often exhibit more antioxidant activity compared to hydroxybenzoic acids like protocatechuic derivatives or ellagic acid. The possible reason for this is that the –CH=CH-COOH moiety found in derivatives of cinnamylic acid possess stronger capacity to donate hydrogen atom and greater stabilization of phenoxy radicals compared to the carboxylate moiety of hydroxybenzoic acid [53].

3.3. Effect of S. retroflexum Leaf Extract on the FFA of Salad Dressings. Synthetic antioxidants like BHA are generally used to hinder or prevent oxidation in food [14]. However, as consumer demand for natural antioxidants is increasing, it is worthy to note that their efficacy in homogenous solutions like methanol cannot be directly extrapolated to more complex food systems including salad dressing [24]. In this research, the efficacy of *S. retroflexum* leaf extracts in inhibiting oxidation of salad dressings was evaluated through oxidative stability tests like FFA. FFAs, resulting from triglyceride hydrolysis, are more susceptible to autoxidation than intact triglycerides [54]. The FFA contents of salad dressing samples stabilized with BHA (0.15 mg/mL) or *S. retroflexum* extracts at various concentrations (400 mg/mL, 300 mg/mL, and 100 mg/mL) over a period of 12 days accelerated storage in an oven at 65°C are presented in Figure 2. Overall, the FFA content of all samples decreased after 3 days except salad dressing with BHA, which showed a slight increase (0.70%). The observed decrease in FFA content after 3 days was ascribed to the volatilization of FFAs at elevated temperatures [55]. Thereafter, the FFA content increased after 6 days followed by another decrease after 12 days. The highest increase in FFA occurred with the control after 6 days, possibly because of lack of inhibitors in the control sample [55]. The increase in FFA content of the treated salad dressing was attributed to moisture, elevated temperatures, and enzymes including lipase derived from ovalbumin, lactic acid bacteria (LAB), or moulds [24, 56, 57]. Overall, all salad dressings treated with *S. retroflexum* showed decrease in FFA after 12 days, while a slight increase (0.11%) occurred with the control. On Day 12, the salad dressing samples with BHA at 0.15 mg/mL and *S. retroflexum* extracts at 400 mg/mL, 300 mg/mL, or 100 mg/mL exhibited significantly ($p \leq 0.05$) lower percentages of FFA compared to the control sample (no extracts). Additionally, it is important to mention that on Day 12, there was no statistically significant difference ($p > 0.05$) in the FFAs of all salad dressing samples treated with *S. retroflexum* and that of salad dressing sample with BHA. Therefore, it might be said that from Day 12, BHA and *S. retroflexum* extracts at 100 mg/mL, 300 mg/mL, and 400 mg/mL showed a similar inhibition on the generation of FFA in salad dressing. To clarify, *S. retroflexum* extracts at 100 mg/mL, 300 mg/mL, and 400 mg/mL slowed hydrolytic rancidity in salad dressing

TABLE 3: Phenolic profile of *Solanum retroflexum* leaf extracts (μg/mL) analyzed by HPLC-DAD.

Rt	Phenolic acids	Molecular weights (g/mol)	*S. retroflexum* (μg/mL)
	Hydroxybenzoic acid		
2.387 min	Gallic acid	170.12	Nd
2.555 min	Ellagic acid	302.20	3649.864
11.484 min	2,4-Dihydroxybenzoic acid	154.12	Nd
3.976 min	Protocatechuate	154.12	58.409
	Hydroxycinnamic acid		
10.179 min	*trans*-Caffeate	180.16	Nd.
15.193 min	4-Hydroxycinnamic acid	164.047	641.444

Abbreviations: Nd: not detected; Rt: retention times.

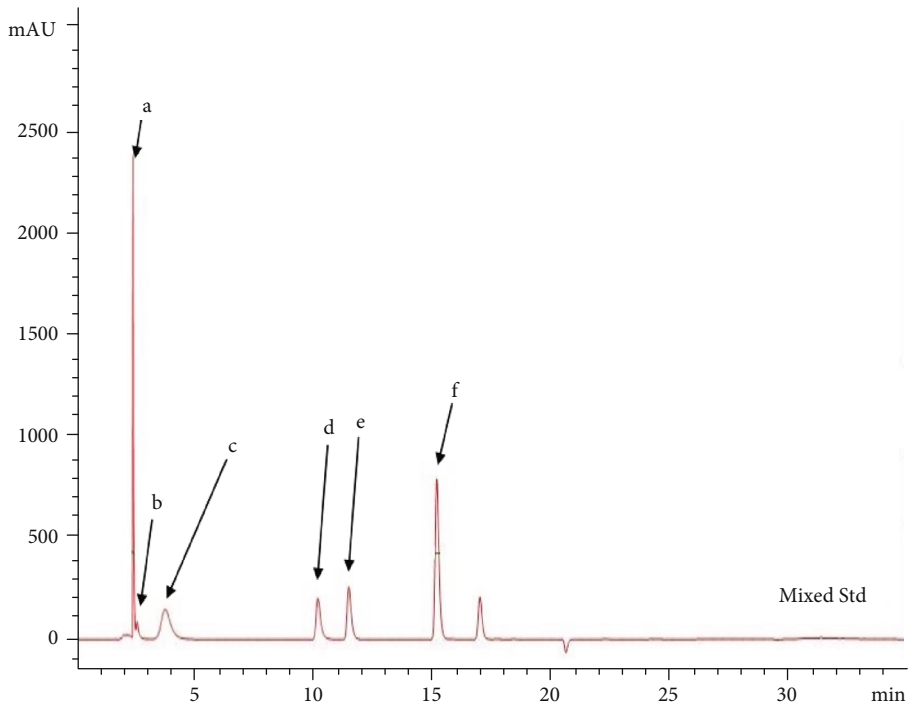

Figure 1: HPLC chromatogram from mixed standards. (a) Gallic acid. (b) Ellagic acid. (c) Protocatechuic acid. (d) Caffeic acid. (e) Dihydroxybenzoic acid. (f) Para-coumaric acid.

during accelerated storage as effective as BHA and, thus, could potentially extend its shelf-life. It is also essential to note that the delaying effect in hydrolytic rancidity by this extract was not concentration-dependent. The inhibiting effect of *S. retroflexum* leaf extracts may be linked to the elevated levels of polar phenolcarboxylic acids like ellagic acid or 4-hydroxycinnamic acid, present in the aforementioned extracts. Indeed, prior studies have documented the lipase-inhibiting properties of polar phenolcarboxylic acids, including *trans*-caffeate and chlorogenate, which were detected in the methanol extracts of *S. retroflexum* and identified by Daji et al. [37, 58]. Elsewhere, Zia-Ur-Rehman et al. [59] revealed that soyabean oil containing benzine *S. tuberosum* peel extracts at different concentrations (800, 1600, and 2400 ppm) showed similar FFA inhibition to that of soyabean oil with BHA (200 ppm) after 15 days storage at 45°C. Similarly, *S. retroflexum* leaf extracts of this study compared well with BHA despite the differences in test concentration and extracts.

Interestingly, it was additionally observed that on Day 3, salad dressing comprising 300 mg/mL *S. retroflexum* extract exhibited a substantially ($p \leq 0.05$) lower percentage of FFA in contrast to all other samples. This suggests that after 3 days, the best FFA inhibition occurred with 300 mg/mL *S. retroflexum* extract in salad dressing. A possible explanation for this result could be that higher concentration of extract in salad dressing (400 mg/mL) could have led to limited solubility and thus, reduced effectiveness [60].

3.4. Effect of S. retroflexum Extract on the POV of Salad Dressings. POVs were assessed in order to ascertain the degree to which generation of hydroperoxides (primary

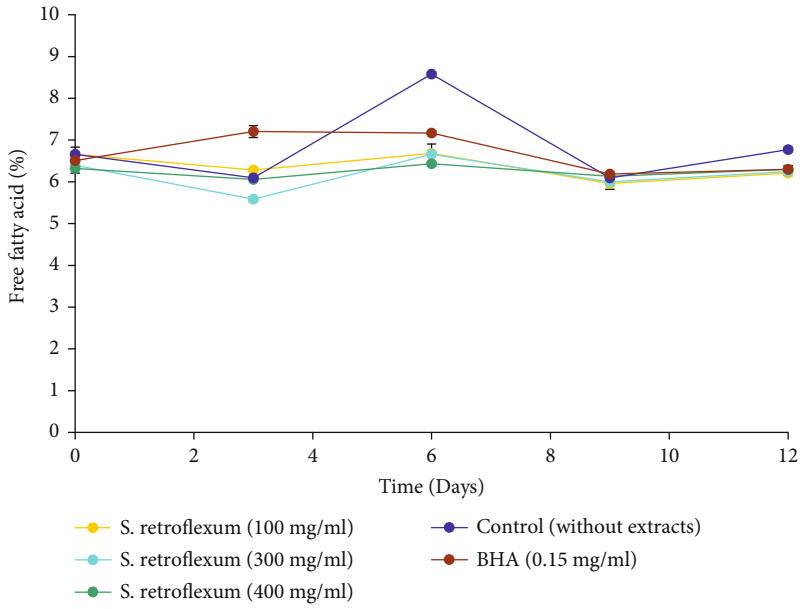

Figure 2: The effect of *S. retroflexum* leaf extracts on the free fatty acid (FFA) of salad dressing during 12-day accelerated storage at 65°C. BHA: butylated hydroxy anisole ($n = 3$).

oxidation byproducts) was hindered by the incorporation of synthetic (BHA) or natural antioxidants [29]. POV may provide valuable information about the initial stages of emulsion oxidation which are referred to as "initiation" and "propagation" [61]. Peroxide may be subject to subsequent oxidation reactions to generate various nonvolatile and volatile secondary compounds [54]. The POVs of salad dressing samples containing BHA and *S. retroflexum* extracts at various concentrations during a period of 12 days accelerated storage in an oven at 65°C are presented in Figure 3. Overall, the POVs of all salad dressing samples rose as storage time increased, primarily as a result of oxidation. Oxidation of salad dressing is facilitated by transition metal ions (especially copper and iron ions), light exposure, high temperatures, exposure to air (oxygen), enzymes (lipoxygenase), and other parameters associated with the dressing composition including pH, size of droplet, or electric charge at the oil–water emulsion interface [61, 62]. The control showed a decrease in POV between Day 3 and Day 6. This might have been due to the volatilization of primary oxidation byproducts especially peroxides [7].

It must be noted that on Day 3, salad dressing samples comprising 100, 300, and 400 mg/mL *S. retroflexum* leaf extracts had substantially ($p \leq 0.05$) lower POVs than those of control. Thus, it can be asserted that on Day 3, *S. retroflexum* leaf extracts retarded lipid oxidation. The relatively low antioxidative effects of *S. retroflexum* extracts (Day 3) on peroxide formation in salad dressings could be explained by the specific structural features and the polarity of phenolic compounds inherent in these extracts because they were aqueous reconstituted and salad dressing's oil-in-water nature. The modes of action underlying phenolics' antioxidant activity are ET and HAT [47]. Methyl alcohol possesses a lower dielectric constant ($\varepsilon = 32.6$ at 25°C) in comparison to that of water ($\varepsilon = 80$ at 25°C), which allows it to dissolve

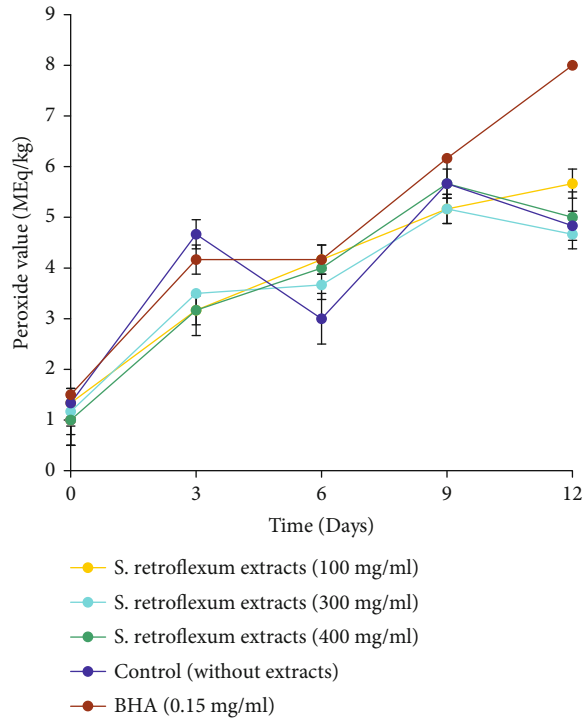

Figure 3: The effect of *S. retroflexum* leaf extracts on the peroxide value of salad dressing during 12-day accelerated storage at 65°C. BHA: butylated hydroxy anisole ($n = 3$).

a greater spectrum of analytes having medium and low polarity than water [63]. Consequently, aqueous reconstituted methanolic *S. retroflexum* extracts would have more polar antioxidants and less nonpolar antioxidants compared to aqueous extracts. Since the "polar paradox" indicates that nonpolar antioxidants are more efficient in more polar

systems such as oil-in-water emulsions (salad dressings) than polar antioxidants, aqueous reconstituted methanolic *S. retroflexum* extracts will therefore be slightly efficient in hindering hydroperoxide generation [61, 62].

Additionally, it is crucial to highlight that the antioxidant efficacy of *S. retroflexum* extracts at 300 mg/mL was generally better in delaying formation of hydroperoxides than that of salad dressing comprising 400 mg/mL of the same extract. This observed pattern aligns with the cut-off theory in emulsion, which suggests that the antioxidant effectiveness tends to increase with antioxidant concentration as the alkyl chain lengthens, until an optimum concentration and threshold in hydrophobicity is attained. Thereafter, increasing the concentration and alkyl length decreases the efficacy of the antioxidant due to antioxidant self-aggregation [64, 65]. In line with this, Franco et al. [66] noticed that ethanolic *S. tuberosum* extracts at 20.37 ppm in soyabean oil, when subjected to accelerated peroxidation conditions (60°C, 15 days), were more effective in delaying the formation of peroxides than *S. tuberosum* extracts at 31.94 ppm in soybean oil under the same conditions.

3.5. Effect of S. retroflexum Leaf Extracts on OSI of Salad Dressing Oils. The effect of *S. retroflexum* leaf extracts and BHA as antioxidants in salad dressing oils was assessed at 110°C by measuring the OSI induction time, which represents the duration it takes for lipids to undergo a fast increase in autoxidation rate. In other words, this OSI value is the point where maximal oxidation rate change is reached, triggered by increased conductivity as volatile organic acids (secondary oxidation compounds produced at the terminal stage of oxidation) are formed [46]. A longer induction time is associated with prolonged shelf-life and conversely [67]. The induction time of salad dressing oils with *S. retroflexum* extracts decreased as the extract concentration increased (Table 4). The longest induction time occurred with BHA and control, while the least occurred with salad dressing containing 400 mg/mL *S. retroflexum* extracts. The observed low induction time at the highest (400 mg/mL) *S. retroflexum* leaf extract concentration implies that the extract increased the pace of accelerated phase of oxidation. To put it simply, *S. retroflexum* extracts had a pro-oxidant effect in salad dressing oil. Overall, the addition of *S. retroflexum* extracts did not improve the induction time. Similarly, the induction time for the BHA containing salad dressing was also identical ($p > 0.05$) to that of the control. In line with the current findings, a study by Ribeiro and Jorge [68] that evaluated the effects of BHA (200 mg/kg oil) as well as hydroalcoholic extract from coffee husk (200 mg/kg oil) on the oxidative stability of soybean oil using Rancimat at 100°C also found no significant differences among induction periods of the control, BHA, and coffee husk extracts between 0 and 5 days. The lack of effectiveness of the hydroalcoholic extracts of plants including *S. retroflexum* and coffee husk in salad oil may be linked to the high concentration of polar phenolcarboxylic acids notably *trans*-caffeate, ellagic acid, and 4-hydroxycinnamic acid in these extracts [68]. As stated by the polar paradox, these polar antioxidants are less efficient in oil such as sunflower oil than nonpolar antioxidants [62].

TABLE 4: Effect of *S. retroflexum* extracts on the oxidative stability index (OSI) of salad dressing oil at 110°C.

Samples	Induction time (hours)
Control (without extracts)	7.93 ± 0.93^{c}
BHA (0.15 mg/mL)	8.00 ± 0.35^{c}
Solanum retroflexum extract (100 mg/mL)	7.73 ± 0.15^{bc}
Solanum retroflexum extract (300 mg/mL)	7.40 ± 0.53^{bc}
Solanum retroflexum extract (400 mg/mL)	5.07 ± 0.25^{a}

Note: Values represent means of three determinations ± standard deviation. Means with different superscripts within the same column indicate a significant difference ($p \leq 0.05$).

By law, salad dressing shall possess a shelf-life of 6 months at refrigerated temperatures [69]. According to the predictions table of shelf life from OSI and Rancimat Induction times for oils, induction time results ranging between 6 and 12 h equate to a commercial shelf life of 6 months at 21°C [61]. All salad dressing oil samples except the sample having 400 mg/mL *S. retroflexum* extracts exhibited induction times higher than 6 h. Therefore, these oil samples except the sample having 400 mg/mL *S. retroflexum* extracts conformed to the legal shelf-life requirement of 6 months.

In fats and oil products such as salad dressing, the maximum levels of POV, acid value (AV), and percentage of FFA are 10 milliequivalents/kg oil, 4 mg KOH/g fat, and 2.01%, respectively (Codex [70]). All salad dressings samples in this study had POVs lower than 9 but their percentage FFA were substantially higher than 2.00% which suggests that samples were within acceptable level for POVs but not within acceptable levels of FFA, perhaps due to their moisture content.

4. Conclusions

This study found that reconstituted aqueous methanolic *S. retroflexum* extracts at 300 mg/mL can slightly improve the oxidative stability of salad dressing under accelerated storage at 65°C, due to their rich polar phenolic content (ellagic acid, 4-hydroxycinnamic acid, protocatechuate, and gallic acid). These extracts (100, 300, and 400 mg/mL) also slowed hydrolytic rancidity in salad dressing, as effective as matched BHA (150 mg/mL), because of their high concentration of lipase inhibitors (*trans*-caffeate, chlorogenate, ellagic acid, and 4-hydroxycinnamic acid). However, following the polar paradox theory, these extracts did not enhance salad oil stability at 110°C, given their high polar phenolic content. Based on these findings, *S. retroflexum* extracts show promise as natural antioxidants for stabilizing food systems like salad dressing. The antioxidant effects of these extracts in salad dressings can be enhanced through advanced extraction techniques, including ultrasound-assisted extraction and high-pressure processing, combining antioxidants with other extracts, microencapsulation, and storage conditions (dark and cool environments) [71–74]. Further research

could examine how extracts affect other oxidation markers using other methods (anisidine value and gas chromatography mass spectrometry), optimize their effects in salad dressing emulsions through manipulating various factors (interfacial area of oil-in-water emulsion droplets, pH, packaging conditions, and temperature), and conduct thorough shelf-life studies including microbial tests and sensory evaluation.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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