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Editorial

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Dear readers,

As another year comes to a close, we have seen time and time again that science remains at the forefront of the world. Climate change, instability in the world, scientific misinformation, and everchanging biomedical concerns all require scientific advancements. Because of this reality, funding for scientific research and science communication are vital for continuing groundbreaking discoveries in the sciences to make this world a better place.

The Chemist is an interdisciplinary general audience journal that publishes scientific articles in all fields of chemistry. The journal also publishes articles that are submitted by the Chemical Pioneer and Gold Medal Award winners offered by the American Institute of Chemists (AIC). Keeping with our commitment to diversity, equity, and inclusive excellence (DEI), we publish scientific articles from authors coming from all regions of the world with every continent represented. Many of our authors come from the Middle East, Asia, and Africa and have published interesting articles on natural products that come from this part of the world.

This year, we have a common theme amongst published articles that involve the synthesis and extraction of natural compounds. Many of these articles include a submission by Atolani et al, which details "Chemical Characterization, Cytotoxicity, Anti-inflammatory, Antioxidant, Antimicrobial and Anti-toxoplasmosis of *Chrysophyllum albidum* Seed Oil." Another article by Drakli et al details "Antioxidant, Chemical Composition of Syrian Essential Oil's *Juniperus excelsa* Fruit and Leaves."

More studies in food analysis include Oboladze et al's "Development and Feasibility Evaluation of the Extraction Methods for Obtaining Quercetin from Onion Peel." Continuing with this common motif, Aliyu et al discussed "Characterization, Chemical Composition and Cream Formulation from the Seed Butter of *Mangifera indica* L." It is obvious that the chemical analysis of foods, liquids, and extraction of chemical compounds from these substances is of vital importance for the greater scientific community and lay general audience as well. In the fields of food preservation, Tella et al offer an interesting review on innovations and food preservation that could have long-reaching and lasting effects on the food supply chain and ensuring individuals have a reliable and safe food source as described in "Modern Innovations in Food Preservations: Mini-review."

The analysis of chemical biomarkers in biological fluids also play a crucial role in chemical diagnostics and treatments of various diseases and disorders. Diabetes is a disease, which results in a dysregulation of glucose homeostasis. However, other analytes also serve as crucial biomarkers of diabetes apart from glucose. In their pivotal work, Kareem et. al analyze "Diabetes Status and Aldose Reductase in Serum of Patients with Peripheral Neuropathy Diabetic." Continuing with this theme, Robert Moran offers a more general view of basic blood laboratory testing "Harmonization of Blood Electrolyte Concentration Results: Are Values 'Watered Down'?" that provides a more wholistic view of blood testing and analysis.

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Chemical manufacturing and processing are also important considerations to take into account in the fields of chemistry and chemical engineering. The field of green chemistry has grown in recent years, where natural products have been used for synthetic and industrial purposes and applications. Kamal et al discuss in their study “Starch and Coniferyl Alcohol Based Polymer: A Step Ahead Towards Green Polymers” where natural products can be used to form green, ecological-friendly polymers. Hassan Seyran also provides a book review of “Peptide Synthesis Methods and Protocols” that discusses solid phase peptide synthesis and other related techniques.

The free exchange of scientific ideas and publication of research articles in peer-reviewed journals will remain absolutely crucial to the development of knowledge and advancement of human nature. Journals such as The Chemist published by the American Institute of Chemists will continue to play a great role in the dissemination of chemical information and science communication. We sincerely hope that you enjoyed this issue and look forward to providing another one in the near future. We greatly thank you for your time and consideration of this issue.

Wishing you all the best and happy holidays and a joyous New Year!

Sincerely,

Alexander G. Zestos and Nayiri M. Kaissarian
Co-Editors of The Chemist

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Chemical Characterization, Cytotoxicity, Anti-inflammatory, Antioxidant, Antimicrobial and Anti-toxoplasmosis of *Chrysophyllum albidum* Seed Oil

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Abstract: *Chrysophyllum albidum*, an important tropical fruit with seeds, was investigated for its chemical composition, cytotoxic free radical scavenging, anti-inflammatory, antimicrobial (on clinical isolates) and antiparasitic properties for prospective application in the cosmeceuticals and food sectors. The physicochemical results of the *C. albidum* oil were 179.52±2.80 mg KOH/g saponification value, 3.93±0.56 mg KOH/g acid value, 1.58±0.11% free fatty acid and 39.34±2.54 g I_2/100 g iodine value, suggesting the suitability of the oil for industrial purposes, such as in skin care production. *C. albidum* seeds contained linoleic acid (33.12%), oleic acid (31.81%), palmitic acid (17.68%) and stearic acid (5.30%) as the major fatty acids. The IC_{50} value estimated for the anti-lipooxygenase potential of the oil (0.56±0.09 µg/mL) was close to that obtained for the standard indomethacin (0.53± 0.07 µg/mL). The IC_{50} obtained for the DPPH (4.74±0.83 µg/mL) and ABTS (0.03±0.11 µg/mL) of the oil was significant but relatively low compared to that of ascorbic acid (0.34±0.04 and 0.54±0.04 µg/mL) used as standards for the DPPH and ABTS free radical scavenging activities, respectively. The oil had significant activities against all the clinical isolates, with *A. niger* showing a significant MIC of 12.5 mg/mL. The seed oil of *C. albidum* possesses numerous biological activities that can be harnessed. The presence of essential fatty acids is

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partly suggestive of the observed bioactivities. Hence, the oil of *C. albidum* seeds with significant health benefits holds potential for possible incorporation into food, pharmaceutical and cosmeceutical products.

Key Words: *Chrysophyllum albidum*, oleic acid, lipoxygenase, antioxidant, cytotoxicity, antiparasite

1. Introduction

Seeds are the major sources of natural oil, known as fixed oil. Fixed oil belongs to a class of hydrolysable lipids that dissolve completely in nonpolar organic solvents such as n-hexane but are insoluble in aqueous media. The major component of fixed oil is triglycerides, making up 85% of the total percentage of its constituents. The minor components of fixed oil are mono-/diglycerides; tocopherol (vitamin E), which is often responsible for the antioxidant property of the oil; chlorophyll/carotenoids, which are responsible for the colouration of the oil; and other minor components, such as steroids, triterpenes, and phospholipids, which are responsible for other biological activities [1,2].

Fixed oil is classified into nondrying, semidrying and drying based on the iodine index, which refers to the quantity in grams of iodine consumed per unit of the oil. This classification helps to determine the potential application of oils. Nondrying oil has an iodine value below 100 gI_2/100 g and is suitable for consumption (vegetable oil) and industrial purposes, such as in the production of soaps. Semidrying oil has iodine values greater than 100 gI_2/100 g and less than 250 gI_2/100 g, and this oil is suitable for the production of lubricant and brake fuel in the automobile industry. Drying oil has an iodine value greater than 260 gI_2/100 g. These types are more applicable for the industrial production of paint, ink, etc. [3,4].

Chrysophyllum albidum, of the family Sapotaceae [5] and is a common plant throughout the Asian and African tropics. The ripe fruit is ovoid with a pointed apex and bears two to eight seeds. The fruit contains seeds with hard lustrous brownish seed coats. The fruits are produced between January and March annually [6]. Phytochemically, stems and seeds reportedly contain secondary metabolites, including phenolics, flavonoids, glycosides and alkaloids [7]. Methanol extracts of *C. albidum* cotyledon seeds reportedly contain eleagnine, tetrahydro-2-methylharman and skatole [6]. Extracts from the root and seed are used as wound healing agents [7]. The plant reportedly possesses good free radical scavenging activities [5,8]. In most regions where the plant thrives, the numerous seeds of the plant are often discarded and grossly underutilized. To establish the pharmacological potential of bioactivities, this work investigated the chemical compositions, radical scavenging, inhibition of inflammation progression, and antiparasitic and cytotoxic activities of highly underutilized *C. albidum* seeds for possible application in the food or cosmetic/pharmaceutical industries.

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2. Experimental and Method

Chemicals

Trolox, ascorbic acid, 1,1-diphenyl-2-picryhydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), antioxidant and anti-inflammatory assay kits, which were high purity grades, were obtained from Santa Cruz Biotechnology, USA. Indomethacin, staurosporine, and fatty acid standards were obtained from Sigma Reagent Company.

Plant Material

C. albidum fruits from which the seeds were collected were obtained at Ilorin, Nigeria, in December 2016 during the fruiting season. The plant material with voucher number UILH/002/1216 was subjected to standard identification and valid authentication at the Herbarium of the University of Ilorin, Ilorin, Nigeria. The seeds were subsequently subjected to drying under the shade, followed by deshelling and pulverization.

Methods

The *C. albidum* seed oil was obtained via Soxhlet extraction using hexane at 60°C followed by extract concentration at low temperature using an automated recirculation rotary evaporator [9]. The hexane extract was subjected to transmethylation and analyzed using gas chromatography–mass spectrometry. For the antioxidant potential, standard assays of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl were adopted. To evaluate the antibacterial and antifungal activities of the oil, isolates that included *Salmonella typhi, Bacillus subtilis, Klebsiella pneamananae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and fungi*, including Aspergillus niger, Rhizopus stolonifer, Penicillium notatum* and *Candida albicans,* were used. The anti-inflammatory activity was measured using a lipooxygenase assay, while the cytotoxicity potential was measured using mammalian cell assays, with indomethacin and staurosporine used as standards. The antiparasitic activity (anti-toxoplasmosis) was also evaluated.

Transmethylation of the Oils

Initially, the concentrated seed oil (100 mg) was constituted in hexane (10 mL), while an aliquot was further reconstituted in hexane to afford a more dilute concentration as desired following a standard procedure. An internal standard, heptadecanoic acid (C -17:0) (100 µL of 100 ppm) and 1 mL of 2.5% (v/v) methanolic sulfuric acid were added to the dilution. The mixture was incubated in an oven and maintained at 80°C for 1 hour followed by cooling to ambient temperature (25°C). NaCl solution (1.5 mL) of 20% (w/v) was added to the mixture, vigorously shaken and separated by centrifugation. The upper hexane layer (containing the methylated products) was separated, concentrated and

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subjected to elution in standardized gas chromatography equipment. Next, the amount of trans-esterifiable oil was determined and noted.

Spectrometry and Spectroscopy Characterization

Gas Chromatography–Mass Spectrometry (GC–MS)

To profile the fatty acid content of the trans-esterified seed oil, the oil was subjected to GC–MS analysis on an Agilent Gas Chromatograph (6890N) equipped with an autosampler. The elution was performed on a nonpolar column of 30 m by 0.25 x 0.25 µm using helium as the carrier gas. The injection volume, flow rate, injection temperature and split ratio were 1 µL, 1 mL/min, 250°C and 5:1, respectively. The heating temperature was adjusted to 100°C (5 min), then 180°C 5°C per minute (kept for five minutes) and finally raised to 330°C at 8°C per minute (stabilized for five minutes). A coupled mass analyser (5975B, Agilent Technologies) with electron impact mode was adopted to generate the fragmentations, which were used to identify the fatty acid ester in addition to the standard used. The mass spectrometer operating at 70 eV (electron impact mode) had a scan range of 35 to 500 m/z.

Fourier Transform Infrared (FT-IR) Spectroscopy Determination

The fresh seed oil and the trans-esterified seed oil were both subjected to FT-IR analysis to identify functional group transformations using an infrared spectrometer (Nicolet iS5 FT-IR) by preparing KBr pellets.

Ultraviolet–Visible Spectrophotometry Analysis

The absorption maximum was determined on an ultraviolet–visible spectrophotometer (Beckman, UK), while a multiscan spectrophotometer [Spectra Max (Plus) US] was used to measure the absorbance measurements for the assays.

Physicochemical Characterization

The physicochemical parameters, including iodine value (IV), saponification values (SV), free fatty acids (FFA) and acid value (AV), were determined following a standard protocol [4,10,11].

DPPH Assay

The *in vitro* DPPH assay to evaluate the antioxidant potential of *C. albidum* seed oil was carried out following a standard procedure [12,13]. Using α-tocopherol as a stan-

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dard reference, the concentrations of the samples were varied (10 – 500 µg/mL). To each sample concentration (2.4 mL), 0.8 mL DPPH (0.1 mM) solution in methanol was added, thoroughly homogenized and kept in the dark at ambient temperature (ten minutes) before termination. The absorbance of the mixture was measured at 517 nm on a scan absorbance reader (Thermo Spectronic, UK).

ABTS Antioxidant Assay

A standard procedure was adopted for the measurement of the ABTS antioxidant potential of the oil [14]. Radical cations ($ABTS^{\bullet +}$) obtained via the reaction of a stock solution of ABTS (7 mM) with $K_2S_2O_8$ (2.45 mM) for 12 h were diluted with methanol to obtain an absorbance reading of 0.7 ± 0.01 at 734 nm. Using ascorbic acid as a reference, the sample (1 mL) 10 – 500 µg/mL was stirred with $ABTS^{\bullet +}$ solution (2 mL), and the absorbance was read at the same wavelength after sixty seconds.

Determination of Anti-inflammatory Potential: Lipoxygenase Assay

In this assay, nordihydroguaiaretic acid and rutin served as reference inhibitors of lipoxygenase following a standard method [15]. In the absence of inhibitors, the lipoxygenase solution (50 µL) was mixed with sodium linoleate (2.0 mL) at 100 µM. An aliquot (30 µL) of the inhibitor dissolved in dimethylsulfoxide (DMSO) was added to make a solution with 100 ppm. The experiment was carried out in triplicate. The absorbance value was measured on an absorbance spectrophotometer, and the extent of inhibition was determined.

Antimicrobial Assay

Antibacterial and antifungal activities were examined by the disc agar diffusion method as previously described [16]. For the antibacterial assay, inoculates of clinical isolates of McFarland standards (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Klebsiella pneumoniae*) were prepared. Thereafter, the plates were impregnated with each oil solution at 62.5 to 500 mg/mL and were prepared by diluting the oil with distilled water. Thereafter, the air-dried impregnated discs were positioned on the surface of the agar plates.

For the antifungal assay, four clinical isolates (*Penicillium notatum, Candida albicans, Rhizopus stolonifera* and *Aspergillus niger*) were inoculated into mycological peptone. After 1 h of incubation, the inoculant was used to swab Sabouraud’s dextrose agar. Incubation was performed at 37°C for 24 h and 48 h for bacterial and fungal strains, respectively. Reference drugs for antibacterial and antifungal activities included gentamycin and tioconazole, respectively, while discs with water only served as controls. To determine the antimicrobial activity of the oil, the diameter (millimeters) of the clear regions of inhibition were measured. The observable clear inhibition

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regions were taken as evidence of antimicrobial action and vice versa.

Minimum Inhibitory Concentration (MIC)

The MIC of the sample was determined using the disc diffusion method [16]. Briefly, the reconstituted sample in agar medium was applied to the dish. After incubating for 24 and 48 h at 37°C and 27°C for bacteria and fungi, respectively, the clear inhibition regions were determined in mm. While sterile medium was used as a control, the MIC was taken as the sample with the lowest concentration causing complete retardation of microbial growth.

Cytotoxicity and Anti-*Toxoplasma gondii* Assays

The cytotoxicity assay using human fibroblast forekin (HFF ATCC®) cells was performed following a procedure previously reported [17,18], as staurosporine-treated cells served as a positive control drug. The antiparasite (*in vitro* anti-*Toxoplasma gondii*) assay on monolayers of HFF cells was performed using a luciferase-active *T. gondii* RH strain 2F, following a standard procedure we reported previously [19-22]. All assays were performed in triplicate independently in 96-well plates (Nunc; Fisher Scientific, Pittsburgh, PA, USA) unless otherwise stated.

Anti-parasitic Action of the Oils in the Presence of Trolox (Measurement of Intracellular Reactive Oxygen Species)

Following the determination of the antiparasitic potential of the seed oil, the mode of antiparasitic action was also determined via the evaluation of the culpability of reactive oxygen species (ROS) by including Trolox, an antioxidant, in the screening assay. Parasite growth inhibition was determined as described above. Thereafter, the level of ROS in cells was determined on HFF monolayers as previously described [17,23].

Statistical Analysis

Data obtained from the assays were subjected to statistical evaluation using one-way ANOVA (GraphPad Prism Software Inc., San Diego, CA), as the results are reported as the mean of three replicates ± standard error of the mean (SEM). The IC_{50}, the concentration that induced 50% inhibition in parasite and/or mammalian host cell growth, was estimated using a nonlinear regression analysis on a dose–response curve in GraphPad Prism 5.

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3. Results and Discussion

Physicochemical Characteristics of the Seed Oil

The *C. albidum* seed afforded (via Soxhlet extraction) an oil yield of 2% of the total weight of the seed material, of which 95% was trans-esterifiable. The physicochemical parameters of the oil determined following standard procedures are shown (Table 1).

Table 1. Results of Physicochemical Analyses

Parameter	***Chrysophyllum albidum*** **oil**
Oil yield	2 (%)
Saponification	179.52±2.80 (mgKOH/g)
Acid Value	3.93±0.56 (mgKOH/g)
Free fatty acid	1.58±0.11 (%)
Iodine Value	39.34±2.54
Colour	Dark Brown
Physical State at Ambient Temperature	Liquid

* Saponification, Acid, % Free Fatty Acid and Iodine value are expressed as the average ± SEM of two replicates

The oil has a saponification value of 179.52±2.80 mg KOH/g (Table 1). The high saponification values exhibited by this oil suggest that the oil will offer promising benefits in cosmetic and personal care products [24,25]. A high concentration of short-chain fatty acids has been implicated in fats and oils with high saponification value [26].

The seed oil has an acid value of 3.93±0.56 mg/KOH/g (Table 1). Low acid values in oil correspond to relative stability with attendant resistance to rancidity and peroxidation. However, a low acid value is also associated with the presence of phenolics and anti-oxidants [4,27]. *C. albidum* had a relatively low free fatty acid value of 1.58±0.11 (Table 1).

To estimate the level of unsaturation in fatty acids, the iodine value was used. This is achieved by reacting fatty acids with iodine compounds. Lower iodine values correspond to harder texture and reduced propensity for oxidative rancidity. The iodine value and saponification value are complementary. The *C. albidum* seed oil had an IV of 39.34±2.54, confirming it as a nondrying oil.

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4. Characterization

Fourier Transform Infrared Analysis of Raw and Transmethylation Oils

The extent of the transmethylation of the oils was established using infrared analysis, and the important peaks are as indicated (Table 2).

Table 2. FT-IR Value for Transmethylated and Raw *C. albidum* Oil

Raw seed oil (cm^{-1})	Transmethylated oil (cm^{-1})	Infrared bands
3439	-	O-H stretching *(b)*
3009	3008.97	sp^2 C-H Stretching *(w)*
2924	-	sp^3 C-H Stretching *(s)*
2854	-	sp^3 C-H Stretching
2654	-	C-H Stretching (methylene) *(w)*
1745	1742.48	Conj. C=O Stretching *(s)*
1712	-	Conj. C=C Stretching
1626	1626.05	C=C Stretching *(w)*
-	1581.00	
1465	1463.85	CH bending
1377	1381.53	CH_3 bending
1240	1239.05	C-O Stretching
1166	1156.73	C-O *(s)*
1093	1090.24	
909	-	O-H oop
840	-	
722	716.62	Long chain branch with C=C
563	-	Long chain branch

Broad (b), Sharp (s) and Weak (w)

The diminished O-H vibration at 3439 cm^{-1} confirms that trans-esterification was successful. The vibrations at 1745 and 1742 cm^{-1} in the crude and trans-esterified oils, respectively, are typical of carboxylic esters and correspond to carbonyl stretching [28].

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UV–Vis Spectroscopy Results

The UV–visible analysis and the comparison with the infrared are depicted in Table 3. The C=C and C=O bands were recorded at 317 (2.49) and 396 (3.43), respectively.

Table 3. Results of UV–Visible Spectroscopy and FT-IR Analyses of Raw *C. albidum*

Oil sample	UV (nm)	Absorbance	IR (cm^{-1})	Transition	Remark
C. albidum	317	2.498	1626	C=C band ($\pi \rightarrow \pi^*$)	C=C bands
	396	3.432	1745	C=O band ($n \rightarrow \pi^*$)	C=O bands

Chemical Composition of the Trans-esterified Seed Oils

The GC–MS result of the oil is depicted in Table 4.

Table 4. Profiles of Fatty Acids in *C. albidum* Oil

Fatty Acid	Systematic Name	Saturation Extent	Concentration (mg/mL)	Composition (%)
Palmitic acid	Hexadecanoic acid	16:0	69.81	17.68
Palmitoleic acid		16:1	1.08	0.27
	cis-10- Heptadecanoic Acid	17:1	1.04	0.27
Stearic acid	Octadecanoic acid	18:0	20.94	5.30
Trans oleic acid	trans-9-octadecenoic acid	18:1n9t	2.72	0.69
Oleic acid	cis-9-octadecenoic acid	18:1n9c	125.59	31.81
Linolelaidic acid		18:2n6t		
Linoleic aicid	all-cis-9,12-octadecadienoic acid	18:2n6c	130.79	33.12
Gamma-Linolenic acid (GLA)	all-cis-6,9,12-octadecatrienoic acid	18:3n6	2.77	0.70
Alpha Linolenic acid **(ALA)**	all-cis 9,12,15-octadecatrienoic acid	18:3n3	5.17	1.31
Arachidic acid	Eicosanoic acid	20:0	2.37	0.60
Eicosenoic acid	cis-11-eicosenoic	20:1	8.29	2.10
	cis-11, 14-Eicosadienoic acid	20:2	3.33	0.84

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Dihomo-gamma-linolenic acid (DGLA)	all-cis-8, 11, 14-Eicosatrienoic acid	20:3n6	2.07	0.52
Heneicosylic acid	Heneicosanoic acid	21:0	3.30	0.83
Arachidonic acid	all-cis-5,8,11,14-eicosatetraenoic acid	20:4n6	1.13	0.28
Eicosatrienoic acid (ETE)	all-cis-11, 14, 17-Eicosatrienoic acid	20:3n3	1.00	0.25
Behenic acid	Docosanoic acid	22:0	0.89	0.22
	Erucic acid	22:1n9		
Docosadienoic acid	all-cis-13,16-docosadienoic acid	22:2n6	2.64	0.67
Tricosylic acid	Tricosanoic acid	23:0	3.92	0.99
Lignoceric acid	Tetracosanoic acid	24:0	1.74	0.44
Nervonic acid	cis-15-tetracosenoic acid	24:1	2.90	0.73
Docosahexaenoic acid/Cervonic acid	cis-4,7,10,13,16,19-Docosahexaenoic	22:6n3	1.32	0.33
Total Saturated				*26.08*
Monounsaturated				*35.87*
Polyunsaturated				*38.05*
Total unsaturated				*73.92*

The *C. albidum* trans-esterified seed oil subjected to GC–MS analysis revealed prominent fatty acids, which included linoleic acid (33.12%), oleic acid (31.81%), palmitic acid (17.68%) and stearic acid (5.30%) (Table 4). Meanwhile, 26.08% of the fatty acid proportion in the oil of *C. albidum* was saturated.

Linolenic acid (omega-3) and linoleic acid (omega-6) are polyunsaturated fatty acids termed essential fatty acids that are required by humans for nutritional purposes but cannot be synthesized by the body. Unlike omega-3 and omega-6, oleic acid, an omega-9 fatty acid, and palmitic acid are important fatty acids found in plant seeds and are very useful in industries for various purposes, such as in paint production and cosmetic formulation. Linoleic acid is also often applied in cosmetic production, as it is reputable for its ability to moisturize the skin while contributing to wound healing and other dermal conditions. Linoleic acid- and linolenic acid-rich oils have been identified as good natural sources of anti-inflammatory agents for acne treatment [3,29]. In addition, palmitic and linoleic acid deficiency in the human diet manifests in physiological forms such as nail cracking, hair loss, skin dryness and scaling. Linoleic acid is a widely applied fatty acid in cosmetics because it moisturizes the skin, aids in the skin healing process, and reduces sunburns and acne treatment [29,30]. Oleic and palmitic acids reportedly permeate the skin to reach sensitive human parts. Furthermore, other micronutrients present in seed oils may prevent the risk of cardio-

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vascular diseases by reducing cholesterolemia and oxidative stress. These micronutrients include phytosterols and various antioxidants, such as polyphenols, tocopherols and coenzymes. Seed oils are widely used in folkloric medicine worldwide, as they serve as potential sources of phytochemicals with antibacterial, antifungal and antiparasitic properties. Therefore, the chemical composition of the oils of *C. albidum* suggests that it may be applicable in food and cosmeceutical preparation, as it may also find application in drug and supplement production if well harnessed.

Anti-lipoxygenase Evaluation

Lipoxygenases (LOXs) are important enzymes that convert arachidonic acid, linoleic acid and other polyunsaturated fatty acids into bioactive metabolites that are responsible for inflammatory and immune responses. These enzymes are key in the biosynthesis of leukotrienes and play vital roles in several inflammation-related diseases, such as cancer, allergic reactions, asthma, colitis ulcerosa, rheumatoid arthritis, and psoriasis [31-34]. The inhibition of leukotriene biosynthesis via the LOX pathway with natural products may have occurred by potential therapeutic means. The anti-LOX activity (anti-inflammatory activeity) of the seed oil of *C. albidum* was determined and compared with a standard drug, indomethacin, following a standard procedure already described. A dose response activity comparable to that of the standard drug was obtained (Table 5 and Figure 1).

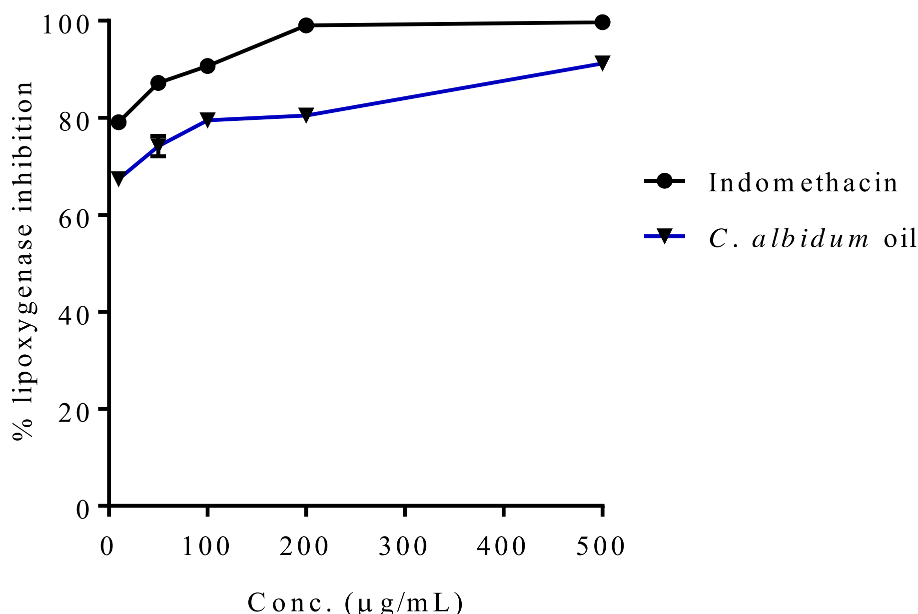

Figure 1. Lipoxygenase Inhibition Potential of the *C. albidum* Seed Oil

The results in Table 5 show that the inhibition of the oil is dose dependent, and the corresponding IC_{50} values of both the oil and indomethacin were 0.56 ± 0.09 and 0.53 ± 0.07 µg/mL, respectively. Seed oil exhibited

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potent LOX inhibitory activity and is suggested to possess anti-inflammatory activity.

Table 5. Results of Anti-lipoxygenase Assay

Concentration (µg/mL)	Indomethacin (% Inhibition)	*C. albidum* oil (% Inhibition)
10	79.10±0.20	67.35±0.67
50	87.20±0.13	74.14±1.22
100	90.68±0.27	79.47±0.47
200	99.05±0.07	80.50±0.75
500	99.68±0.023	91.18±0.36
IC_{50}	**0.53± 0.07**	**0.56±0.09**

Antioxidant Activities

For the evaluation of the free radical scavenging capacity of the *C. albidum* seed oil, two antioxidant assays (DPPH and ABTS) were adopted. Precisely, the oil showed a DPPH dose–response scavenging action (IC_{50} = 4.74±0.83 µg/mL), which was lower than the activity observed in the standard, ascorbic acid (IC_{50} = 0.34±0.04 µg/mL), as depicted in Table 6 and Figure 2.

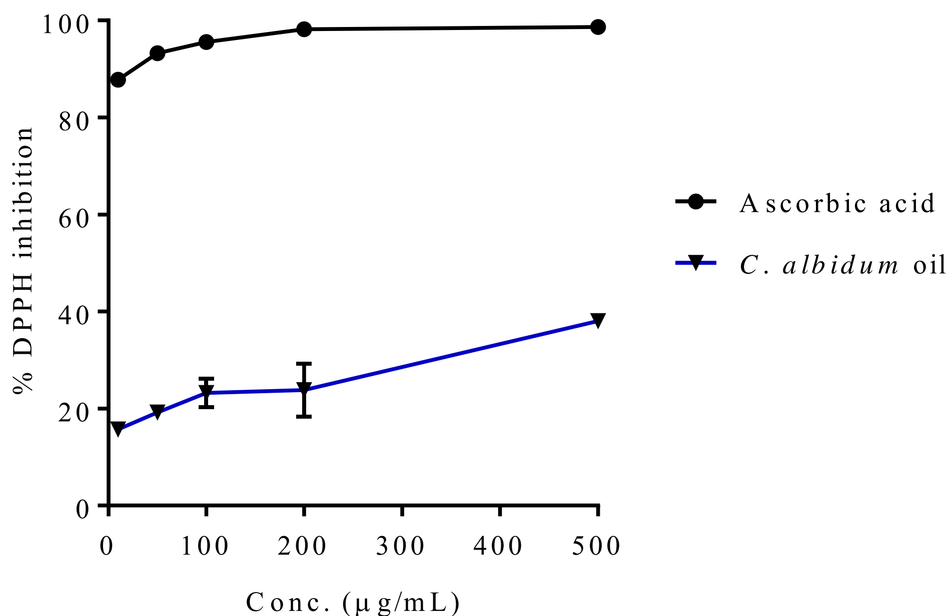

Figure 2. DPPH Scavenging Activities of *C. albidum* Seed Oil

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Table 6. DPPH Scavenging Activities of *C. albidum* Oil

Dose (µg/mL)	**% Inhibition** (Ascorbic acid)	**% Inhibition** (*C. albidum oil)*
10	87.76 ± 0.044	15.73 ± 0.23
50	93.25 ± 0.016	19.22 ± 0.58
100	95.55 ± 0.096	23.19 ± 1.70
200	98.22 ± 0.007	23.84 ± 3.18
500	98.67 ± 0.001	38.03 ± 0.82
IC_{50}	**0.34 ± 0.04**	**4.74 ± 0.83**

Values are representations of the average ± SEM of three evaluations.

ABTS Radical Scavenging Potential of the *C. albidum* Seed Oil

The ability of the oil to scavenge ABTS radicals was evaluated using a standard procedure. The oil did not exhibit concentration-dependent properties (Table 7, Figure 3) but was significant with a value (IC_{50} = 0.03±0.11 µg/mL) when compared with ascorbic acid (IC_{50} = 0.54±0.04 µg/mL).

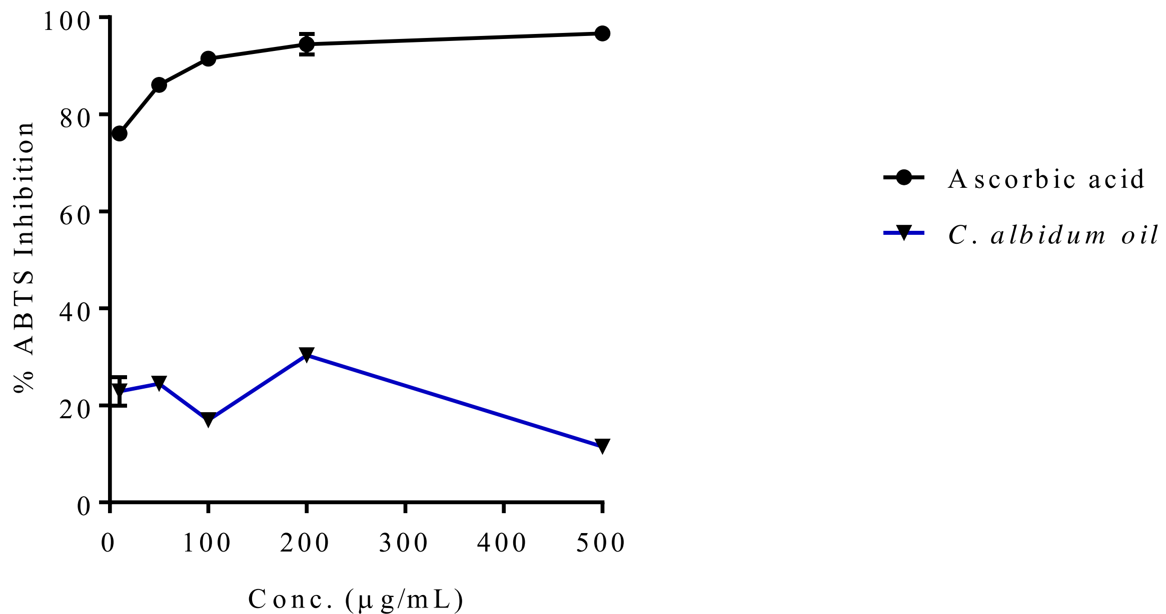

Figure 3. ABTS Percent Inhibition by *C. albidum* Oil

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Table 7. ABTS Scavenging Activities of *C. albidum* Oil

Dose (µg/mL)	**% Inhibition** (*C. albidum* oil)	**% Inhibition** (Ascorbic acid)
10	22.91±1.69	76.05±0.66
50	24.50±0.29	86.04±0.44
100	16.95±0.21	91.49±0.39
200	30.34±0.44	94.43±1.20
500	11.51±0.25	96.68±0.16
IC_{50}	**0.03±0.11**	**0.54±0.04**

Values are representations of the average ± SEM of three evaluations.

From the antioxidant assay results (Figures 2 and 3), the oil exhibited some potential to scavenge DPPH and ABTS radicals.

Antimicrobial Evaluation

The oil exhibited appreciable inhibition (200 mg/mL) of the growth of all the test organisms (Table 8 and Figure 4). *E. coli, P. aeruginosa* and *A. niger* at 200 mg/mL exhibited the highest antimicrobial sensitivity (17±1 mm) to the *C. albidum* seed oil, while *R. stolonifer* exhibited the lowest sensitivity (13±1 mm).

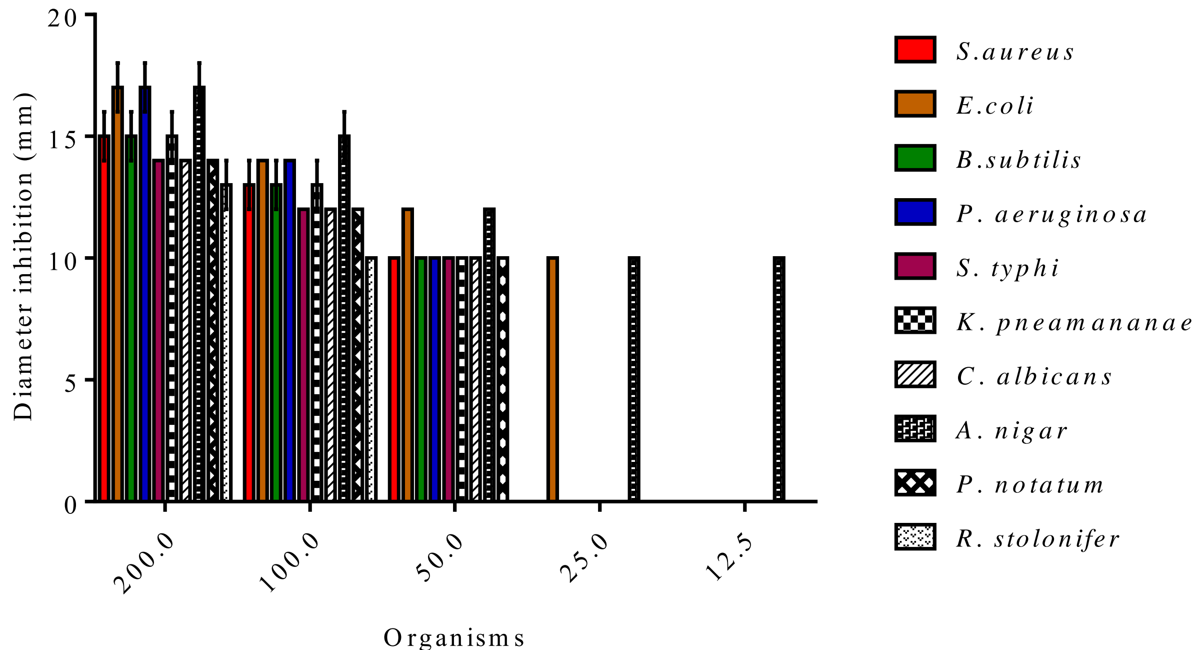

Figure 4. Evaluation of *C. albidum* Oil Microbial Inhibition

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The lowest MIC was recorded against *A. niger* at a concentration of 12.5 mg/mL and an inhibition diameter of 10 mm. The *C. albidum* seed oil had significant activity, which was lower than the activity recorded for gentamicin. Various natural products have shown good antimicrobial activities with potential for further development [11,35-39].

Table 8. Results of Microbial Inhibition of *C. albidum* Oil

	Concentration (in mg/mL)					Standard	
	200	100	50	25	12.5		MIC (mg/mL)
Tested organism	Inhibition (mm)						
Bacteria						Gentamin (10 mg/mL)	
Klebsiella pneumoniae	15±1	13±1	10	-	-	34	50
Staphylococcus aureus	15±1	13±1	10	-	-	34	50
Pseudomonas aeruginosa	17±1	14	10	-	-	36	50
Escherichia coli	17±1	14	12	10	-	36	25
Salmonella typhi	14	12	10	-	-	36	50
Bacillus subtilis	15±1	13±1	10	-	-	36	50
Fungi						Triconazole 70%	
Rhizopus stolonifer	13±1	10	-	-	-	28	100
Candida albicans	14	12	10	-	-	28	50
Aspergillus niger	17±1	15±1	12	10	10	26	12.5
Penicillium notatum	14	12	10	-	-	26	50

Values are reported as the average ± SEM of duplicate determinations.
MIC - Minimum Inhibition Concentration

Cytotoxicity Assay

The *in vitro* cytotoxic potential of *C. albidum* oil on HFF mammalian cells was determined. The cellular toxicity (Table 9) was lowest at 250 µg/mL, with cell viability (128.64%), while at 1000 µg/mL, cell viability was 0.03%. As shown in Figure 5, *C. albidum* revealed dose-dependent cytotoxic activities against HFF cells. Dégbé et al. [40] demonstrated the low cytotoxic effect of an ethanolic extract of *Vernonia amygdalina* on mammalian cells as well as moderate antioxidant properties.

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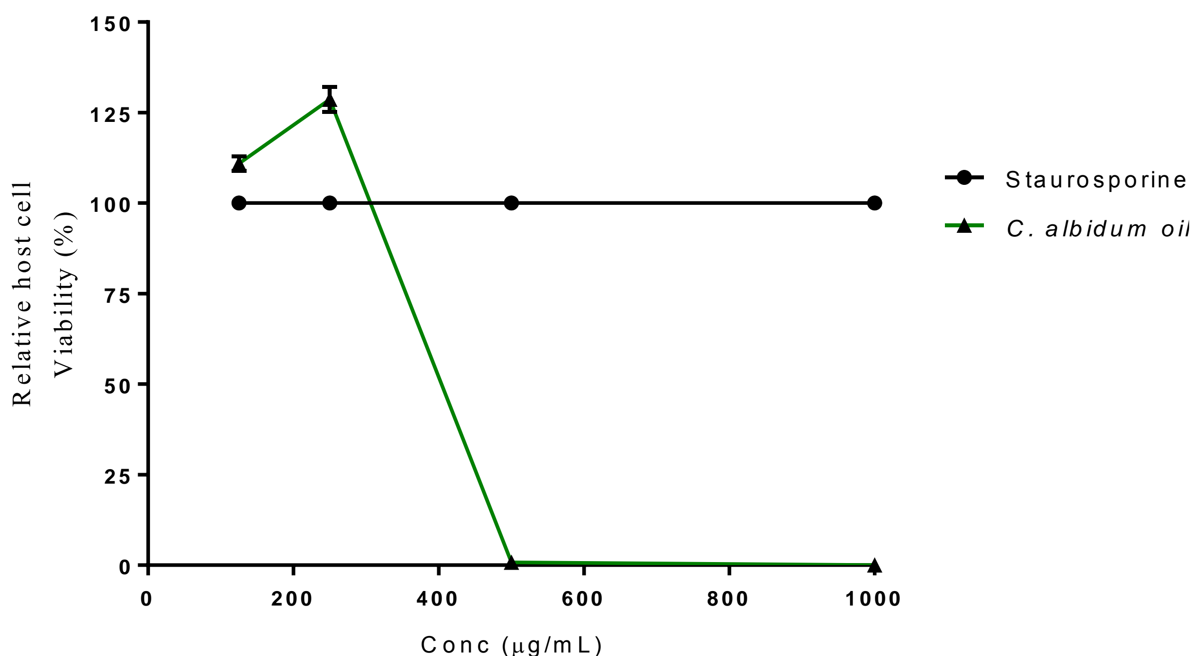

Figure 5. Evaluation of the Viability of the Cells on HFF Monolayers. Values are representations of the average ± SEM of three evaluations, and the experiment was performed three times independently. As part of the validation, the control was spiked with staurosporine (1 µM)

Table 9. Cellular Toxicity Evaluation of *C. albidum* Oil

Dose (µg/mL)	***C. albidum*** (% Viability)
125	110.97±1.99
250	128.64±3.40
500	0.79±0.02
1000	0.03±0.00

Anti-parasite Activity of *C. albidum*

T. gondii, a common protozoan parasitic agent responsible for toxoplasmosis, is known to possess the capability to infect nearly all warm-blooded animals [17,18]. In more than the 1 billion human populations that it infects, it often has lethal outcomes in pregnant women, although it is asymptomatic in other healthy individuals. *T. gondii* human infection occurs through the consumption of uncooked or improperly boiled infected meat [41,42]. In this study, *C. albidum* exhibited concentration-dependent activities against *T. gondii* parasite growth (Table 10). Precisely at 125 and 250 µg/mL, the oil had 97.89% and 113.39% parasite growth, respectively (Figure 6).

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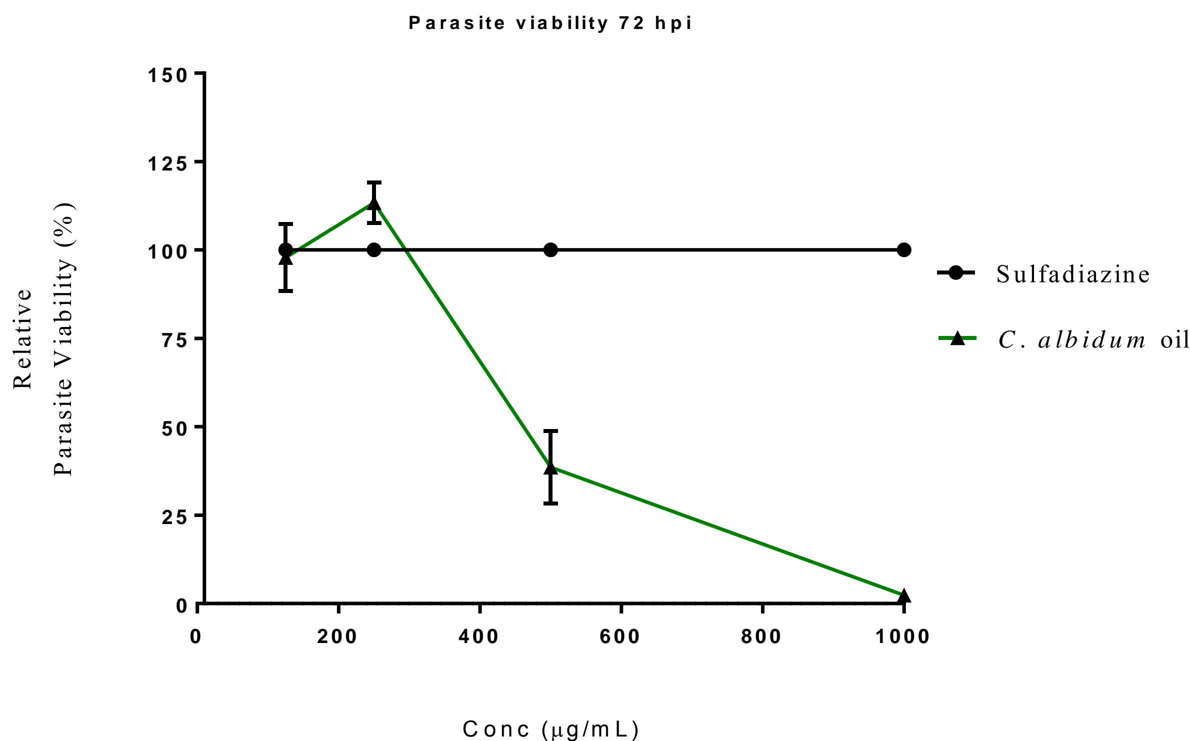

Figure 6. Parasite Inhibition Potential of *C. albidum* Oil. Values are representations of the average ± SEM of three evaluations, and the experiment was performed three times independently. As part of the validation, the control was spiked with staurosporine (1 µM)

Conversely, at 500 and 1000 µg/mL, the oil exhibited intense inhibition of *T. gondii* parasite growth. The *in vitro* antiparasitic action exhibited is attributable to the quality of the phytochemicals constituting the seed oils. In previous studies, the ethanol extract of *Vernonia amygdalina* reportedly inhibited *T. gondii* activity [40]. Similarly, *P. betle* has been shown to be a potential candidate in the treatment of toxoplasmosis by inhibiting parasite growth *in vitro* in HFF cells and promoting survival in an experimental model [43]; the growth inhibitory properties of *Lepidium perfoliatum* and *L. sativum* against *T. gondii* tachyzoites have also been reported [44].

Table 10. *T. gondii* Inhibition Activity

Dose (µg/mL)	***C. albidum*** **(% parasite growth)**
125	97.89±9.49
250	113.39±5.81
500	38.59±10.38
1000	2.38±0.11

Values are representations of the average ± SEM of three evaluations of the experiment performed three times independently. As part of the validation, the control was spiked with staurosporine (1 µM)

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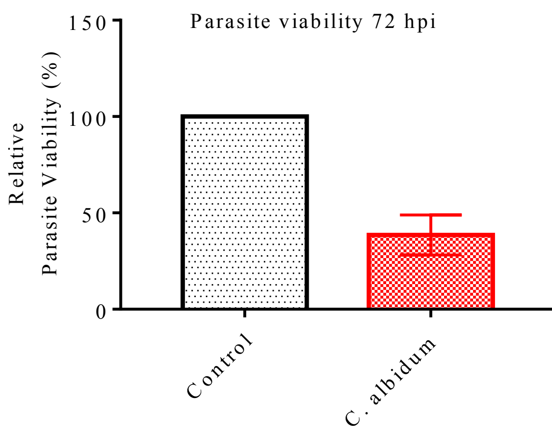
Parasite Inhibition Potential of *C. albidum* Oil in the Presence of Trolox

Following the establishment of the parasite inhibition potential of the oil, we sought to determine the likely mode of antiparasitic action. Therefore, we investigated the culpability of ROS in the inhibition potential by the inclusion of Trolox, an antioxidant at a concentration of 100 µM, in the screening.



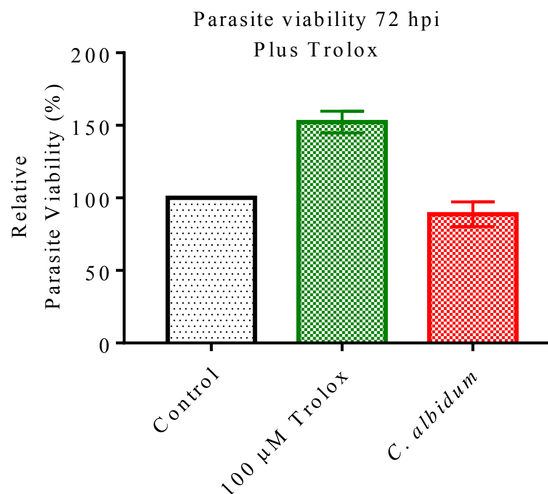


Figure 7 a-b. *T. gondii* Inhibition Potential of *C. albidum* in the Inclusion/Exclusion of α-Tocopherol (Trolox). Values are representations of the average ± SEM of three evaluations of the experiment performed three times independently.

As seen in Figure 7a-b, the Trolox inclusion in the screening assay induced a significant reduction in the inhibition potential. The results obtained for ROS analysis revealed that the *C. albidum* oil did not boost the ROS secretion both at the inclusion and exclusion of *T. gondii* infection (Figure 8a-b). In contrast, *C. albidum* oil impaired the fluorescing capacity of the cellular mitochondrial membrane both when *T. gondii* infection was absent (Figure 8c) and present (Figure 8d). The emerging observation is suspected to be a result of *T. gondii* infection leading to the disruption of the physiological integrity of cells.

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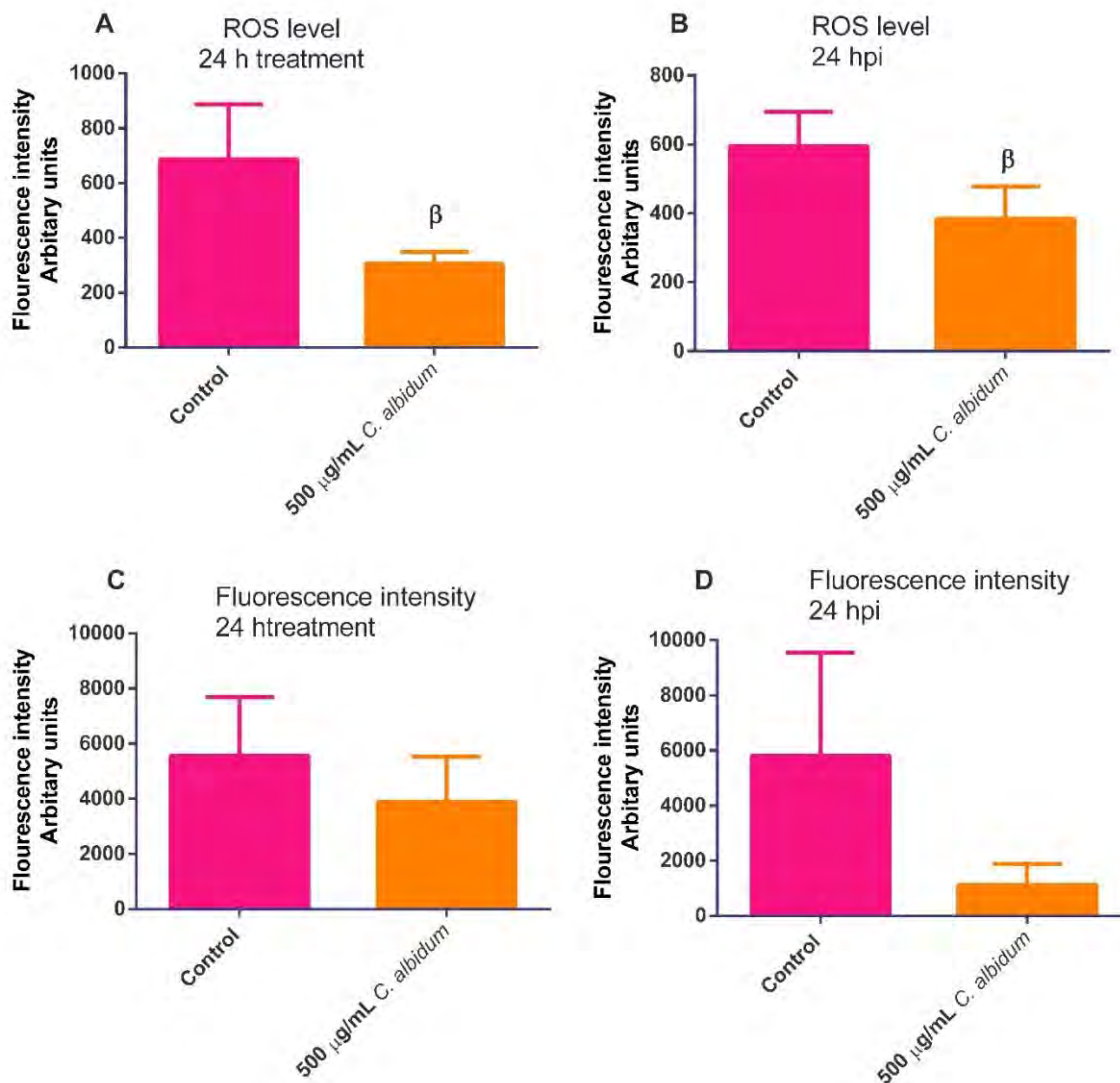

Figure 8a-d. Results of ROS Level and MitoRed Fluorescence Intensity After 24 h Introduction of *C. albidum* Seed Oil; (**a**) in the absence of *T. gondii* infection; (**b**) in the presence of *T. gondii* infection; (**c**) in the absence of *T. gondii* infection; (**d**) in the presence of *T. gondii* infection. Values are representations of the average ± SEM of three evaluations of the experiment performed three times independently. In comparison with the control, β is significant at $p<0.05$.

Selectivity Index

To determine whether the inhibition of *T. gondii* growth by *C. albidum* oil was a result of specific or general toxicity, the selectivity index (SI) of the sample was estimated by

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calculating the ratio of the cytotoxicity (IC_{50}) in mammalian cells to the antiparasite activity (EC_{50}). As shown in Table 11, the SI (<1) of *C. albidum* was found to be lower than that of the standard drug sulfadiazine (<4). While the *C. albidum* oil showed good inhibition potential against *T. gondii* growth, it could not exhibit satisfactory selectivity toward the parasite versus the host cells. The results obtained in this study revealed that the inhibitory activity of the *C. albidum* seed oil was due to general cellular toxicity or by an underlying antiparasitic mechanism beyond the scope of the present study.

Table 11. Selectivity Index of *C. albidum* Seed Oil

Samples	Host cell cytotoxicity (IC_{50} (µg/mL)	Anti-parasite activity EC_{50} (µg/mL)	Selectivity Index (SI): IC_{50}/EC_{50}
C. albidum	≤350	≥400	<1
Sulfadiazine	≤500	≤150	<4

5. Conclusions

The anti-LOX activity (anti-inflammatory activity) of the seed oil of *C. albidum* has a dose response activity comparable to that of the standard drug (indomethacin). The DPPH dose–response scavenging action of the oil (IC_{50} = 4.74±0.83 µg/mL) is lower than the activity observed in the standard, ascorbic acid (IC_{50} = 0.34±0.04 µg/mL), while in ABTS analysis the oil extract shows a significant activity higher than the ascorbic acid. The different in the antioxidant activities is due to different mechanism of action of the assay.

In this study, we showed that *C. albidum* seed oil contained good amounts of industrially useful fatty acids, of which oleic acid, linoleic acid and palmitic acid are major. The oil exhibited several biological properties, including anti-lipooxygenase, antioxidant, anti-inflammatory, anti-toxoplasmosis, cytotoxic and antimicrobial potential. Of important attention are the antimicrobial, anti-lipooxygenase and anti-toxoplasmosis potential, which hold prospects for further development. The exploration of many underutilized tropical seed oils in recent times has opened a new channel of research for further investigation. *C. albidum* seed oil, which is often discarded, holds promise for industrial utilization. Considering these results together, the biological activities shown by the grossly underutilized seed oil of *C. albidum* could be effectively exploited in food processing, nutraceutical and cosmeceutical development. For example, if the oil extract of *C. albidum* is incorporated into personal care products applied topically, it may prevent or clear skin infections and inflammation-related conditions.

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6. Acknowledgments

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Diabetes Status and Aldose Reductase in Serum of Patients with Peripheral Neuropathy Diabetic

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Abstract: The purpose of this research was to evaluate diabetic risk factors and aldose reductase in patient with diabetic peripheral neuropathy disease. This work was done on 81 subjects, which were classified into 27 with diabetic peripheral neuropathy, 27 with diabetic type 2, and 27 healthy, considered as a control group. The collection period was from (10 October 2021 to 20 December 2021). All patients and control groups were collected from the medical City Al-Imamian, Al-Kadhmiyain-Baghdad-Iraq. Regression analysis was used to investigate potential risk factors leading to diabetic peripheral neuropathy in diabetic type 2 patients. Statistical analysis used mean±SD and significant difference P value ($P<0.05$). The spread of peripheral neuropathy in diabetes type 2 patients was 50% from all studied patients. Most of the participants in the study were smokers and had a family history of DPN and T2DM receptivity. The comparison in duration of DPN was a more extended period than the T2DM group. Ages and BMI were strongly correlated with the level of aldose reductase activity. The mean value of aldose reductase was a significant increase ($P<0.05$) between the studied groups. The mean value of glucose, HbA1c, for diabetic peripheral neuropathy participants was highly significantly ($P<0.05$) higher than in type 2 diabetic and controlled healthy groups. On the other hand, patients with type 2 diabetes can develop complicated strategic approaches through an essential aldose reductase biomarker, which can be inferred by monitoring aldose reductase levels.

Key Words: Diabetic peripheral neuropathy, aldose reductase, hyperglycemia, glycosylated hemoglobin, micro complications of type 2 diabetes

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1. Introduction

Diabetic polyneuropathy "DPN" is among the most common causes of chronic problems of type-2 diabetes, affecting upwards of half of those with the disease. [1]. In Basrah, southern Iraq, a study reported that the main risk factors for foot ulcers are peripheral neuropathy, impaired wound healing process, decreased defense mechanisms, and impaired immunity, which can result in amputation and death. The large majority of Iraqi type 2 diabetes patients who have more extended periods of DM and hyperglycemia have a greater possibility of ending with diabetic foot ulcer outcomes [2]. DPN symptoms include numbness and tingling pain, weakness, loss of senses, and ataxia. [3,4]. Aside from the significant morbidity, mortality, and decreased quality of life experienced by patients, DPN is regarded as the most important initiating health risk for diabetic foot ulcers and non-traumatic lower leg surgical removal [5-7].

Diabetic nerve damage is caused primarily by elevated glucose levels in the blood (hyperglycemia), which disturbs the capacity of nerve cells to interact, resulting in a weakness of the bloodstream providing nutrients and oxygen to the nervous system [8,9]. Furthermore, axonal vitality is lost as a result. Thus, inflammatory cytokines can undoubtedly cause nervous dysfunction as well as, ultimately, death of cells [10]. Other risk factors include body mass index (BMI), smoking, diabetes period (time span), age, hypertension, fasting glucose, and HbA1c levels [11-16]. Diabetic peripheral neuropathy (DPN) is one of the most prevalent and disabling complications of diabetes mellitus (DM), which occurs in more than half of affected individuals. Some previous studies reported that more than 25% patients with T2DM may develop DPN, although up to half of them may remain asymptomatic. Clinically, the diagnosis of DPN often depends on the presence of patient-reported symptoms and physical signs, thus delaying the detection of subclinical diabetic peripheral neuropathy (sDPN). Therefore, identifying sDPN at an early stage and detecting the predisposing factors for its development may slow, stop, or even reverse the progression of DPN and avoid the occurrence of DPN-related morbidity and complications (Risk Factors for Subclinical Diabetic Peripheral [1] Neuropathy in Type 2 Diabetes Mellitus). Aldose reductase is indeed a cytosolic NADPH-dependent oxidoreductase, which stimulates the decrease of aldehydes and carbonyls, including monosaccharides, with the primary function of converting glucose to sorbitol [17]. It is found in non-uniform levels in most mammals with high levels in the eye (retina, cornea, and lens), peripheral nerves, kidney, and myelin sheath tissues, which are frequently involved in diabetes-related complications [18]. The development and progression of diabetic neuropathy are thought to be influenced by four distinct pathways. These pathways are named intracellular production of advanced glycation end products, "AGE" precursors, hexosamine pathway, protein kinase C "PKC" activation, and polyol pathway, and they depict metabolic abnormalities in nerve physiology. High glucose levels activate the polyol paths (the enzyme aldose reductase converts glucose to sorbitol). Aldose reductase consumes NADPH when glucose is converted to sorbitol, reducing glutathione production and increasing susceptibility to intracellular oxidative stress. The increased activity of the polyol pathway lessens myoinositol, which leads to peripheral neuropathy [19-22]. According to the current research, hyperglycemia could perform a crucial role in the pathophysiology of com-

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plications via various mechanisms such as aldose reductase (AR) related polyol path, increased formation of advanced glycation end products, and excessive oxidation stress.

2. Materials and Methods

Subjects

The study includes 81 subjects: adult males ages ranging from 33-78 years, twenty-seven (27) healthy subjects as a control, and 54 patients with diabetic type 2. The patient population was divided into 2 groups: diabetic type 2 group (27 T2DM) and Diabetic peripheral neuropathy group (27 DPN). The study was conducted at Mustansiriyah University College of Science, Chemistry Department from 10 October 2021 to 20 December 2021. All patients and the control were collected from Baghdad, Iraq, Al Imamian, Al Kadhmiyain, Medical City. Both the patients and the control were evaluated using biochemical tests, and immunological and biomedical laboratory tests.

Exclusion criteria: all subjects were without any noticeable inflammatory anomalies, tumors, or long-term illnesses. Patients' baseline characteristics and control data were collected from patient health registers. The following information was gathered: age, sex, DM duration, smoking status, family history, and hypertension.

The identification of DPN was confirmed by previous medical reports from the Specialized Endocrine and Diabetes Center of Iraqi Ministry of Health, and positive results were obtained from neurologic medical tests and peripheral nerve tests. In addition, a medical evaluation involving a review of each patient and clinical signs and symptoms of psychological examination was recorded (tingling pins and needles or numbness in the hands and feet; sharp, burning severe pain, especially at night; loss sensitivity to touch; muscle weakness).

Sample Collection

Four milliliters of blood were taken from fasting patients, and the control group's blood samples were obtained during the hours of 9:00 am to 12:00 pm. Each blood sample was divided into two parts. The first one: 2 milliliters of blood was transferred into EDTA-containing tubes for HbA1C measurements. Second, 2 milliliters were transmitted into gel tubes for biochemistry measurements (glucose and aldose reductase). For 30 minutes after that, the serum was centrifuged for 10 min at 3000 rpm, divided into Eppendorf, and kept at -20°C until the related parameters were measured.

Sample Analysis

The body-mass-index (BMI) of the subjects separated the research-measured data into normal (19≤ 24.9) and overweight (25≤ 29.9). Using the formula: BMI= Weight (kg) / Height (m^2) [24]. Additionally, fasting glucose level and enzymatic hemoglobin A1C (HbA1c) were calculated utilizing an automated clinical chemistry analyzer

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(Atellica-CH930-Siemens, German), while aldose reductase was calculated by sandwich ELISA method using (AR ELISA Kit MyBioSourse\USA).

Statistical Analysis

The statistical impacts of the study were estimated by The SPSS version 26 ANOVA statistical software to calculate the quantitative variables for parameters reported in the research. It comprised the mean, standard deviation (SD), and p-values (ANOVA) and using an analysis of variance, Pearson's correlation is calculated. Conducted to determine significant differences in different variables among all groups, data analysis significance was found at p-value <0.05 and extremely significant at (p-value <0.01).

3. Results and Discussion

The findings in Table 1 describe the age, hypertension, BMI of patients and healthy subjects, which shows the range for all study groups was between 35-82 years for the three groups. The mean ± SD of their ages were: 53.450±8.0126 and 55.125±10.754 and 50.250±10.460 for the three groups, respectively, with a significant p-value (p>0.05). The mean hypertension (mmHg) with a standard deviation (SD) of control individuals is 1.851±0.3620 ±7.441, while the mean ± SD of hypertension (mmHg) of T2DM patients is 1.555±0.506. Additionally, the mean±SD for DPN patients was 1.407±0.500 with a significant p-value (p>0.01). The BMI mean values for patients and control groups were found to be approximately similar with a non-significant p-value (p>0.05).

Table 1. Demographic Values for the Participants

Groups	Control No (27)	T2DM No (27)	DPN No (27)	P-value
Parameter	Mean±SD	Mean±SD	Mean±SD	
Age (years)	49.814±9.603	55.740±11.591	52.888±6.885	0.081
Hypertension (mmHg)	1.851±0.362	1.5556±0.50637	1.407±0.500	0.002
BMI (kg\m**2)	25.574±3.233	27.059±2.585	26.497±2.383	0.143

Type 2 diabetic, T2DM; diabetic peripheral neuropathy, DPN; hypertension levels ≥140 (mmHg) significant if p-value <0.05.

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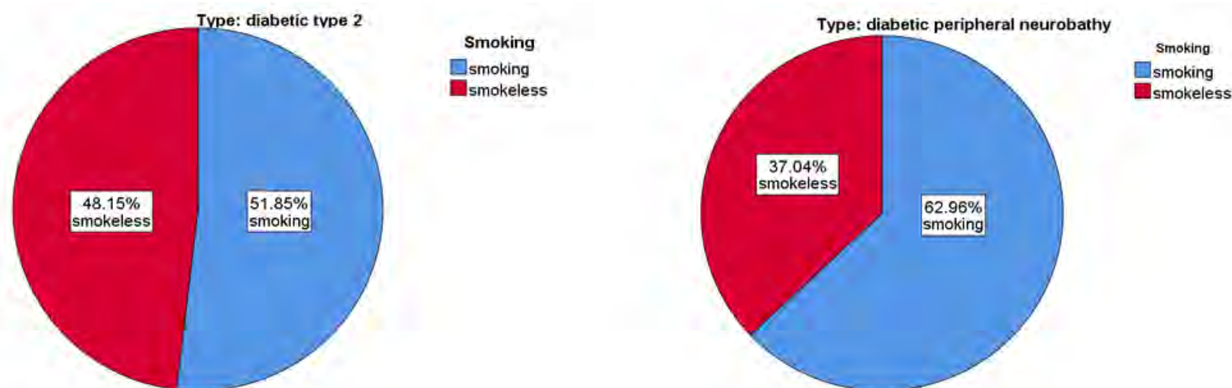

Figure 1. Distribution of Smoking and Nonsmoking According to the Study Groups

Figure 1 shows that most of this study's participants were smokers, 62.96% and 51.85% for DPN and T2DM, respectively. Furthermore, the family history of disease results were 74.07% and 51.58% for DPN and T2DM, respectively [25]. Identification of independent risk factors for diabetic neuropathy progression in patients with type 2 diabetes mellitus. Journal of International Medical Research who had a family history of disease. Other research reported that in every category studied, sick people were much more likely to have a significant history of diabetes and complications such as "DPN". The family history of diabetes and major complications were associated with peripheral neuropathy [26,27].

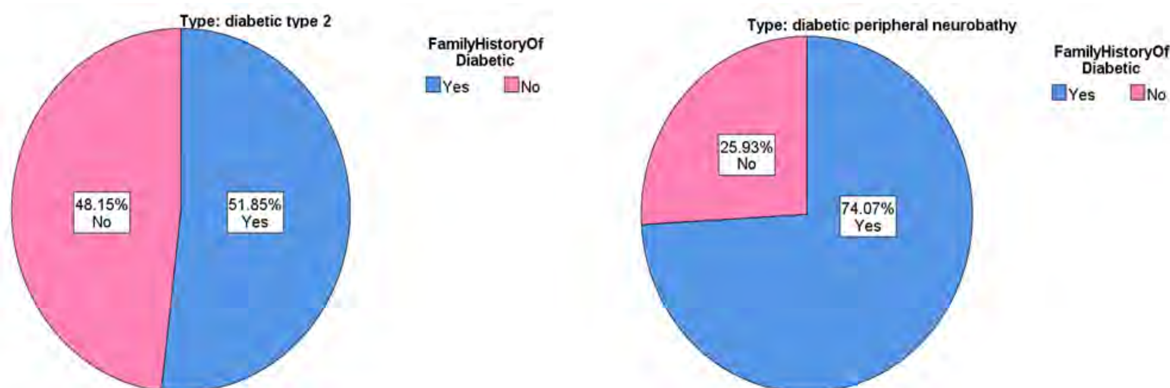

Figure 2. Percentage of the Family History with the Disease Between the Study Groups

In addition, Figure 2 shows that most of the participants in the study were smokers (62% and 51% for DPN and T2DM, respectively). Furthermore, most of them have a family history of the disease for DPN and T2DM (74% AND 51% for DPN and T2DM, respectively). These results agree with (28-30) have a family history of the disease.

Other research reported that in every category studied, sick people were much more likely to have a significant history of diabetes and complications such as "DPN". Family history was linked with peripheral neuropathy patients [31,32].

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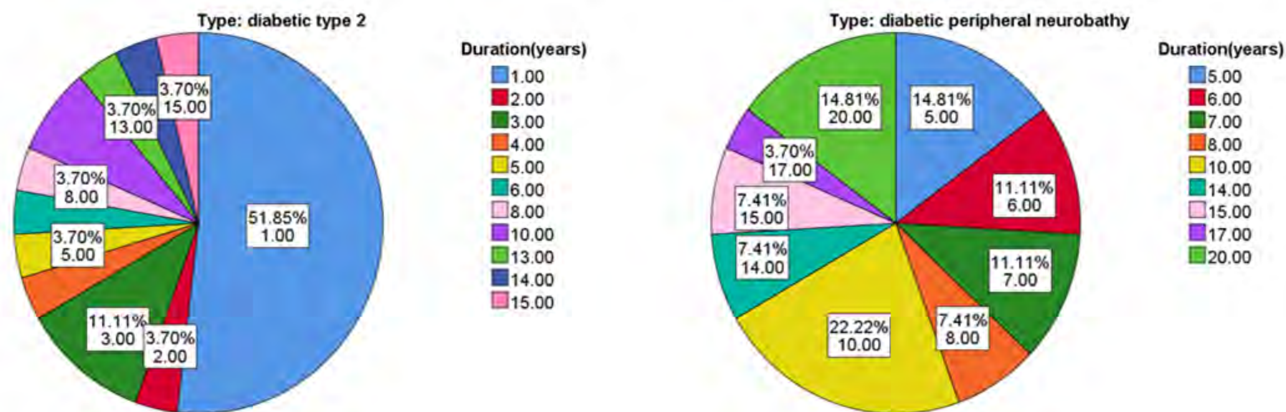

Figure 3. Disease Duration for Both T2DM and DPN

Also, the comparison in duration of disease in Figure 3 showed highly significant differences for the DPN group. It was over a longer period compared to the T2DM group ($p<0.05$) [14].

According to the study's findings, there is also a highly significant increase in the level of hypertension in the DPN group, especially compared to the T2DM and control groups ($p<0.05$). It agrees with previous studies that hypertension and diabetes are independent risk factors and have significant additive interactions with disease [15].

Table 2 illustrates the clinical parameter analysis for the three DPN patients with T2DM and control groups. Analyzing the current data of participants (n=81) showed that the variance of aldose reductase in the analysis was significant. The mean of aldose reductase for T2DM was noticeably higher than for both DPN and control groups ($p>0.05$). Many other studies showed that the relationship between aldose reductase (AR) and hyperglycemia would be cause the development of diabetic type 2 to diabetic peripheral neuropathy. AR levels were significantly increased in T2DM patients, and the accumulation of polyol pathways was mediated by AR and intermediate glycation products or advanced glycation end products (AGE), which induced oxidative stress. Also, modified protein kinase C activities are considered to be responsible for diabetic peripheral development [34,35]. The mean value of fasting glucose level for the DPN group was significantly higher than that of T2DM and control groups ($p< 0.05$), which is similar to other previous studies [16]. The comparison of the HbA1c between the groups showed a significant increase for the DPN group compared to T2DM and control groups. It agrees with other studies that reported that the difference between DPN groups compared to T2DM and control groups was statistically significant ($p<0.05$) [16,33].

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Table 2. Biochemical and Clinical Characteristics of the Participants

Groups	Control No (27)	T2DM No (27)	DPN No (27)	P-value
Parameter	Mean±SD	Mean±SD	Mean±SD	
AR	14.893±2.967	53.761±13.852	31.492±10.389	0.0001
FG (mg\dl)	95.222±8.536	173.493±47.378	285.503±113.428	0.0001
HbA1c	5.439±0.386	7.780±1.342	9.651±1.992	0.0001

AR, Aldose Reductase; FG, Fasting Glucose; HbA1c, Glycosylated Hemoglobin.

The results in Table 3 with Figure 4 show that the AR had a strong positive correlation with Age (years) (r= 0.545**) (p= 0.003) in the T2DM group, however, with a moderate "positive correlation" with hypertension (r= 0.425*) (p= 0.027) and a strong "negative correlation" with BMI in control group (r = -0.554**) (p= 0.003).

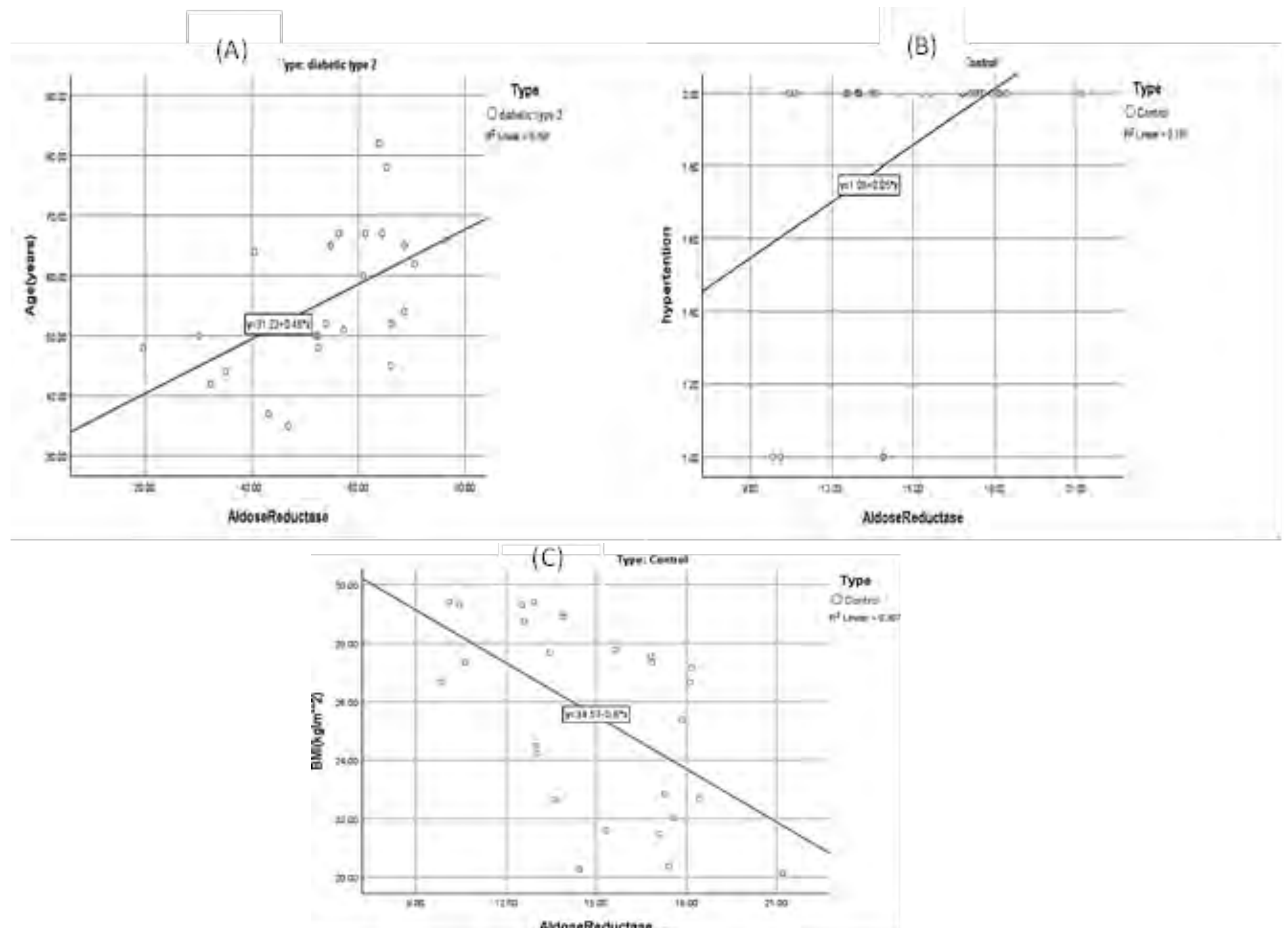

Figure 4. (A) Significant Correlation Between Aldose Reductase and Age (years) (B) Significant Correlation Among Both Aldose Reductase and Hypertension (C) Significant Correlation Between Aldose Reductase and BMI

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Table 3. Correlation Between AR with Demographic and Clinical Values in the Control Group, Diabetic Type 2 Group, and DPN Group

AR						
Group	Control		T2DM		DPN	
Parameter	(r)	(p)	(r)	(p)	(r)	(p)
Glu H (mg\dl)	0.127	0.528	0.190	0.342	-.110	0.585
Hba1c	-0.292	0.139	-0.138	0.493	-.138	0.491
Age (years)	0.030	0.882	0.545**	0.003	-.201	0.316
Smoking	0.193	0.334	0.118	0.559	-0.138	0.491
FHD	-0.050	0.804	-0.007	0.972	-0.031	0.879
Hypertension	0.425*	0.027	0.135	0.502	-0.168	0.401
Duration (years)			0.090	0.654	0.284	0.151
BMI (kg\m**2)	-0.554**	0.003	-0.208	0.298	0.030	0.883

Aldose Reductase (AR), Glucose (Glu H), Family history of Diabetic (FHD), Pearson Correlation (r), P-value (p). ** Correlation is significant at the 0.01 level. * Correlation is significant at the ($p<0.05$) levels.

In this current study, there was also no relationship between the alteration in HbA1c and glucose with alteration in the level of aldose reductase. It corresponded with other studies that reported no statistically significant correlation observed between the level of AR and the average of HbA1c and fasting blood glucose [36,37].

Although there's reasoned evidence that points to the AR as a biologically probable crucial gene for developing type 2 diabetes complication diseases [38], so much research has found that the AR is linked to diabetic retinopathy, neuropathy, and nephropathy, possibly due to an involvement of the polyol pathway in the pathophysiology of naval vessel diabetic complications [39-41]. Furthermore, the alteration in AR levels in the DPN group are an obvious characteristic that control group. A future study is needed to fully understand the relationship between the serum AR and DPN. Because other studies indicated that there is a high incidence of AR in T2DM patients and is linked to diabetic peripheral neuropathy. Quite a lot of pathological modifications in nerve cells, nerve fibers, and vascular endothelial cells can result in nerve dysfunction and, eventually, death. It has been suggested that nerve damage might have developed during the pre-diabetes stage. Post-load hyper glucose levels may be the primary mechanism causing increased oxidative stress, vascular dysfunction, and stimulation both for the polyol pathway and the protein kinase C, resulting in impaired nerve cell metabolism and damage to DNA [42].

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4. Conclusion

The increase in the mean value of aldose reductase was significant ($p<0.05$) for the DPN group compared to others. The mean value of glucose HbA1c for DPN participants was considerably higher than those in the T2DM and healthy controls groups ($p<0.05$). It can be concluded that the AR level was an important parameter for following the T2DM cases complicated by DPN. This is because the enzyme converts glucose to sorbitol, which can accumulate in cells, leading to oxidative damage. By monitoring AR levels or its activity, clinicians can potentially gain insights into the progression of diabetic complications. Inhibition of aldose reductase is being explored as a therapeutic strategy to prevent or delay these complications.

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Harmonization of Blood Electrolyte Concentration Results: Are Values 'Watered Down'?

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Abstract: Analysis of physiologic specimens such as blood, urine, and cerebrospinal fluid for electrolytes, especially sodium and potassium, began more than a century ago as physician-biochemists tried objective ways of assessing the most basic aspects of physiology-water-electrolyte and acid/base balance. Of the types of specimens, whole blood and the liquid serum or plasma[I] separated from the formed elements[II] became the medium in which these ions were measured, first with gravimetry, later by emission/absorption spectroscopy. Each reported results as a concentration per volume of plasma (mg/dL or mEq/L). Expected or normal ranges were established, which in the case of sodium were quite narrow relative to the average (140 +/- 5mmpl/L). The advent of ion selective electrode (ISE) technology made it possible to measure them directly in whole blood with minimal specimen preparation, but ISE's gave results consistently higher than the established norms by an amount equal to the 'normal' range. Since most initial evaluative blood work on a patient admitted to a hospital or clinic includes the electrolytes, physicians had become accustomed to the values and looked at the 'high' ISE values as 'errors'. In fact, it was the ISE that was measuring what the body's sensors detected and they specifically allowed differentiation in certain serious but less common disorders, a fact that was obscured by the inherent difference in values on normal subjects. Technically the matter is simple (though there are some complex nuances) – the electrolytes are dissolved in the water fraction of the plasma, not the plasma as a whole. Changing well established physician perspectives, however, was (and is) a different story. And now the rest of the story….

Key Words: Ion selective electrode (ISE)

[I] Serum-the liquid portion of blood after natural coagulation is complete; plasma the liquid portion resulting when coagulation has been inhibited, typically by additives in the collection vessel. We will use the term plasma to represent both in this report.

[II] Formed elements-blood cells*Erythrocytes-RBCs, leucocytes-WBCs, and platelets, each metabolically active.

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1. Introduction

Measurement of sodium and potassium in both urine and in blood has been an established practice in support of clinical decision making for more than a century. Early methodology for each measurand[III] involved quantitative dilution of an aliquot of plasma[IV] from a specimen of blood to precipitate proteins, followed by use of the clear diluted filtrate to quantitatively precipitate (more dilution) the selected ions, with gravimetric measurement of the precipitate-basic quantitative analysis.

The process of collection, preparation and measurement could take many hours. The measurements were physiologically informative and enabled the establishment of an expected or 'normal' range for healthy subjects (Sodium:135-145 mEq/L, Potassium: 3.5-4.8 mEq/L of plasma). Once it was realized that the measurements could be used to detect various imbalances of metabolism and aid in the management of renal disease, diabetes, and other clinical conditions, it became a desirable laboratory procedure[V] [1].

The physiologically narrow 'normal' range and the observed significance in the acutely ill led clinical biochemists to seek out methods of measurement that allowed a smaller specimen size and shorter processing time to get equally reliable results. Consequently, among other analytical approaches, the qualitative 'flame' test for dissolved ions was quantitatively adapted to the electronics becoming available in the early-mid 20th century. This methodology dramatically increased the clinical utility of sodium and potassium measurements since it was sensitive, reliable, rapid, and easy to perform. Now the tests could be performed in a matter of a few minutes on collected blood specimens of <5 mL of blood instead of 10-20mL.

2. Flame Atomic Emission Spectroscopy in Clinical Laboratories: Method Characteristics

Two major characteristics of the flame atomic emission spectroscopy (FAES)[VI] analytical process as applied to blood are salient to this report. First, as with gravimetry, FAES of blood

[III] Measurand: entity intended to be measured (i.e., you measure the millivoltage that resulted from the sensor that detected the pH in the buffer, which pH was due to the carbon dioxide tension – thus the **measurand** is **carbon dioxide**, in this case the **kind of quantity** or characteristic of the carbon dioxide being measured is in this example, tension or partial pressure).

[IV] Earlier still was a similar process in which 'whole blood', that is the mixture proteins of blood cells, and the liquid fraction was precipitated, followed by treatment to isolate the ions.

[V] Today, it is estimated that hyponatremia affects about 30% of the hospitalized population in the United States.

[VI] Clinical lab colloquial-flame photometry.

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plasma, requires separation of collected blood plasma from the blood cells. Each subsequent step must be quantitative. The accurate measuring of a plasma aliquot and a quantitative dilution to both bring the light intensity of the excited electrolyte ions into a suitable range for the photodetectors and to reduce the amount of plasma protein and lipids in the specimen as processed through the flame. Second, to get quantitative results, it is necessary to compare flame emission intensity of the diluted plasma specimen with the emission intensity of a standard concentration. Standards with known concentrations in aqueous solution, were then treated/diluted the same as the plasma/urine patient specimen. This gave excellent comparison to the 'gold standard', gravimetry.

For several decades, this analytical approach became the norm with countless clinical and analytical reports and medical texts and external quality assurance schemes [1], being based on measurements using the technology. During this period, atomic absorption spectrometry (AA) became more commonly available but offered no practical advantage over the FAES for processing clinical specimens.

3. Method of measurement establishes reference ranges:

Both the gravimetric and FAES/AA methods use specimens of plasma that are diluted, then incorporate standards prepared in aqueous medium having Na/K values bracketing the range of Na/K seen in gravimetry-makes sense, right? These would then be diluted in the same sequence applied to the plasma specimen. Both resulted in 'normal' patient values of 135-145 mEq/L (mmol/L). So, everything analytically lined up with the expected, despite the interesting fact that during the same period, so-called isotonic saline for infusion was recognized as 0.9% NaCl, which gives a value of 153 mmol/L for sodium and chloride.

Ion Selective Electrodes-New Technology Arrives! Ion selective electrodes (ISEs) measure either voltage or amperage. Their selectivity comes from at least two components of their design: 1) a 'membrane to separate the electronic components from the blood cells and plasma protein, and 2) a characteristic selectivity for the measurand to be detected. For some measurands these are combined, for others the selectivity may come from additional reactants[VII] in the internal characteristics of each 'electrode'[VIII].

[VII] Arguably the first ISE was the carbon-dioxide electrode of Severinghaus and Bradley, which used a silastic membrane to separate the blood from the electronics, but which allowed passage of CO_2 gas. The gas in turn changed the internal pH which was detected by the internal pH sensitive glass.

[VIII] For some systems these 'electrodes' are in appearance nothing like what one is accustomed to, but are integrated into a circuit board, include multiple sensors, flow paths for the specimen, 10 or more analytes in the space of a pinky fingernail!

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'Indirect' ISE Measurement: The ISE can be used to measure in an environment like that in which the FAES/AA measurement occurs. In that situation the separated plasma specimen(s) are diluted/treated and processed continuously. This is operationally convenient and economical for exceptionally large volume operations in which the electrolytes are just a part of an overall set of measurements for health or metabolic disorder. Without statistical quibbling, the results obtained should be the same as for flame emission or other means of measuring sodium/potassium.

Direct Measurement using ISE's: The advent of ISE's also allowed direct measurement of sodium and potassium (and later other electrolytes/measurands). However, it was immediately recognized that ISEs immersed in plasma got different values than the 'standard' (diluted) methods. Typically, 'direct' ISE results were about 6-8 percent higher → ~150mEq/L for sodium. The first thought was a method error between the traditionally measured sodium and the ISE, values at least were consistent.

Note the percent bias of the sodium (the one easiest to spot because of its magnitude). For those unfamiliar with blood plasma values, that bias corresponds identically to the mass percent of plasma protein[IX].

Blood Gases by ISE: A clinically related measurement set to the electrolytes is the group of tests called 'blood gases. Just as electrolytes are among the most critical tests in initial evaluation of critically ill patients, so are the blood gases (some would say they can be even more critical since they relate to the patient's oxygen status). Blood gas analysis (BGA) consists of pH (acid-base), as well as the measurement of oxygen and carbon dioxide tension (pO_2 and pCO_2), both essential in assessing breathing (the most basic of body functionality), are also measured using electrodes that are themselves ion or gas selective. They necessarily measure **directly in whole blood**.

Using ISEs for **direct measurement** of electrolytes on undiluted whole blood made it clearly feasible to incorporate the two sets of critical tests together on one device – the 'enhanced' blood gas analyzer (eBGA). Over the past quarter century, this has been the most common clinical use of the direct measurement technology using ISE's, with the three largest manufacturers of BGA's having 'flagship' models of their portfolio being the eBGA's (the blood gas component), since it is analytically required, the electrolyte component because it was fast dependable and convenient.

Now, in addition to being able to directly measure whole blood or plasma for electrolytes, the ISE has an **additional advantage**. Since they measure an undiluted specimen, they sense what the body's homeostatic system senses - the **activity of the ion in plasma water** (pw or p_w).

[IX] There are several other minor contributors (junction potential gradient, etc.) to the bias (N. Fogh-Andersen, Personal communication, 2024)

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What had been previously measured in both the 'gold standard' gravimetry or using FAES/AA was a different quantity, the **ion's concentration in the liquid phase** – a liquid containing a colloidal suspension of various proteins plus potentially of lipoproteins and free lipids – none of which contain significant amounts of either sodium or potassium.

Fortunately, the total protein concentration of blood serum/plasma is consistent, so for many clinical conditions it is not an issue. However, for those patients that have low or high protein levels in the plasma, if unrecognized, the induced protein-plasma water error could push the apparent sodium/potassium values outside the normal range to the extent of requiring electrolyte therapy.

An analogous situation arises with hyperlipidemia. The lipid fraction of the liquid plasma/serum contains no electrolytes. If that fraction is significant, as in diabetes, pancreatic diseases, or other lipid disorders, even hyperalimentation, an analytically accurate measure could easily be misleading.

4. Standardizing and Reporting Blood Electrolytes

Given the dilemma of physician expectations developed over decades versus the real advantage of ISE measurement directly, there seemed to be two choices 1) keep the familiar (to physicians) concentration of the ions in the total plasma volume or 2) make a change and report the ion activity in the aqueous phase [converted by convention into concentration units (mmol/L) and familiar to electrochemists and system developers].

To change or not to change-the 'watering down?' It is not my point to re-argue which approach is best, but rather to cite what two internationally respected organizations [3,4] have recommended. This recommendation is: for purposes of harmonizing results for sodium and potassium, such that direct, undiluted ISE systems might be correlated to agree with the FAES/AA reference methods [5,6] or alternatively, to a reference material developed in accordance with the procedures outlined by the NCCLS/CLSI standard C29A. The processes that resulted in these conclusions and recommendations involved substantial input from an agreement by manufacturers and thought-leader-users of ISE systems as well as governmental representatives.

Further than just these 'paper' standards or recommendations, the National Institute for Standards and Technology **(NIST)** in the USA and the Health Care Technology Foundation (HECTEF) in Japan have actually developed Certified Reference Materials (CRMs) to allow more convenient harmonization of the various ISE systems, so that they can all report the same value across a range of Na/K analyzers. An enormously successful effort to make harmonization of results for these analytes is now feasible and, in fact, possible.

While there have been issues of widespread availability of the CRMs to clinical laboratories, the process for preparing reference materials as described quite clearly

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in NCCLS C29 - A2 has been available for manufacturers for some time – only minor changes were necessary in the description of that process as the standard went through the proposed, tentative and now the final approved level publication. Then, we should all be able to have quantitatively comparable results or **Na/K** using an ISE system.

5. Whole Blood vs. Plasma vs. Plasma Water:

In whole blood specimens using direct ISE's, all the electrolytes are determined similarly, in that they exist in the water fraction of the plasma and their concentrations in that plasma water are measured in that 'whole blood' specimen. While water fractions vary (e.g., hyperlipidemia,Hyperproteinemia), electrolyte balance is focused on the water fraction where these electrolytes exist. The sensors involved in this 'whole blood' specimen measurement sense what the body's own sensors do – the concentration in the plasma water. Those ISE-based systems using undiluted specimens of either whole blood or plasma and based on this, report a value for sodium that is 'normalized' for average plasma water concentration. The clinical implication of this is that in cases of lowered plasma water fraction (hyperlipidemia, hyperproteinemia) sodium (and potassium) levels are indicative of real electrolyte status rather than being artifactually low.

The conundrum comes into play when interpreting clinical results. As stated earlier, most clinicians are more accustomed to results obtained from the mass-specimen processing instrumentation (indirect ISE) and when confronted with a result from both an acute care facility (probably a direct measurement) and a routine central laboratory are more likely to first consider their usual source of electrolyte results! As we have shown, a potentially bad move, one with potentially profound consequences diagnostically and therapeutically.

6. Conclusion

Where, then, do we stand? The promise of clinical utility without confusion, especially in the Critical Care and Point-of Care areas as brought about by eBGA's measuring electrolytes on whole blood has been realized [7]. In the cited study both the whole blood measurement and measurement of the plasma from the whole blood specimen on the same analyzer give the same results. The hyponatremia shown by the indirect (central laboratory) measurement on diluted plasma is shown for what it is – **pseudo**hyponatremia. Any measurement using dilution without considering the proportion of plasma that cannot contain the ions such as sodium, potassium and chloride is subject to this. While the cited study references the Radiometer system, because of personal knowledge, I would expect the same sort of performance for the Corning-Chiron-Bayer-Siemens BGAs. It is probably true for the other major manufacturers who participated in the development of the CLSI/NCCLS standard C29A and the NIST SRM.

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On the other hand, there exist several unconfirmed reports that are less clear in methodology and especially their assumptions based on anecdotal observations. These require confirmation and more extensive study, but they were more general in nature and simply made gross comparisons of central laboratory vs. the BGA 'correlation' and frequency of errors/discrepancies.

This gets us to one of the critical challenges I have written [8] and spoken [9] about in the past-one of communication among all interested parties-even those that speak the same language but in different dialects such as lab-speak, nurse-speak, doctor-speak, and the most difficult dialect of all, computer-speak. We may in fact have in the results an example of either crosstalk or failure to talk about the way in which direct ISE's can be and are measuring sodium and how they are and can be linked to a recognized NIST-SRM. The 'problem' was solved by C29A and the NIST SRM, but was it communicated both internally at all ISE manufacturers and externally to the end user (laboratory).

The question remains, however, as to the status of electrolytes in blood. The problem is technically solved but there may still be significant confusion as to what differences mean. If we decide based on the agreement of the experts such as IFCC, CLSI and NIST, we could easily conclude that systems have been harmonized by the, at least, two major manufacturers of BGA/Electrolyte analyzers. The 'watering-down' of the standardization process to get 'harmonized' results has worked [10]. More on the anomalies later, however.

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Development and Feasibility Evaluation of the Extraction Methods for Obtaining Quercetin from Onion Peel

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Abstract: Onion (*Allium cepa* L) is a worldwide food resource due to its nutritional and medicinal value. Increasing demands and production of onions have begun generating increased amounts of onion byproducts during processing, such as peel, and their disposal poses a burden on the environment. The processing of the onion generates a vast amount of onion peel or skin that is thrown away as a waste, which is rich in bioactive compounds such as free quercetin. Quercetin is one of the major plant-derived bioflavonoids and has garnered significant attention due to its wide range of pharmacological properties. The aim of the present work was to develop various alternative methods capable of efficiently reprocessing onion peel, combined with the purification dry column vacuum chromatography and analytical spectrophotometric procedures for obtaining high-purity quercetin, that would be easy to perform, fast, effective, selective, reproducible, time- and energy-saving, eco-friendly, and cheap without the use of toxic solvents. The concept of the present work was not only to allow us to obtain the target quercetin in laboratory conditions but also that would be capable of and appropriate to be transferred to an industrial scale, and contribute to the application of onion peel as an agro-industrial waste. The ultrasound-assisted and boiling with heater extraction techniques were used to extract the target compound from onion peel and the effect of critical parameters (ultrasonic power, extraction time, solvent nature, solvent volume, temperature, and the amount of raw material on the extraction process) were investigated. The dry column vacuum chromatography technique was used for purification to remove unwanted non-polar and polar impurities from the target bioactive compound; a new, specific and rapid UV-spectrophotmometric analytical procedure was developed to evaluate the pruity of the dry powdered extract obatiend with the developed methods. The percentage contents of quercetin in the dried and powdered samples of onion peel vary from 0.170 mg/g to 2.850 mg/g. A certain part of the work was devoted to an extensive discussion of the economic feasibility of each developed procedure. Based on the feasibility evaluation of the 11 different developed methods, there is selected a fast, effective, selective, reproducible,

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time- and energy-saving, eco-friendly, and cheap extraction method that should be suitable for scale-up and can be transferred to the industry.

Key Words: Extraction, onion peel, spectrophotometry, quercetin

1. Introduction

Onion (*Allium cepa* L) is a worldwide food resource due to its nutritional and medicinal value. World onion production has increased by at least 25% over the past 10 years with current production being around 44 million tonnes annually, making it the second most important horticultural crop after tomatoes. According to the Food and Agriculture Organization of the United Nations, in 2021, the global production of onion was 107 million tons [1]. Increasing demands and production of onions have begun generating increased amounts of onion byproducts during processing, such as peel, and their disposal poses a burden on the environment. The processing of the onion generates a vast amount of onion peel or skin that is thrown away as a waste. Dry onion peel is rich in bioactive compounds such as polyphenols, flavonoids, tannins, and other secondary metabolites, especially free quercetin. The composition of onion peel changes based on different varieties, agronomic conditions of the region in which they were cultivated, and the extraction techniques used [2-3]. Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one), found abundantly in onion peel and containing more than 77-fold that found in the edible part of the onion, is one of the major plant-derived bioflavonoids. This compound mainly exists in dry onion peel in aglycone form (67-86% of the total quercetin), with only a small proportion of quercetin appearing in the glycoside form [4]. This natural compound is interesting due to its wide range of bioactive activities and shows not only antioxidant properties like all the bioflavonoids but also anti-inflammatory, antiviral, antibacterial, anticarcinogenic, hepatoprotective, cardiovascular and anti-platelet effects. It has been known to have anti-obesity, antidiabetic, and antihypertensive effects in animal and human studies as well. Quercetin could also sensitize resistant cancer cells to chemotherapy and synergize the effects of drugs on nonresistant cancer cells [5-13].

Due to the antioxidant characteristics of onion peel, this material can be useful in many food-related industrial aspects where prevention of oxidative damage or free radical formation is involved. Therefore, food quality, shelf-life extension, and intelligent packaging could be improved or maintained by developing these characteristics [2]. In general, the scale-up production of valuable bioactive products from agro-industrial wastes is generally troublesome. The main reason is the large gap between laboratory and large-scale production, and the development of laboratory standard operating procedures for kilogram-scale production is challenging nowadays. Also, the synthesis of derivatives requires chemically pure bioactive compounds. Thus, the elaboration of simple, rapid, cost-saving, and eco-friendly extraction, purification, and analytical procedures for routine preparation or semi-industrial production of high-purity natural bioactive compounds including quercetin can be considered a relevant way of solving the problem.

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Extraction is one of the essential stages in investigating desired bioactive compounds, recycling agro-industrial wastes and their application in the nutraceutical and pharmaceutical industries. One of the pioneering conventional methods for extraction is dependent on the polarity of the target compound and solvent systems. Solvents such as a mixture of water and ethanol, water, ethanol, and methanol have been most commonly utilized to obtain extractable compounds from onion peels. Recently, to reduce the consumption of solvents, make the process eco-friendly, and at the same time enhance the efficiency of extraction, the use of different methods such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pulsed electric field (PEF) extraction, enzyme-assisted extraction (EAE), supercritical fluid extraction (SFE), subcritical water extraction (SWE), and pressurized liquid extraction (PLE) has been encouraged. With respect to extractions of bioactive compounds from onion peel or waste, various methods, such as conventional extraction and nonconventional extraction methods, such as SWE, UAE, and MAE, have been reported by several authors. The usage of conventional solvents for extraction leads to adverse effects on the environment, considering their volatility, nondegradable nature, and toxicity. These methods are time- and/or solvent-consuming, are not selective, and are targeted at total phenols rather than quercetin. However, these green processes reduce the harmful impacts on the environment by minimizing or eliminating the use of solvents and improving extraction efficiency [14-23].

The aim of the present work was to develop various alternative methods for obtaining high-purity quercetin from onion peel that would be easy to perform, fast, effective, selective, reproducible, time- and energy-saving, eco-friendly, and cheap without the use of toxic solvents. The concept of the present work included the development of a method that would not only to allow us to obtain the target quercetin in laboratory conditions but also that would be capable of and appropriate to be transferred to an industrial scale, and contribute to the application of onion peel as an agro-industrial waste. The various protocols capable of efficiently reprocessing onion peel, combined with the purification dry column vacuum chromatography (DCVC) and analytical spectrophotometric procedures for obtaining quercetin in the form of the extracted dried product, are proposed in the present paper by the authors. The different extraction techniques, solvents, sequence of stages, temperature range, and extraction time were investigated during the experiment. A certain part of the work is devoted to an extensive discussion of the economic feasibility of each developed procedure, evaluated according to various parameters. As a result, there was determined various methods intended for use, met our goals and the predetermined target profile.

2. Materials and Methods

Materials

The ethyl acetate, n-hexane, acetone, ethanol, methanol, petroleum ether, white spirit, sodium sulfate, Merck silica Gel 60 – 0.015-0.040 mm - №1.15111.1000 Celite® 545 particle size 0.02-0.1 mm were purchased from Merk. The certified analytical reference standard of quercetin dihydrate (89.1% calculated on anhydrous quercetin) was

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purchased from United States Pharmacopoeia (USP).

Instrumentation

The Milli Q Adventage A10 purification system (Millipore, France), dual-frequency ultrasonic bath DW-5200DTS (China), Vortex-Genie™ 2 Mixer (USA), Magnetic Stirrer IKA C-MAG HS 10 (China), pH-meter Hanna Instruments HI 2211 (USA), analytical balance ALX-210 (USA), Biobase small capacity rotary evaporator (China), GFL water bath (Germany), Hermle Z200A centrifuge (Germany), laboratory mill SM-450C were used for sample preparation. The spectrophotometric analysis was performed using UV-Vis spectrophotometry UV-1900i Shimadzu (Japan). All the measuring equipment was appropriately calibrated.

Methods

The ultrasound-assisted and boiling with heater extraction techniques were used to extract the target compound from onion peel. 20 g of the powdered dried sample of onion peel was transferred to a 250 mL round-bottomed (conic) flask. Various volumes of the following extraction solvents – ethyl acetate, acetone, ethanol and purified water were used. Experiments were carried out by ultrasonication at 40 kHz and boiling with a heater at different times and various temperatures. The obtained organic extracts during the extraction process were dried through sodium sulfate. After sufficient evaporation of the solvent, 3 g of celite was added to the wet sample. In order to evaporate the solvent during the extraction process, a rotary evaporator at 30-50°C under reduced pressure was used to give a dried homogeneous mixture. The obtained dried solid sample was used for the purification stage. The purification of the obtained dried extract was performed using the DCVC with ethyl acetate/hexane, ethyl acetate/petroleum ether and ethyl acetate/white spirit as an eluent solvent, which was composed of a cylindrical sintered glass funnel (height – 10 cm, diameter – 4 cm), a separating funnel, and a glass joint connecting these two with a sidearm to apply a vacuum and aspirator pump. Merck Silica Gel 60 – 0.015-0.040 mm – Merck № 1.15111.1000 was used as an adsorbent (height – 7 cm).

The reference standard of quercetin dihydrate was dissolved in methanol and diluted with the same diluent to obtain a standard solution at 0.004 mg/mL concentration. In order to prepare the test solution, the dried extracted product was dissolved and diluted in the same manner as the standard solution to obtain the same concentration (100 %). The obtained solution was filtered through a 0.45 μm through 0.45 μm polyvinylidene difluoride (PVDF) microporous membrane filter. The quantification was performed using the external standard method. Methanol was used as a blank solution.

The absorbances of the standard and the test solutions were measured at a wavelength of 375 nm. The UV-Vis spectra of solutions were scanned in the wavelength range of 190–600 nm.

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Calculations

The concentration of quercetin – C_S, mg/mL in the test solution was calculated by the following equation (1):

$$C_s = \frac{Abs_S \times W_{St} \times D \times P}{Abs_{St} \times 100} \quad (1)$$

where, Abs_{St} – the absorbance of the standard solution; Abs_S – the absorbance of the standard solution; W_{St} – the weight of the standard of quercetin dihydrate reference standard, mg; D – the dilution factor of the standard; P – the purity of the standard of quercetin dihydrate calculated on anhydrous basis, %.

The percentage content of quercetin (purity) – X, % in the extracted product was calculated in equation 2:

$$X = \frac{C_S \times V_S \times 100}{W_S} \quad (2)$$

where, W_S – the weight of the extracted product sample, mg taken for analysis; V_S – the dilution of the extracted product sample, mL.

The percentage content of quercetin – x, %, in the onion peel (the percentage yield of the extraction procedure) was calculated in equation 3:

$$x = \frac{w \times 100}{m} \quad (3)$$

where, m – the weight of the dried sample of onion peel taken for the extraction, mg; W – the weight of the extracted product, mg;

The content of quercetin (assay) – A, mg per 1 g of the onion peel was calculated in equation 4:

$$A = \frac{w \times X \times 1}{M \times 100} \quad (4)$$

where, M – the weight of the dried sample of onion peel taken for the extraction, g.

The percentage recovery - R, % was calculated by the following equation (5):

$$R, \% = \frac{(W_d - X) \times 100}{W_a} \quad (5)$$

where, W_d – the determined amount (added and indigenous) of quercetin, mg in the dried sample of onion peel added analytical standard; W_a – the amount of standard of quercetin, mg to the dried sample of onion peel, mg.

The similarity factor – Sf, % for two standard solutions was calculated by the following equation (6):

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$$Sf = \frac{W_{st1} \times Abs_{st2} \times 100}{W_{st2} \times Abs_{st1}}$$

where, Abs_{St1} – the absorbance obtained with the standard solution I; W_{St1} – the weight of the standard for the standard solution II, mg; Abs_{St2} – the absorbance obtained with the standard solution I; W_{St2} – the weight of the standard for the standard solution II, mg.

3. Results and Discussion

Extraction procedures were developed by taking into account the following factors: the selectivity and yield of the extraction procedure, and the purity of the extracted product. To enhance the efficiency of the extraction process and increase the recovery of target compound for extracting compound from onion peel, various critical parameters – ultrasonic power, extraction time, solvent volume, solvent nature, temperature, and the amount of onion peel – were investigated and selected. The effect of key parameters for quercetin UAE extraction was evaluated by varying the temperature (25°C, 50°C, 60°C), the extraction time (30-60 minutes) and the extraction solvent (ethyl acetate, acetone, ethanol and water). The extraction temperature of 50°C was determined to be the optimal operating temperature for volatile organic solvents, 60°C for purified water. An increase in the temperature slightly increases the yield of the target product but drastically decreases selectivity; lowering the temperature decreases the yield of extraction. The optimal extraction time for UAE was 1 hour. The selectivity decreased significantly if the extract was stored after the extraction, even for 30 minutes. The priority of solvents toward selectivity was arranged as follows: ethyl acetate>acetone>water>ethanol. The solvent priority in the evaporation stage from a technological point of view was ethyl acetate>acetone>ethanol. Eleven extraction methods with their purification procedures were developed and established in the form of eleven standard protocols described below. These methods can be represented in the general scheme given in Figure 1. For purification, the DCVC method was used, which was a fast and efficient alternative technique.

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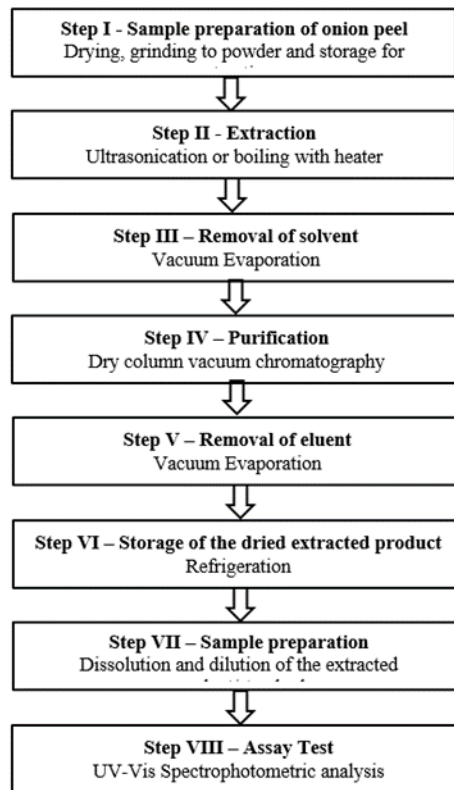

Figure 1. The General Scheme of Stepwise Method

The elution gradient was initially set to be 100% hexane, petroleum ether, white spirit and the percentage of ethyl acetate was gradually increased with 5% from 0 to 100%; The volume of each eluent portion was 20 mL; initially, the obtained fractions (1-20 fractions) were collected in 25 mL test tubes. The UV-Vis spectrophotometer was then used to detect the presence of quercetin in each fraction. Fractions containing the same compounds were then combined, and the solvent evaporated to dryness on a rotary evaporator. The eluent priority in the DCVC purification stage was ethyl acetate/white spirit>ethyl acetate/petroleum ether/hexane>ethyl acetate/hexane [19,24].

Protocol 1: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 200 mL of ethyl acetate was added and ultrasonicated for 30 minutes at 25-30°C. The extract was stored for half hour, filtered on filter paper; 3 g of celite was added to the filtrate and solvent was evaporated on a rotary evaporator. The obtained powder was stored in a refrigerator for further purification. The DCVC purification: eluent system – ethyl acetate/hexane with gradient 5%.

Protocol 2: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 200 mL of ethyl acetate was added and ultrasonicated for 1 hour at 25-30°C. The extract was stored for 2 hours, filtered on filter paper; 3 g of celite was added to the filtrate and solvent was evaporated on a rotary evaporator. The obtained powder was stored in a refrigerator for further purification. The DCVC purification: eluent system – ethyl acetate/hexane with gradient 5%.

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Protocol 3: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 200 mL of acetone was added and stored for 24 hours at 25-30°C. The extract was filtered on filter paper; 3 g of celite was added to the filtrate and solvent was evaporated on a rotary evaporator. The obtained powder was stored in a refrigerator for further purification. The DCVC purification: eluent system – ethyl acetate/hexane with gradient 5%.

Protocol 4: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 200 mL of ethanol was added and ultrasonicated for 1 hour at 25-30°C. The extract was stored for 2 hours and filtered on filter paper; 3 g of celite was added to the filtrate and solvent was evaporated on a rotary evaporator. The obtained powder was stored in a refrigerator for further purification. The DCVC purification: eluent system – ethyl acetate/hexane with gradient 5%.

Protocol 5: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 400 mL of purified water was added and ultrasonicated for 1 hour at 60°C. The extract was stored for 30 minutes. Filtered and extracted with ethyl acetate (50×3mL). Ethyl acetate phase was dried for 1 hour and 3 g of celite was added. Solvent was evaporated on a rotary evaporator, and the obtained powder was stored in a refrigerator. This procedure had been repeated on the same sample of onion peel. The obtained powdered samples were combined for further purification. The DCVC purification: eluent system – ethyl acetate/petroleum ether with gradient 5%.

Protocol 6: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 200 mL of ethyl acetate was added and ultrasonicated for 1 hour at 25-30°C. The extract was filtered on filter paper; filtrate was evaporated, and the powder stored in a refrigerator. 200 mL of ethyl acetate was added to the same sample of onion peel and ultrasonicated for 1 hour at 50°C. The extract was stored 2 hours filtered on filter paper; filtrate was evaporated. The powdered samples were combined, 100 mL of purified water was added, and the mixture was ultrasonicated for 1 hour at 25-30°C, filtered, and the filtrate was extracted with ethyl acetate. Solid phase was dissolved in ethyl acetate. Ethyl acetate phases were combined, 3 g of celite was added, solvent was evaporated on a rotary evaporator, and crude quercetin was stored in a refrigerator for further purification. The DCVC purification: eluent system – ethyl acetate/hexane with gradient 5%.

Protocol 7: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 800 mL of purified water was added and heated 1 hour at 90-100°C. The extract was stored for 30 minutes, then filtered and again stored for 72 hours. The formed precipitate was filtered on filter paper and filtrate was extracted with ethyl acetate (50 mL×3). Ethyl acetate phase was dried on Na_2SO_4 for 1 hour and 3 g of celite was added. Solvent was evaporated on a rotary evaporator, and the obtained powder was stored in a refrigerator for further purification. Precipitate was immersed in a conic flask, 150 mL of ethyl acetate was added and ultrasonicated for 30 minutes at 25-30°C, the solution was stored for 30 minutes, and filtered on filter paper. 3 g of celite was added, solvent was evaporated on a rotary evaporator, and the powder was stored in a refrigerator for further purification. The DCVC purification: eluent system ethyl acetate/petroleum ether with gradient 5%.

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Protocol 8: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 400 mL of purified water was added and ultrasonicated for 1 hour at 60°C. The extract was stored for 30 minutes, then filtered and extracted with ethyl acetate (100 mL×3). Ethyl acetate phase was dried for 1 hour and 3 g of celite was added. Solvent was evaporated on a rotary evaporator, and the obtained powder was stored in a refrigerator for further purification. The DCVC purification: eluent system – ethyl acetate/white spirit with gradient 5%.

Protocol 9: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 400 mL of purified water was added and ultrasonicated for 1 hour at 60°C. The extract was stored for 30 minutes, then filtered and extracted with ethyl acetate (50 mL×3). Ethyl acetate phase was dried for 1 hour and 3 g of celite was added. Solvent was evaporated on a rotary evaporator, and the obtained powder was stored in a refrigerator. The DCVC purification: eluent system – ethyl acetate/hexane with gradient 5% for fraction 1-10 and gradient 2.5% for fraction 11-17.

Protocol 10: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 400 mL of purified water was added and boiled for 30 minutes. The extract was stored for 30 minutes and filtered on filter paper. The filtrate was extracted with ethyl acetate (50 mL×3). Ethyl acetate phase was dried on Na_2SO_4 for 1 hour and 3 g of celite was added. Solvent was evaporated on a rotary evaporator, and the obtained powder was stored in a refrigerator for further purification. The DCVC purification: eluent system – ethyl acetate/hexane with gradient 5% for fraction 1-10 and gradient 2.5% for fraction 11-17.

Protocol 11: The extraction procedure: 20 g of dried onion peels were ground, weighed and placed in a conic flask; 200 mL of ethyl acetate was added and ultrasonicated for 1 hour at 25-30°C. The extract was filtered on filter paper; 3 g of celite was added to the filtrate and solvent was evaporated on a rotary evaporator, and the obtained powder was stored in a refrigerator for further purification. The DCVC purification: eluent system – ethyl acetate/hexane with gradient 5% for fraction 1-10 and gradient 2.5% for fraction 11-17.

Six individual extractions were performed according to each protocol. Each method with analytical procedure was verified with respect to the following performance parameters: specificity, precision and accuracy according to the appropriate guideline and the methodologies reported by the authors [25-28]. All the extracted dried products containing quercetin, obtained with the developed methods, were analyzed using UV-Vis spectrophotometric procedure and measured absorbances (at 375 nm) of standard and tests solutions. Accordingly, the concentrations of quercetin, expressed in mg/mL in test solutions – C_s, the percentage content of quercetin (purity) – X, % in the extracted product, the yield of the extraction procedure – x, % and the content of quercetin in onion peel, mg per 1 g of the waste material – A were evaluated by calculating equations 1-4, respectively. The results are given in Table 1.

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Table 1. Analytical Data

Protocol №	Precision	Accuracy	Test Solution		The purity of the extracted product – X, %	The yield of the method – x, %	The content of quercetin in onion peel – A, mg/g
	RSD, % (n=6)	Percentage recovery – R, %	Absorbance (n=6)	Concentration – C_s (n=6), mg/mL			
1	1.704	97.50	0.069	0.00119	29.27	0.110	0.322
2	4.255	95.12	0.173	0.00298	73.09	0.107	0.782
3	3.774	98.11	0.154	0.00264	64.83	0.105	0.681
4	2.202	96.75	0.090	0.00154	37.83	0.045	0.170
5	2.809	95.33	0.114	0.00197	48.25	0.233	1.124
6	3.364	95.09	0.137	0.00236	57.79	0.117	0.676
7	3.541	95.97	0.144	0.00248	60.82	0.389	2.366
8	2.958	96.12	0.120	0.00207	50.82	0.213	1.082
9	2.050	98.52	0.083	0.00144	35.21	0.358	1.261
10	3.913	98.11	0.159	0.00274	67.21	0.424	2.850
11	3.113	97.74	0.208	0.00358	87.83	0.083	0.729
Average value	**3.408**	**96.76**	**0.139**	**0.00239**	**55.72**	**0.199**	**1.095**
Maximal value	**4.255**	**98.52**	**0.208**	**0.00358**	**87.83**	**0.424**	**2.850**
Minimal value	**1.704**	**95.09**	**0.069**	**0.00119**	**29.27**	**0.045**	**0.170**

The specificity test of the analytical procedure was checked by scanning the background control - blank (methanol), standard and test solutions in the wavelength range of 190–600 nm. According to the obtained UV-Vis spectra, there was no interference, and the blank solution had no absorbance at 375 nm. There was a very strong spectral similarity between standard and test solutions.

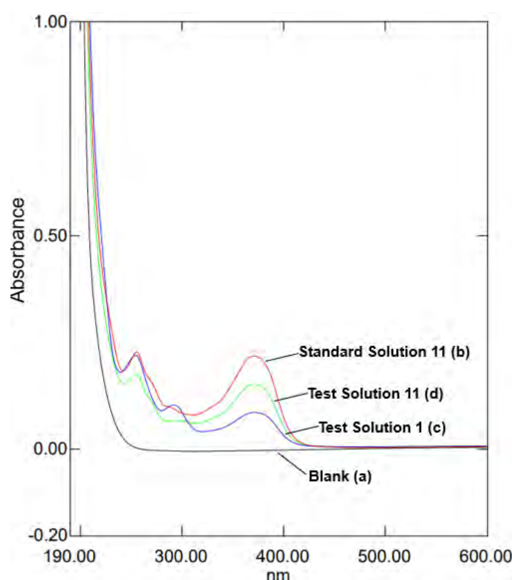

Figure 2. Overlapping UV-Vis Spectra Obtained with the Blank (a), Standard Solution (b), Test Solution 1 (sample 1 with lowest purity) (c), Test Solution 11 (sample 11 with highest purity) (d) Scanned in the Wavelength Range of 190–600 nm

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Figure 2 depicts the overlapping UV-Vis spectra obtained with the blank, standard solution and test solutions scanned in the wavelength range of 190-600 nm. Hence, this analytical spectrophotometric procedure could specifically measure the absorbances of the analytes.

The precision of each method was estimated by preparing the two standard solutions and six test solutions according to each protocol. This parameter was checked by calculating the RSD of six determined concentrations (mg/mL) of quercetin (Cs) in the test solutions (acceptance criteria: ≤5.0%). The similarity factor – Sf, % between the absorbances obtained with two standard solutions was calculated by the equation 5 and should be within 98.0 %-102.0% (acceptable criteria). The calculated value of the Sf was 100.11%. The results of the precision test are given in Table 1 which show that all the developed procedures have a good precision.

The accuracy test was assessed by performing recovery studies using the standard addition method by spiking known amount of analytical standard in the test solution at 150% of quercetin. The spiked test solution was prepared according to the following procedure: the analytical standard of quercetin was mixed with the dried and powdered sample of onion peel to obtain 150% of quercetin in the spiked test solution before starting the extraction procedure. The accuracy was expressed as a percentage recovery which was the percentage of standard compound recovered from the spiked test solution (sample + standard) and calculated using equation 6. The recovery – R, % should be within 95.0-105.0% (acceptance criteria).

The results given in Table 1 show that all eleven procedures have a good recovery rate. Based on the analytical results, all eleven developed methods described in the above-mentioned protocols are specific, characterized by good precision and accuracy and respectively considered to be verified procedures.

As the results show, the determined percentage contents of quercetin in the extracted products are different. Protocol 11 is characterized with the highest purity (87.83%) of the extracted product, and the lowest purity (29.27%) is observed in case of protocol 1. The UV-Vis spectra (Figure 3) confirms that the highest purity of the extracted product was obtained with protocol 11, and its spectral similarity to the standard is equal to 0.999.

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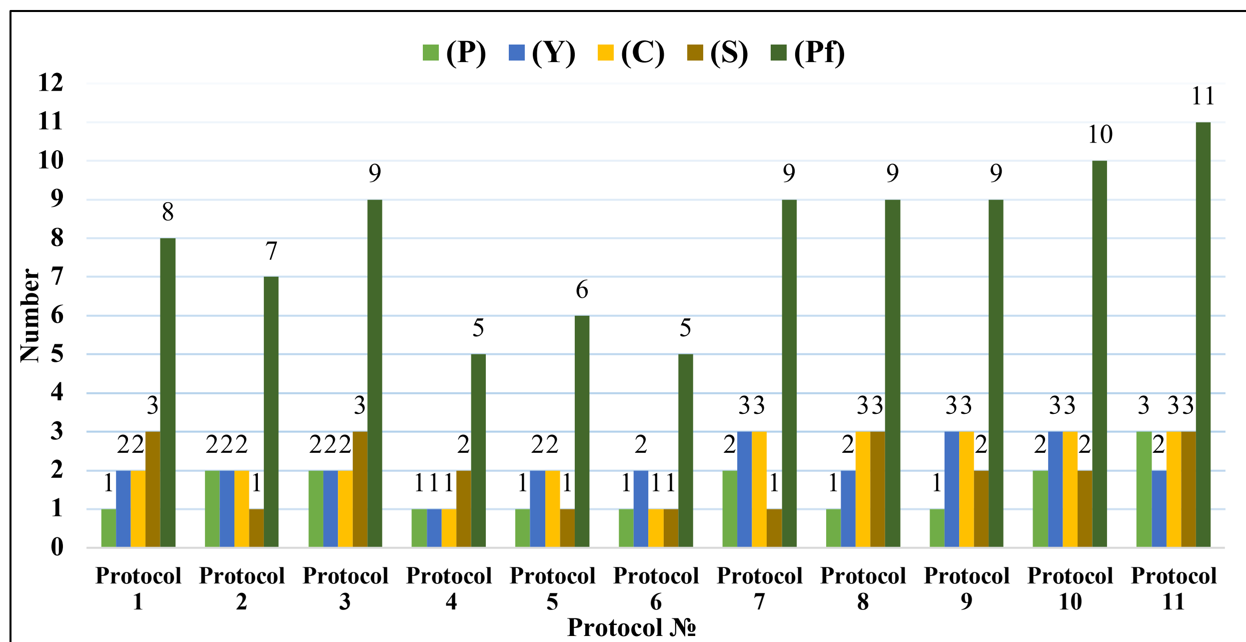

Figure 3. The Chart of the Feasibility Evaluation of the Developed Methods Described in the Protocols

A different picture is observed if we compare the developed procedures in terms of the extraction yield. The highest yield (0.424%) was obtained with protocol 10, although the purity of the product is high and equal to 67.21% but relatively lower than the result obtained with protocol 11.

Despite the acceptable yields of the extraction observed, the methods described in protocols 1, 4, 5, 6, 8, and 9 do not provide the possibility of obtaining high-purity quercetin; therefore, it is necessary to use additional purification procedures of the obtained product. The extracted product with the highest purity of quercetin can be obtained with protocols 2 and 11, and the highest extraction yield of quercetin can be obtained with protocols 7, 9, and 10. The extraction procedures described in protocols 2, 10, and 11 are characterized by an acceptable yield, and the purity of the extracted products obtained using them is relatively higher. Therefore, these three proposed procedures are eco-friendly, effective, and selective extraction methods based on ultrasonication that can be used successfully in laboratory conditions.

To evaluate feasibility and select an optimal method for preparative purposes in industrial scale, the following four parameters were used: the purity of the extracted product (P), yield of the method (Y), costs of goods (C), and simplicity of the method (S). The evaluation was carried out using a quantitative method using the mentioned parameters. Each parameter had a 3-point scale (Table 2). Finally, the number of the priority factor (Pf) was calculated by the simple equation [Pf = (P)+(Y)+(C)+(S)]. The higher the calculated number of the priority factor, the higher the priority and feasibility of the developed method. The results of the feasibility evaluation of the methods are given in Figure 3. The highest number of the priority factor was observed in case of protocol 11; additionally, the purity of the extracted

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product was higher (87.83 %). Therefore, the method is appropriate and suitable to be used for preparative purposes and to be transferred from laboratory conditions to industrial scale.

Table 2. Criteria of the Parameters and the Ranges of Numbers of the Priority Factor with Corresponding Categories

Priority category	Point	Purity of the extracted product, % (P)	Yield of the method, % (Y)	Costs of goods, 1 mg US cent (C)	Simplicity of the method (S)	Number of the Priority Factor (Pf)
High	3	>85	>0.3	<5	Simple to perform	10-12
Medium	2	60-85	0.05-0.3	5-10	Simple to perform and 2 steps and more	7-9
Low	1	<60	<0.05	>10	Moderately difficult to perform and 2 steps and more	4-6

Based on the analytical data, the percentage contents of quercetin (the purity of the extracted product) – A, expressed in mg per 1 g of the dried and powdered samples of onion peel, vary from 0.170 mg/g to 2.850 mg/g, calculated on the anhydrous basis. The purity is equal to the purity of the standard, which was also confirmed by the presented UV spectrum (Figure 2).

4. Conclusion

It has been shown that onion peel is a rich source of quercetin through the application of advanced eco-friendly and low-cost extraction techniques with the use of non-toxic solvents. The proposed three different, effective, eco-friendly, reproducible, and selective ultrasound-assisted extraction methods, combined with suitable and verified analytical spectrophotometric procedures for obtaining quercetin from onion peel, are alternative laboratory methodologies that provide a high-quality target quercetin in the form of the dried powdered extracted product. In addition, these proposed methods can be successfully used by scientific and quality control laboratories to quantitatively determine quercetin in onion peel and other phyto-extracts. Also, there is proposed a fast, effective, selective, reproducible, time- and energy-saving, eco-friendly, and cheap extraction method based on the feasibility evaluation that should be suitable for scale-up and can be transferred to the industry in order to obtain quercetin in kilogram-scale production from onion peel as an agro-industrial waste material. Further studies should be aimed at standardizing and validating the proposed method in order to obtain a pilot product as an ingredient for later development of a commercial production line of food or nutraceutical formulations.

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5. Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Antioxidant, Chemical Composition of Syrian Essential Oil's *Juniperus excelsa* Fruit and Leaves

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Abstract: In this work, the chemical composition determined the antioxidant activity of essential oils obtained from the leaves and fruits (seed cones) of *Juniperus.excelsa*, which grows in the Khashaa area of the Qalamoun mountains in Syria. The yield of *J. excelsa* essential oil extracted by Clevenger-type was 1.38% for leaves and 0.8% for fruits. Gas chromatography-mass spectrometry (GC-MS) was applied to investigate the chemical composition of *Juniperus excelsa* essential oil (JEO) for leaves and fruit. The results showed that the main constituents were α-pinene (52.99% – 81.42%), delta-Cadinene (8% – 1.26%), dl-Limonene (1.38% – 1.311%), gamma-Elemene (2.15% – 1.53%), delta-3-careen (4.97% – 0.43%) and gamma-Gujunene (4.53% – 2.55%) and α-Cadinol (3.82% – 0.525), respectively. The antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and the results showed RSA= (55% – 38%), respectively.

Key Words: *Juniperus excelsa*, essential oil, GC-MS, antioxidant activity

1. Introduction

Juniperus is one of the major generals in the Cupressaceae family, it includes about 50-75 species distributed throughout the world, depending on the taxonomic classification [1], and it has more than 220 varieties [2]. *Junipers* belong to the Pinophyta (Conifers) division of plants, producing many biologically active metabolites, which contributes to their various biological activities [3]. *Juniperus excelsa* is an evergreen tree with a narrow, pyramidal crown when young, becoming more spreading with age; it can grow 4 - 12 meters tall, sometimes reaching 20-25 meters. The bole on more giant trees can reach 150-250 cm in diameter [4]. It grows on dry rocky slopes in hills and

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mountains at an altitude of 150-2700 m [5]. *Juniperus excelsa*, commonly called the Greek juniper or Grecian juniper, is a juniper found throughout the eastern Mediterranean, from northeastern Greece and southern Bulgaria across Turkey to Syria and Lebanon, Jordan, the Caucasus mountains, and southern coast of Crimea [6]. This species of *Juniperus excelsa* has been divided into 2 subspecies (subspecies: M.Bieb and subsp. polycarpus) by vargon: one with a distribution in southeastern Europe, the Crimea, southern Turkey mainly to Lebanon; the other, a continental component extending from northern Turkey to Kyrgyzstan and Pakistan [7]. The *Juniperus excelsa* M.Bieb strain is the subject of this paper, grows in temperate regions throughout the eastern Mediterranean mountains and is widespread in Lebanon. In Syria, it exists in the Qalamoun mountains and in the Latakia mountains. It is locally known as "lezzab" or "chajarit al bakhour" [8]. *Juniperus excelsa* was formerly treated in a looser sense to include the species *Juniperus polycarpos* and *Juniperus seravschanica*. The three are now generally seen as distinct, though they are all similar and have similar properties. It can be challenging to separate out the various uses attributed originally to *Juniperus excelsa* to any of these three species and, certainly on the traditional level, they are very likely to apply to all three. All the medicinal uses we know of are listed here (where possible, we point out if they were specifically attributed to what is now one of the three species) [9].

The reference number [10] mentioned that seed cones of *Juniperus polycarpos* are edible raw or cooked. Native people in Iran eat the uncooked cones with rice (the purplish green to blue globose cones are about 7-12 mm in diameter and take 2 years to mature on the tree [11]. As for medicinal uses, since ancient times, juniper has been known as folk remedies against various diseases. The first information about the therapeutic properties of juniper is found in the Egyptian Ebers papyrus (ca. 1500 BC), which mentioned the use of juniper against tapeworms and roundworms [12]. An excellent review by Hartwell (1967-1971) on medicinal plants mentioned the use of *Juniperus virginiana* L. (red cedar) leaves for the treatment of genital warts [13]. Some studies have reported the use of juniper berry essential oil in aromatherapy formulations for perfumes and cosmetics, as well as in folk medicine for various diseases such as bronchitis, arthritis [14] and several parasitic diseases [15]. The Himalayas plant (*Juniperus seravschanica*) is considered useful as an antihypertensive, stimulant, appetizer, diuretic, carminative and anticonvulsant. It is used in folk medicine to treat a range of conditions including abdominal spasm, diarrhea, asthma, fever, headache, gonorrhea and leukorrhea. Used in Lebanese and Turkish folk medicine (this could refer to both *Juniperus excelsa* and *Juniperus polycarpos*), the seed cones are used to treat cutaneous diseases such as eczema and skin rash. It is used to treat a wide range of respiratory tract diseases like asthma, cough, common cold, pneumonia, throat inflammation and tuberculosis, urinary tract inflammations, rheumatism, and to remove renal and gall bladder stones [10]. In various regions of the world, the plant is used to treat dysmenorrhea, jaundice, and tuberculosis, to induce menses and expel a fetus [16].

The studies on the Syrian juniper tree (*Juniperus excelsa*) found in the Qalamoun region are very limited and almost unavailable, so this work aims to determine the chemical composition of essential oils contained in the leaves of *Juniperus excelsa* to determine the antioxidant activity. Figure 1 shows *Juniperus excelsa* M.Bieb trees.

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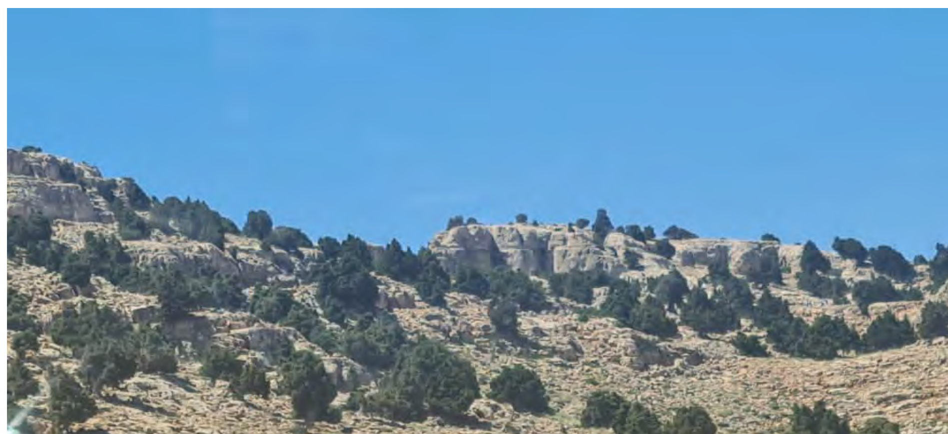

Figure 1. ***Juniperus excelsa*** **in the Khashaa Area (Qalamoun Mountains), Syria**

2. Materials and Methods

The preparation of solutions and the conduct of chemical analyses were carried out at the laboratories of the Private Syrian University. The analysis of antimicrobial activity was carried out at the National Commission for Biotechnology in Damascus, while the analysis of essential oil by gas chromatography was carried out at the Atomic Energy Commission in the Syrian Arab Republic.

Collection and Preparation of Plant Leaves

The plant, *Juniperus excelsa* M.Bieb, was collected precisely on 14 July 2023 from the Al-Khushaa region in the Al-Qalamoun mountains, Ras al-Ma'arra village, Yabroud area, Damascus countryside, Syria.

The fresh leaves and green fruits were separated from the twigs and washed with tap water, then the drying process was carried out for 24 hours in a dark and dry place at room temperature.

Figure 2. ***Juniperus excelsa*** **M.Bieb Leaves and Branches Bearing Berry Fruits**

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The grinding process was carried out with an electric grinder immediately before work until a grain size of less than or equal to 0.2 mm diameter was reached. Figure 2 shows *Juniperus excelsa* M.Bieb leaves and branches bearing berry fruits.

Preparation of the Essential Oil

The steam distillation method was employed using a Clevenger-type apparatus to extract the essential oil. The process was conducted in two repetitions. Each time, 100 grams of the ground leaves or fruits were subjected to steam distillation for 4 hours, along with 1000 milliliters of distilled water [17].

DPPH Radical Scavenging Assay

The evaluation of *Juniperus excelsa* essential oil's (EO) antioxidant properties was conducted through the DPPH Radical Scavenging Assay (RSA). To initiate the assessment, a stock solution of DPPH was prepared by dissolving 4 milligrams of DPPH in 100 mL of 99% ethanol. Filtration of the DPPH stock solution with ethanol yielded a usable solution with an absorbance of approximately 0.886 at 517 nm. Subsequently, 1 mL of the workable DPPH solution was mixed with 50 µL of EO in a test tube. The tubes were then placed in complete darkness for a duration of 30 minutes, after which the absorbance at 517 nm was measured. The following formula was employed to calculate the percentage of antioxidants or Radical Scavenging Activity (RSA) [18]:

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

where: Ac—Control reaction absorbance; As—Testing specimen absorbance.

Gas chromatography-mass spectrometry

Essential oils of leaves and fruits of *Juniperus excelsa* were analyzed by gas chromatography with electron-ionization mass-selective detector, according to the previously published procedure [19]. In brief, 1 µL of the sample dissolved in hexane (1;10) was injected into a split/split-less inlet at 240°C, with a split ratio of 1:50. Helium (purity 99.999%) was used as a carrier, with a constant flow of 1 mL/min. The separation was achieved on the HP-5MS 5% Phenyl Methyl Silox column (325°C: 30 m x 250 µm x 0.25 µm) using the following temperature program: start at 40°C, 2°C/min , then 2.5°C/min to 70°C, then 2°C/min to 130°C , then 2.5°C/min to 160°C , then 5°C/min to 200°C , then 10°C/min to 240°C, and hold for 10 min. The eluate was delivered to the mass spectrometer via a transfer line held at 260°C. The ion source temperature was 240°C, electron energy was 70 eV, and the quadrupole temperature was 150°C. Data was acquired in scan mode (m/z range 42–850). The compounds were identified by comparison of mass spectral with data libraries (Wiley Registry of Mass Spectral Data, 7th ed. and NIST/EPA/NIH Mass Spectral Library 05). Relative amounts of components, expressed in percentages, were

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calculated by normalization measurement according to the peak area in total ion chromatogram.

3. Results and Discussion

The resulting essential oil exhibited a very pleasant and strong aroma, while its color appeared transparent without any obvious color in the case of the leaves and fruits. The average percentage of essential oil (extracted by Clevenger type) relative to the raw material of the leaves was 1.38%, while the essential oil yield of the fruits was 0.8%.

This study worked in the identification of antioxidants within the essential oil of *Juniper excelsa* leaves and fruits by evaluating 2,2-diphenyl-1-becquerylhydrazyl (DPPH) radical scavenging activity. DPPH screening is a well-established, cost-effective and effective methodology for measuring antioxidant capabilities. It is based on the use of free radicals to assess the ability of substances, to act either as hydrogen donors or scavengers of free radicals. This technique involves the reduction of a DPPH, which is a stable free radical, and interaction with an unpaired electron leads to intensive absorption at 517 nm, which appears as a purple color. For example, the reactivity of juniper essential oil has been demonstrated by the ability of oils to act as hydrogen atoms or electron donors in the transformation of stable purple to reduced yellow. A free radical scavenging (FRS) antioxidant, for example, reacts to DPPH to form DPPH-H, which has a lower absorbance than DPPH because of the lower amount of hydrogen. It is radical in comparison to the DPPH-H form because it causes decolorization, or a yellow hue, as the number of electrons absorbed increases. Decolorization affects the lowering capacity significantly. As soon as the DPPH solutions are combined with the hydrogen atom source, the lower state of diphenylpicrylhydrazine is formed, shedding its violet color [20]. After the mathematical relationship of the antioxidant activity was applied to the essential oils that were extracted from leaves and fruit of the Clevinger-type (antioxidant activity= $[(Ac-As)\div Ac]\times 100$), it was showed (55% – 38%), respectively. The results can be interpreted by comparison with data from previously published articles as reference [21]; which indicated that the main compounds of oils showing high antioxidant activity were α-pinene (33.7%) in juniper berry, limonene (74.6%) in celery seed, benzyl acetate (22.9%) in jasmine, myristicin (44%) in parsley seed, patchouli alcohol (28.8%) in patchouli, citronellol (34.2%) in rose, and germacrene (19.1%) in ylang-ylang. In the same paper [21], it was noted that the scavenging abilities of various bear root essential oils ranged from 39% – 90% (39 for Angelica seed oil to 90 for jasmine).

The chemical composition of *Juniperus excelsa* leaves and fruit essential oil determined by Gas chromatography-mass spectrometry (GC–MS) GC/MS is presented in Table 1. Twenty-six components for leaves and 21 for fruits, representing total detected constituents, were identified: 100% for leaves and 98.11% for fruits. The terpenes percentages were: monoterpenes (3.84% – 4.76%), and cyclic monoterpenes (23.07% – 28.57%), cyclic sesquiterpenes (46.15% – 57.14%), cyclic sesquiterpenoids (26.94% – 9.53%) of leaves and fruits, respectively.

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Table 1. Chemical Composition of Fruit (Berries) and Leaves Essential Oils from Wild-Growing *Juniperus excelsa* in the Qualamoon Mountains, Syrian

Library/ID	Classification	Leaves %	Fruit %
1R-alpha-Pinene	bicyclic monoterpene	52.9923	81.421
delta-Cadinene	bicyclic sesquiterpene	8.0325	1.2653
delta-3-Carene	bicyclic monoterpene	4.9747	0.4682
γ-Gurjunene	bicyclic sesquiterpene	4.5341	2.5561
α-Cadinol	bicyclic sesquiterpenoid alcohol	3.8224	0.5258
γ-Muurolene	bicyclic sesquiterpene	3.562	0.4525
tau-Cadinol	bicyclic sesquiterpenoid alcohol	2.4306	0.5258
gamma-Elemene	monocyclic sesquiterpene	2.1563	1.526
β-Cubebene	tricyclic sesquiterpene	2.0148	0.6968
Limonene (Nesol)	monocyclic monoterpene	1.3888	1.3112
beta-Caryophyllene	bicyclic sesquiterpene	1.3769	0.9419
δ-Cadinol	bicyclic sesquiterpenoid alcohol	1.3119	-----
tau-Muurolol	bicyclic sesquiterpenoid alcohol	1.2665	0.6109
Elemol	3	1.174	-----
α-Gurjunene	tricyclic sesquiterpene	1.0697	-----
beta-Myrcene	monoterpene	0.8837	1.2558
alpha-Muurolene	bicyclic sesquiterpene	0.8789	0.3576
2-β-Pinene	bicyclic monoterpene	0.7722	1.0817
beta-Cadinene	bicyclic sesquiterpene	0.7084	------
Manoyl oxide	tricyclic labdane diterpenoid	0.6981	----
Aromadendrene	tricyclic sesquiterpene	0.6799	------
Camphene	bicyclic monoterpene	0.6728	0.4161
delta-Elemene	monocyclic sesquiterpene	0.6722	0.9142
α-Terpinolene	monocyclic monoterpene	0.6622	0.4011
Kaurene	tetracyclic diterpene	0.6561	-----
Ledol	bicyclic sesquiterpenoid alcohol	0.6324	------
beta-Elemene	monocyclic sesquiterpene	0.6314	0.7237
Sclarene	bicyclic sesquiterpene	0.2763	-----
beta-Maaliene	tricyclic sesquiterpene	-------	0.252

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The main constituents of the essential leaves and fruits oils of *J. excelsa* were α-pinene (52.99% – 81.42%), delta-Cadinene (8% – 1.26%), dl-Limonene (1.38% – 1.311%), gamma-Elemene (2.15% – 1.53%), delta-3-careen (4.97% – 0.43%), gamma-Gujunene (4.53% – 2.55%), and α-Cadinol (3.82% – 0.525), respectively. Figure 3 shows the chemical formulas of the main compounds.

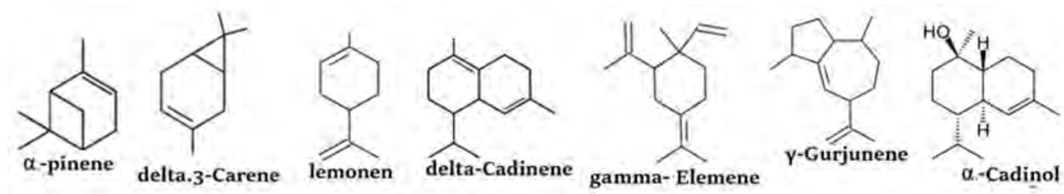

Figure 3. Chemical Formulas of the Main Compounds

The results of this study did not completely agree with the published data of previous studies. At the same time, the compound α-pinene is consistent with most studies [8,21,22], as it constitutes the most abundant compound in the essential oil. In contrast, some studies, which are very few, indicated that sabonene was the most abundant [23], while in other studies, limonene had the highest percentage [24], the largest in existence. A comparison of the numbers and quantities of components found in the essential oils of these plants grown in different parts of the world indicates that the oil composition of individual plants may vary significantly due to climate, growing region, time of collection, etc. These differences are very common.

4. Conclusion

In previous studies, researchers explored the different natural products that plants make. They focused on terpenes, a significant group of substances in conifer trees. Terpenes help protect plants from harmful invaders like pathogens and plant-eating animals. Some terpenes also work as antioxidants, which can reduce damage from stress. Terpenes are affected by various stresses like lack of water, changing temperatures, pollution, and attacks from pathogens. However, how they react to stress depends on the type and strength of the stress. Each conifer species has its typical terpene mix, but even trees of the same kind can have different terpene patterns.

5. Recommendations

Alpha and gamma terpenes found in extracts of this plant have been reported to have high antioxidant properties. In addition, some studies have shown that the monoterpene components of juniper essential oil enhance resistance to oxidative stress of organisms, and the essential oil is also strongly antifungal where the main compound responsible for these antifungal activities is delta-3-carene. Therefore, we recommend studying this type of Syrian juniper as an antifungal and antibacterial.

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6. Author Contributions

Investigation, project administration, supervision, and software: D. Mariam. Writing–review & editing, formal analysis, and methodology: A. H. Manar.

All authors have read and agreed to the published version of the manuscript.

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8. Data Availability Statement:

Data is contained within the article.

9. Conflicts of Interest:

The authors declare no conflict of interest.

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Characterization, Chemical Composition and Cream Formulation from the Seed Butter of *Mangifera indica* L.

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Abstract: Many individuals worldwide aspire to maintain beautiful skin and hair, making the use of safe cosmetic products essential. The objective of this study was to assess the viability of utilizing mango seed butter, a byproduct of juice production, for the environmentally friendly production of cosmetic items. The study examines the composition of fatty acids and the ability of mango seed butter to reduce inflammation in a laboratory setting. We acquired the oil using solvent extraction, using n-hexane as the solvent, and then underwent transesterification via methanolysis to yield fatty acid methyl esters. The fatty acid composition was determined using gas chromatography-mass spectrometry (GC-MS). The oil contains four primary fatty acids: oleic acid (35.715%), palmitic acid (29.365%), stearic acid (25.397%), and docasadienoic acid (11.905%). Topical cream was prepared using seed butter and its ability to reduce albumin denaturation was tested. The results showed that both the seed butter and formulations had dose-dependent effects similar to the standard indomethacin, especially at lower seed butter concentrations. The findings suggest that mango seed butter, which is typically considered a waste product, shows potential in the development of both safe and cost-effective natural cosmetics.

Key Words: *Mangifera indica L*, cream, transesterification, methanolysis, anti-inflammatory, formulation

1. Introduction

Organic cosmetics that incorporate bioactive phytochemical substances provide significant beauty and pharmacological benefits while minimizing harm to users and the environment. Researchers also found that herbal cosmetics incorporate natural bio-

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active substances like antioxidant, anti-cancer, and antibacterial properties, which may aid in a variety of skin ailments. Phytochemicals, including vitamins, proteins, tannins, terpenoids, and other bioactive compounds, have the ability to revitalize, refresh, and safeguard the skin against different skin conditions [1,2]. On a daily basis, nearly all individuals use cosmetics, a widely-used chemical preparation [2].

Lately, there has been a shift in focus towards natural goods due to the harmful consequences of synthetic cosmetic products. The use of natural goods is steadily increasing worldwide due to their perceived enhanced safety and fewer negative side effects compared to synthetic alternatives [3]. Organic products contain potent antioxidants, vitamins, and other bioactive substances derived from natural sources, which effectively decelerate the skin aging process [4].

Mango, scientifically referred to as *Mangifera indica* L., is a highly significant tropical fruit belonging to the *Anacardiaceae* family. The increasing consumer demand led to a continuous expansion of global output, reaching its highest point to date at 47 million metric tons in 2016 [6]. People extensively grow the mango tree, a tropical fruit tree, because of its juicy and flavorful characteristics. However, beyond its well-known fruits, the mango tree offers a multitude of other components that remain largely unexplored in terms of their potential applications. An example of such a component is the seed, which makes up a substantial proportion of the fruit's weight. Traditionally, the fruit processing sector has viewed mango seeds as byproducts and disposed of them without realizing their untapped potential [6].

The chemical composition of mango seed butter suggests that it has the ability to function as an emollient, moisturizer, and enhancer of the skin's barrier. The cosmetics business is highly interested in these features as it strives to address the growing consumer demand for safer and more ecologically friendly goods. This industry is progressively seeking more natural and sustainable components [8,9]. Consumer tastes in the worldwide cosmetics and skincare sector have changed to favor products containing natural and plant-based components. Concerns about safety and the environmental effects of synthetic substances drive this movement [4].

The current trend has opened up possibilities for investigating the use of mango seed butter in skincare products, specifically creams and lotions. Nevertheless, despite the increasing interest, there is a scarcity of extensive studies that investigate the chemical makeup of mango seed butter and its potential advantages in beauty applications. Studies have demonstrated that using mango seed butter can improve skin moisturization and flexibility, both of which are essential elements in skin health preservation. Creams and lotions are essential elements of skincare routines, including moisturization, shielding, and specific therapy. The development of these goods entails meticulous consideration of chemical selection, such as emulsifiers, stabilizers, thickeners, and active substances. Attaining the appropriate equilibrium among these constituents is crucial for producing goods with favorable sensory characteristics, durability, and effectiveness [14].

Comprehending the scientific principles underlying cream composition is essential for integrating components such as mango seed butter into skincare products. This study aimed to analyze the properties of an underused mango seed oil, assess its potential

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for cosmetic applications, and measure its anti-inflammatory effects.

2. Materials and Methods

Sample Collection and Preparation

The mango seed (*M. indica* L.) was obtained from Ilorin, Kwara State, Nigeria, during the fruiting season in February 2022. We sent the plant sample to the herbarium of the Department of Plant Biology at the University of Ilorin in Ilorin, Nigeria, for standard identification and authentication. Mr. Bolu Ajayi assessed the sample and assigned it a voucher specimen number, UILH|001|969|2023. Following the verification process, we removed the outer shell of the seed material. We then dried the inner part, known as the endocarp, at room temperature (26-28ºC), crushed it into a powder, and stored it for future use.

Oil Extraction

The *M. indica* seed material was finely ground and then underwent many rounds of thorough Soxhlet extraction at a temperature of 60ºC using n-hexane. We condensed the consolidated extract using a vacuum rotary evaporator at a lower temperature to yield the oil.

Physicochemical characterization

The oil's physicochemical properties, including color, iodine value, and acid value, using standard methods and necessary minor adjustments, were made when needed [2,12].

GC-MS Analysis

Analyzing the fatty acid content of the trans-esterified oil was done using an Agilent Technology 7890A gas chromatograph GC-FID, which was equipped with a fused silica capillary column. We determined the fatty acid profile of the FAMEs by comparing their MS spectra with data from the National Institute of Standards and Technology (NIST, 2008) database. The relative proportions of the constituent chemicals were calculated as percentages derived from the peak regions obtained from the gas chromatography (GC) analysis, using the total ion chromatogram (TIC).

Formulation of Cream

The cream was formulated using bee wax as the emulsifier, tocopherols, water, and glycerine. Bee wax was heated with tocopherols, water, and glycerine in a beaker using a hot plate before adding the mango butter. The experiment was replicated using varying quantities of mango butter seed oil. We transferred the concoctions into various receptacles to solidify and preserve them (Table 1). The products underwent a stability test and evaluation for a duration of 70 days.

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Table 1. Formulation of Cream from Mango Seed Oil

Formulations	Bee Wax (g)	Water (ml)	Tocophenols	Glycerine (ml)	Mango Butter
F1	1.0	5.0	0.2	4.0	1.0
F2	1.0	5.0	0.2	4.0	2.0
F3	1.0	5.0	0.2	4.0	3.0
F4	1.0	5.0	0.2	4.0	4.0
F5	1.0	5.0	0.2	4.0	5.0

3. Results and Discussion

The physicochemical characteristics of different formulated creams made from *Magnifera indica* seed butter are shown in Table 2. As shown in the table, the seed has a moderate oil yield of 14%. The low yield suggests blending the oil with other oils for cream production. The low saponification value of 78 mgKOH/g indicates its low ability to be used in soap making and justifies its use in cream making. The low saponification value can also be attributed to the presence of higher molecular weight fatty acids (this was confirmed in Table 4, showing the fatty acid composition of the seed oil). The presence of docasadienoic acid contributes to the obtained low saponification.

Table 2. Physicochemical Properties of *Magnifera indica* Seed

Parameters	Mango butter
Colour	Pale Yellow
% Oil Yield	14
Saponification value (MgKOH/g)	78
Acid value (MgKOH/g)	20.98
Iodine value (g of I_2/100g)	61.50
Free fatty acid (MgKOH/g)	10.49
Physical State at room temperature (26-28ºC)	Solid

A high iodine value is an indicator of a high degree of unsaturation. The fact that the oil shows a low iodine value of 61.50 g of I_2/100g was equally confirmed by instrumental analysis done using GC-MS, which confirmed the oil is highly saturated with a degree of saturation of 54.762% (Table 4). The low free fatty acid content of 10.49 mgKOH/g is a good indication of the quality of the oil and its shelf life [16]. Table 3 shows the stability test results for the prepared cream throughout the 70-day evaluation. The cream consistently displays a smooth texture and retains its pale-yellow coloration. This

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indicates the cream's stability, which the natural bioactive components of the seed oil may contribute to. This demonstrates that seed oil has good viability as a base oil in cream production.

Table 3. Cream Stability Test Results

Oil conc.	Production Date	Observation Date	Texture	Color
1g	June 12, 2023	14 days	Smooth	Pale yellow
2g	June 12, 2023	28 days	Smooth	Pale yellow
3g	June 12, 2023	42 days	Smooth	Pale yellow
4g	June 12, 2023	56 days	Smooth	Pale yellow
5g	June 12, 2023	70 days	Smooth	Pale yellow

Table 4 gives the fatty acid composition of the seed oil. Oleic acid accounts for a large percentage of the seed oil at 35.715%, followed by palmitic acid (29.365%) and stearic acid (25.397%). The oil shows a high degree of saturation (54.762%), which may explain why it is solid at room temperature (26-28ºC). Oleic acid, which is the most abundant fatty acid in the seed oil, has been reported for its ability to enhance smoothness and softness of the texture as well as maintain skin moisture [17,18]. The oil may have consistently demonstrated a smooth texture throughout the 70-day stability test periods due to its presence. The ability to prevent the denaturation of albumin is a good indicator of anti-inflammatory action. It is a good quality for a cream to have a mild ability to reduce inflammation on the skin. The bioactive principles in seed oil often confer the same medicinal properties on the products; research by [15] shows seed oil products often retain their pharmacologic properties, hence why the prepared oil was screened for their anti-inflammatory properties.

Table 4. Chemical Composition GC-MS Analysis of Fatty Acid in *Magnifera indica*

R.T. (min)	Fatty acid	Saturations	% Composition
18.32	Palmitic acid	16:0	29.365
20.34	Docasadienoic acid	22:2	11.905
20.45	Oleic acid	18:1	35.715
20.82	Stearic acid	18:0	25.397
	Total Saturate		**54.762**
	Total Unsaturated		**45.238**

RT: Retention Time

Table 5 shows the anti-inflammatory potentials of formulated mangoes seed oil cream and indomethacin drug used as Standard control. Figure 1 gives a better graphical illustration of the analysis.

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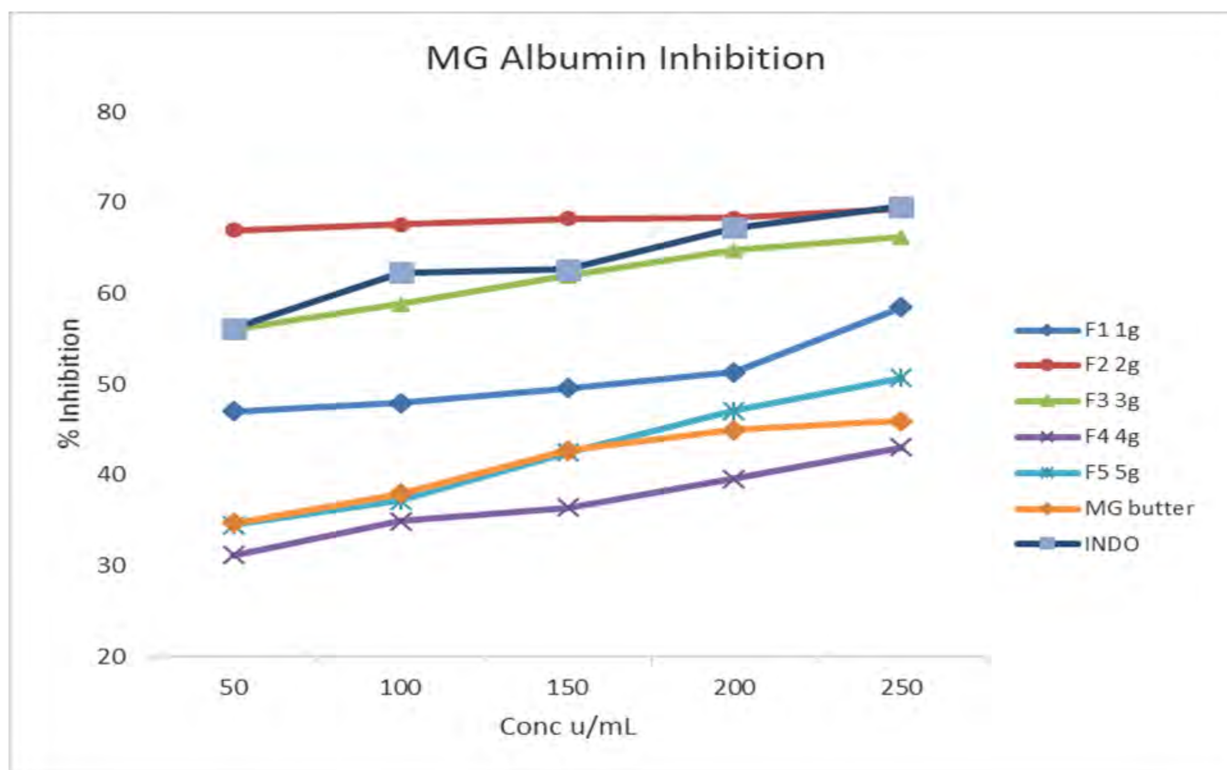

Figure 1. Percentage of Inhibition

From the results obtained, F2 cream gave the best ability to reduce skin inflammation when used as a cream, better than indomethacin used as the control, while the abilities of F3 and F1 are comparable to indomethacin used as the standard. This implies that mango seed oil works best when incorporated at a ratio of 1-3 g and that the cream has a good viability as an anti-inflammatory cream.

Table 5. Albumin-denaturation Inhibition Activity of Formulated Cream

Conc. u/mL	F1	F2	F3	F4	F5	MGB	INDSTD
50	47.02±1.79	66.10±2.02	56.05±0.39	31.14±0.91	34.53±0.69	34.70±0.52	56.15±0.41
100	47.95±0.50	67.60±1.37	58.84±0.54	34.92±0.42	37.21±0.29	37.99±1.09	62.28±0.39
150	49.59±0.53	68.25±1.62	61.96±0.14	36.39±0.14	42.40±0.77	42.75±0.57	62.67±0.39
200	51.34±0.39	68.31±0.66	64.81±1.13	39.63±0.11	47.13±0.82	44.99±1.66	67.26±0.81
250	58.51±0.71	69.29±0.74	66.17±2.67	43.08±0.21	46.25±0.14	45.97±1.07	69.56±0.14

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4. Conclusion

Different variety cream blends were produced and screened from *Magnifera indica* L. seed butter for their potential to reduce skin inflammation when used as body cream from their albumin-denaturation inhibition activity. When administered as a cream, F2 cream showed superior efficacy in reducing skin inflammation compared to the control, indomethacin. The efficacy of the F3 and F1 creams was comparable to the standard indomethacin. This suggests that *Magnifera indica* L. seed butter is a viable alternative as a base oil in body cream production. Its use will reduce year-round seed waste and improve the community's economic activities with good mango tree reserves.

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Modern Innovations in Food Preservations: Mini-review

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Abstract: The development of sustainable food preservation techniques and technologies to mitigate food losses and wastage during the harvest season has become a trending topic of global interest. This paper concisely summarizes the modern trends in food preservation as an indispensable element in the emerging food industry. The need to ensure the integrity of food preserved over a prolonged period is imperative to ascertain the quality of food consumed by humans. The food industry has recently been inundated with innovative techniques for better efficiency of food preservation technique that sustains the nutritive value and dietary content. Since the most widely adopted techniques include chemical, biological and physical methods, emphasis has been placed on this discussion in this review. While a series of other conventional and non-conventional techniques of preservation has been developed to achieve a longer shelf life of food, chemical preservation techniques seem to be more widely adopted essentially due to their ease of use, affordability and tendency to maintain the nutritional quality of food products for a prolong period. Despite the disadvantage of toxin introduction by chemicals among other associated hazards, chemical preservative techniques enjoy superior patronage. The presence of moisture in food, allowing microbes to thrive and their elimination via thermal means, is often a great choice. Chemical preservation receives more attention because of the impossibility of eliminating moisture via thermal means in many cases. Modern, advanced, innovative food preservative techniques such as the use of high-pressure processing, thermal processing, microwave, ultraviolet, infrared, ionizing, biosensors, vacuum packaging, high electric discharge, high hydrostatic processing, magnetic field, pulsed electric field, ultrasonication, nanotechnology means need further investigation and adaptation for seamless application.

Key Words: Food preservation, wastage, conventional techniques, toxin, shelf-life

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1. Introduction

The ever-increasing global population, coupled with the increasing complexities associated with food safety and security, has placed serious pressure on available food resources [1,2]. The preservation of food has been a critical element of the food industry for a long time, as it is necessary to ensure the safety and quality of food products over a prolonged duration. While drying, salting, freezing, fermentation, smoking, and some others (Figure 1) have been the traditional methods of preservation for centuries, recent technological advancements and evolving consumer requirements have led to the emergence of novel and innovative food preservation techniques [3,4].

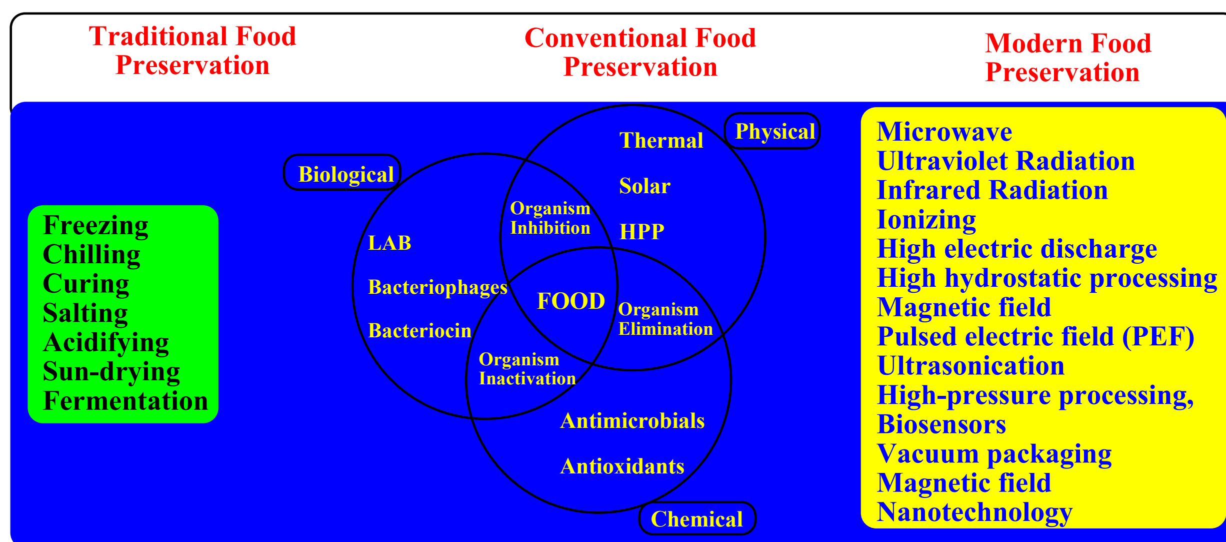

Figure 1. Basic Classification of Food Preservation Techniques

The food preservation industry has been transformed by new and innovative techniques, leading to improved efficiency and effectiveness. This is accomplished by the emergence of new preservation techniques generally categorized as chemical, biological, and physical methods. Since the preservation techniques have overlapping features, classifications have become complex. Chemical preservation techniques are particularly popular due to their ability to maintain the nutritional and sensory quality of food products for an extended period [3]. These methods are often combined with refrigeration and freezing to achieve optimal results [5,6].

New and advanced packaging techniques have been developed to further extend the shelf life of food products. One such technique is modified atmosphere packaging (MAP), a process that changes the atmospheric condition inside a package to slow down the deterioration process of food products. Another technique is active packaging, where active agents are added to the package to prevent microbial growth and oxidation [7]. The reduced oxygen and carbon dioxide content in the enclosure alters the metabolic process of microbial activities significantly and thereby extending the shelf-life of the packaged food [8,9]. These innovative packaging techniques have been proven to be effective in preserving a variety of food products, such as fruits, vegetables,

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meats, and dairy products. By using these techniques, the quality of the food products is maintained for a longer period, resulting in less food waste and increased available food products for consumers.

Incorporating innovation into food preservation has significant implications for the food industry. In addition to extending the shelf life of food products, it has the potential to decrease food waste, enhance food safety, and augment the availability of food products. By preventing the spoilage of food products, more food can be supplied to regions that may lack access to fresh food, thereby improving food security and combating food scarcity [7]. Food preservation techniques have significantly brought about a variety of effective methods that have helped address challenges in the food industry. This review examines the impact of innovations on food preservation, with a focus on emerging techniques and their transformative impact on the food industry. The review provides a quick insight into some latest innovations in food preservation and their implications in the food industry.

2. Preservation Techniques

Chemical Preservation Method

Chemical preservation techniques have significantly impacted the food industry by providing effective means to preserve food products for extended periods while maintaining their nutritional and sensory quality. Kalem et al. (2018) [3] submitted that chemical preservation techniques have gained tremendous popularity in the food industry due to their ability to preserve food products while maintaining their quality. The chemical preservatives used in food products are typically antimicrobial agents that inhibit the growth of microorganisms responsible for spoilage and food-borne illnesses [10,11].

Sodium benzoate is a chemical preservative commonly utilized against molds, yeasts, and specific bacteria [12]. Sulphur dioxide, on the other hand, has become a popular preservative in fruits and vegetables such as wine and dried fruits due to its antioxidant and antimicrobial effects [3]. Nitrites and nitrates are also frequently employed in meat preservation to prevent bacterial growth and preserve colour and flavour [3]. Other chemical preservatives that have been used to inhibit microbial growth in food include sorbate, hydrogen peroxide, parabens and benzoates.

The use of chemical preservatives in food products has caused concerns among consumers and health experts due to potential health risks associated with some of these preservatives. Research has shown that some chemical preservatives, including sodium nitrate and nitrite, may increase the risk of certain cancers [12] besides having harmful implications on the freshness and sensory properties of certain food [13]. This has led to rising demand for natural and organic preservatives in the food industry as consumers increasingly seek healthier and safer food options. The use of chemical preservatives in food products has thus raised concerns about their safety and health effects. As such, there is a need for continued research and development of natural and organic preservatives to meet the changing needs and preferences of consumers. Apparently, the issues of consumers' trust and acceptance of preservative method and

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technology cannot be overemphasized [14]. Recent surveys have indicated that most consumers, particularly those that prioritizes health over economic gain, prefer thermal preservation to others, including chemical means, for fear of incorporation of xeno-biotics in the process [15].

Biological Preservations Techniques

Biological preservation techniques have revolutionized the way food products are preserved, offering a more natural and safer alternative to chemical preservatives. These techniques involve the use of naturally occurring microorganisms or enzymes to prevent the growth of harmful bacteria and spoilage microorganisms in food products. Ktari et al. (2020) [16] noted that biological preservation techniques provide several advantages over chemical preservatives, including the ability to maintain the natural quality of food products and reduce the risk of chemical contamination [17].

One of the mostly adopted biological preservation techniques is the lactic acid bacteria (LAB) used to ferment and preserve food products such as meat, dairy, and vegetables. LAB produces lactic acid as a by-product of fermentation, which creates an environment that inhibits the growth of spoilage microorganisms by lowering the pH of the food product. This process not only preserves the food but also enhances its flavour and nutritional value. The use of LAB is effective in preserving a wide range of food products, including cheese, yogurt, sausages, and pickles, among others [16]. Another interesting biological preservation technique is the use of bacteriophages. Bacteriophages are a type of biological preservation technique that has been used in the food industry to control the growth of harmful bacteria responsible for foodborne illnesses, such as *Salmonella* sp. and *Escherichia coli* by infecting and killing specific bacteria [18]. Although these techniques offer several advantages over chemical preservatives, they also have limitations. For instance, the use of LAB may not be suitable for all food products, and bacteriophages' effectiveness may be affected by environmental factors such as temperature and pH [16]. Despite the advantages of biological preservation techniques over chemical preservatives, these methods suffer some setbacks with respect to environmental impact [19]. Specifically, the use of antilisterials as biocontrol of *L. monocytogenes* in foods have the demerit of intolerance in unfavorable environmental conditions. The low-stress accommodation potentials and the alteration of food taste are a major setback. Most importantly, the lysogenic phages could be vehicles for horizontal gene transfer and also, some bacteriocins may induce changes in the diversity of intestinal microbiota in different regions of the gastrointestinal tract [20]. As a result, additional research is required to enhance the effectiveness and suitability of these techniques for various food products and settings [6].

Physical Preservations Techniques

Physical preservation techniques refer to the application of physical means to prevent food spoilage. These methods have been developed as alternatives to chemical and

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biological preservation techniques, mainly to overcome their limitations that affect food quality and safety. This technique is often preferred because it is devoid of the incorporation of chemical or biological agents into the food. The technique has been adopted in the preservation of milk and meat products among others [21]. High-pressure processing (HPP) is one of these physical preservation techniques, which entails exposing food products to high levels of pressure to inactivate microorganisms and enzymes that induce spoilage [22]. HPP is efficient in preserving the safety and quality of diverse food products like juices, meat, seafood, and fruits, while retaining their nutrient content and flavour [23-25].

Thermal processing is another physical preservation technique that involves using high temperatures to eliminate microorganisms and enzymes in food products [26]. This technique is commonly used in the canning industry to preserve food products such as fruits and vegetables for extended periods. Thermal processing can also be used to sterilize dairy products and milk, which are susceptible to bacterial contamination [27-31].

Although physical preservation techniques provide several benefits over chemical and biological techniques, such as the absence of chemical residue and the preservation of nutritional quality and flavour, they also have limitations. For example, some physical preservation techniques like thermal processing, which makes use of external heat, can cause significant changes in food quality, taste and texture [26]. Additionally, the effectiveness of physical preservation techniques may be influenced by various factors, such as food composition, processing conditions, and packaging materials [22]. Since food with fresh-like characteristics, a high nutrient content, and preserved sensory quality traits is consumers' preference, these qualities are rarely maintained in sufficient ration when food is subjected to heat processing, as the heat treatments diminish sensory qualities such as flavor, color, and vitamins. The diminished qualities often warrant the inclusion of more supplements following the heat treatment [32,33]. Incredibly, thermal techniques of food preservation have received continuous modifications and improvement for optimum product preservation thereby delivering food products with better quality [34]. The advantages and disadvantages of the three conventional methods are discussed in Table 1.

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Table 1. Comparison of Conventional Food Preservation Techniques

Method	Advantages	Disadvantages	Limitations/Threats
Physical preservation technique	Absence of chemical residue and preservation of nutritional quality	Thermal processing causes significant changes in food quality, taste and texture	Influenced by factors such as food composition and packaging materials
Biological preservation techniques	Quality of food is maintained and no chemical contamination	May not be applicable to all food materials	Use of LAB is limited to certain foods and environmental factors can affect bacteriophages' effectiveness
Chemical preservation techniques	Inhibit the growth of microorganisms responsible for food spoilage and food-borne illnesses	Potential health risks associated with some of these preservatives	Safety and health concerns

Other Preservation Techniques

The concept of food preservation has continued to receive attention as more innovations are being introduced. Some other modern innovative techniques being adopted in recent times include the use of microwave, ultraviolet, infrared, ionizing, high electric discharge, high hydrostatic processing, magnetic field, pulsed electric field, ultrasonication, nanotechnology and some others [35,36].

The low thermal conductivity of food makes conventional heating of food relatively slow. Microwave technology has been reported as a food processing technique which enables processing within a short period of time compared to conventional heating. Hot air-drying is usually employed by industries as the main dehydration technique. This method, however, presents several problems such as poor quality of the dried product. The rate of drying reduces with time and water removal becomes ineffective. Thus, severe conditions are required for conventional hot air drying, resulting in frequent overheating and over-drying of the food materials. These reasons led to the microwave heating method to be developed and proposed as a new drying technique for food preservation [22].

The ultrasound preservation technique has been presented as an innovative method for food preservation. It is employed in the food industry for a number of purposes such as food analysis and food processing (homogenization, freezing, degassing, cutting, drying, antifoaming and extraction). Some advantages of the ultrasound method as a food processing technique includes effective mixing, reduced temperature, reduced energy and increased production rate. The ultrasound method also enables the elimination of microorganisms without destruction of nutrients of foods in food preservation. In addition, low-power ultrasound is reported as an attractive and non-thermal technique which could be employed to avoid problems such as physical and chemical changes,

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nutrient loss and organoleptic property change [37].

The infrared radiating heating technique has been reported to be one of the best ways of reducing heating time for food processing. This method gives a significant advantage over conventional heating including the possibility of achieving uniform heating, less loss of quality, and the absence of solute migration in food materials. In addition, significant energy is saved by utilizing this method [38].

Nanotechnology is concerned about atoms, molecules, or macromolecules with size range of approximately 1–100 nm. The nanomaterials possess dimensions and internal structure which are within 1 to 100 nm scale, allowing the observation and material manipulation in the nanoscale region. The presence of bioactive components in functional foods makes the foods prone to degradation and eventually leads to inactivation when subjected to a hostile environment. Nano-encapsulation of these food materials can extend the shelf-life of food products. Degradation of the food materials is usually slowed down or prevented when encapsulated in nanostructures for delivery to target sites. New nano-antimicrobials have shown promising effects in safeguarding food from deterioration. Nano-based food packaging has major advantages over conventional packaging because it provides better materials with improved mechanical strength, barrier properties and anti-microbial films [39].

3. Impact of Food Processing Innovation

The Impact of Food Processing Innovation on Food Safety

The development and implementation of novel technologies and protocols in the food preservation sector has had a substantial impact on food safety and security. Food-borne illnesses are still a major worldwide health problem because they cause high rates of morbidity and mortality in addition to extensive economic loss [40].

Innovative technologies such as nanotechnology, biosensors, and blockchain have been found to improve food safety and quality [41]. The use of biosensors in food packaging can detect the presence of harmful microorganisms or pathogens in real time, enabling prompt intervention to prevent foodborne diseases. Similarly, nanotechnology can enhance the effectiveness of food processing and preservation while reducing the risk of contamination [42]. Blockchain technology has also boosted supply chain transparency and traceability, thereby enabling faster identification and recall of contaminated food products [43].

Furthermore, food innovation has sparked the development of innovative, safer, and more nutrient-dense food products. For example, it has been determined that plant-based ingredients used in meat substitutes are healthier than animal products, which lowers the risk of foodborne illnesses [44]. The development of probiotics and functional foods has also been identified as an effective way to enhance gut health and boost immunity, thereby reducing the risk of foodborne diseases. Innovations in the food industry have the potential to transform food

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safety by introducing new technologies, processes, and products that enhance the safety and quality of food products. Nonetheless, it is crucial to subject these innovations to rigorous testing and regulation to guarantee their safety and effectiveness. Compared to traditional heat processing processes, Pulsed Electric Field, for instance, is thought to be a highly energy-efficient and ecologically benign procedure. Ultraviolet radiation preservation methods are environmentally benign, safe for use on both solid and liquid foods, and effective in preventing recontamination when applied to pre-packaged food goods. Furthermore, it has a reduced processing time and excellent food penetration without posing a significant risk to customers' health [45].

The Impact of Food Processing Innovation in Reducing Food Waste

Food innovation has been instrumental in reducing food waste through various means. New technologies, processes, and products have been developed to extend the shelf life of food products, reduce spoilage, and improve supply chain management [16]. By extending shelf life, active food packaging lowers food waste while protecting and preserving food content through processes brought on by intrinsic and acquired variables such as the incorporation of antioxidant and antimicrobial agents during packaging or as packaging materials [46]. Global estimates indicate that between 27% and 39% of the entire yearly harvest of fish is lost largely due to poor processing and preservation, thereby making fish waste a major global concern [47]. This underscores the imperativeness of innovative and smart food preservation. Modified atmosphere packaging (MAP) is an example of such innovation, which alters the air surrounding food products to prolong their shelf life [22]. MAP has been successfully used in preserving various types of food products, including meat, fish, fruits, and vegetables. Smart packaging technologies, such as time-temperature indicators, are also an innovative approach to reducing food waste by providing real-time information about the condition of the food product and preventing spoilage and waste [31]. Additionally, the development of apps and software that facilitate food donation and redistribution has assisted in connecting surplus food products with food-deficient areas thereby reducing waste [43].

Food waste is being adopted as a resource in new product formulations and processes that have been developed as a result of innovations in the food sector, which has decreased food waste. For instance, food waste can be turned into biofuels to reduce its negative environmental effects and offer a reliable supply of energy [22]. Similarly, food waste can be used as a raw material in the production of new food products, such as upcycled snacks and beverages, which can create new markets and reduce waste [16]. Food innovation has significantly reduced rapid degradation and waste through the development of innovative technologies, processes, and products that improve food preservation, supply chain management, and utilization of food waste as a resource. Nonetheless, it is necessary to continue research and development to enhance these innovations and tackle the global challenge of food waste.

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4. Conclusion

The use of innovative technology and methods to increase the quality, safety, and shelf-life of food products has revolutionized the food industry. Although conventional techniques of chemical, biological and physical methods of preservation have been used to achieve a longer shelf-life of food, chemical preservation techniques seem to be more widely used partly due to their availability, affordability and tendency to maintain the nutritional quality of food products for prolonged periods. These innovations, such as high-pressure processing, thermal processing, biosensors, and nanotechnology, have resulted in improved food safety and decreased food waste. Nevertheless, it is necessary to carry out further research and development to confirm the safety and efficiency of these technologies. Biosensors, thermal processing, high-pressure processing, nanotechnology, and other advances have led to reduced food waste and increased food safety. However, further study and improvement are required to verify the efficacy and safety of these technologies. Globally, food innovation has changed consumers' perceptions about food presservation, and with more improvement, it may be able to solve pressing problems with sustainability and food safety. The Sustainable Development Goals (SDGs), especially SDG 2, which aims at ending hunger while minimizing food loss and negative environmental impacts, can be fulfilled in part by using the emerging cutting-edge, novel food preservation techniques. It is also emphasized that the adoption of new food preservation innovations, customer preference and safety must be prioritized. To ensure that customer expectations and demands are met in an efficient and useful way, the application of innovation must be periodically evaluated together with safety throughout the processing and preservation stages.

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Starch and Coniferyl Alcohol Based Polymer: A Step Ahead Towards Green Polymers

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Abstract: Enzymatic polymerization can be regarded as an environment friendly synthetic process of developing polymeric materials, providing a good example of green polymer chemistry. The present study depicts the polymer based on coniferyl alcohol and starch by using the enzyme in *Pleurotus ostreatus* mushroom powder, which acted as a versatile peroxidase to catalyze the reaction of polymerization. To observe the changes in bond formations, we take account of a comparison between IR spectra of starch and obtained samples of polymer. The IR characterization of the polymer synthesized shows some extra bands at 860 cm^{-1} and 709 cm^{-1}, which shows the presence of some extra bond formation due to additives. The thermo-gravimetric curve shows continuous loss of mass with an increase in temperature. The DSC curve of polymer presented the glass transition temperature at approximately 80°C. The SEM analysis of starch film and the polymer shows the change in morphological pattern.

Key Words: Coniferyl alcohol, starch, biodegradability, polymer, thermo-gravimetric analysis

1. Introduction

Environmental concerns are rapidly growing over the large amounts of accumulating waste. Interest in biodegradable polymers has also risen sharply. Natural polymers can be sourced abundantly from renewable resources, while synthetic polymers originate from finite resources. Biodegradation of polymeric biomaterials involves cleavage of hydrolytically or enzymatically sensitive bonds in the polymer. Biodegradable polymers constitute a distinct category of polymers that undergo bacterial decomposition, yielding natural by-products like gases, water, biomass, and inorganic salts,

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and can be sourced from both natural and synthetic origins.

A vast study of literature shows that Vega et al (1996) performed the study of starch-based blends by thermo-gravimetric analysis and introduced a model that facilitates a precise understanding of material degradation exhibiting a symmetric curve in weight loss derivative concerning temperature. This model serves as a valuable instrument for analyzing blends in thermo-gravimetric studies, especially those comprising elements with significant temperature overlap during degradation [1]. Further study was carried by Onnerud et al. (2002) synthesized lignin oligomers. In a novel approach, peroxidase does not directly interact with lignin monomers such as coniferaldehyde and coniferyl alcohol. Instead, manganese oxalate functions as a diffusible redox shuttle. Initially, it is oxidized from manganese, Mn(II) to Mn(III), by a peroxidase, while simultaneously, the lignin monomers undergo oxidation to radicals, forming covalent linkages typical of lignin. Furthermore, a high molecular mass polymer is produced through the oxidation of coniferyl alcohol by Manganese(III) acetate in a mixture of dioxane and water [2]. In 2007, Barikani et al. investigated the synthesis and characterization of starch modified polyurethane and reported that the effect of prepolymer percentage on hydrophobicity was measured through contact angle and it was found that it increased with increasing amount of prepolymer. Glass transition temperature (T_g) is also affected with increasing amount of urethane linkage. By SEM, it was observed that the surfaces of modified starch are rougher and more disordered than the surface of unmodified starch particles, which confirms the grafting and modification of starch [3]. Kaewtatip et al. (2007) performed the preparation of cassava starch grafted with polystyrene by suspension polymerization. This study demonstrated the capability of polymerization of styrene monomer on the granular starch without emulsifier and the synthesis of graft copolymer without gelatinization of starch [4]. Su et al. (2012) presented the characterization of poly-caprolactone and starch blends for potential application within the biomaterials field. The findings revealed notable enhancements in the phases of PCL and starch with the application of MDI. Optimal results were achieved when the MDI content was at 1.0 wt% in conjunction with a starch content of 30 wt%. This proportion yielded satisfactory end products characterized by a smooth microstructure, appropriate mechanical properties, and high dynamic mechanical performance at body temperature scale [5]. Taboada-Puig et al. (2012) depicted the polymerization of coniferyl alcohol by a versatile peroxidase from *Bjerkandera* sp. and effects of H_2O_2 concentration aqueous organic solvents and pH. The aim of this study was to evaluate the ability of one versatile peroxidase and the bio-catalytically generated complex Mn(III) –malonate to polymerize coniferyl alcohol to obtain dehydrogenated polymers(DHPs) and to characterize the DHPs formed. Hydrogen peroxide was used as an oxidant and Mn^{2+} as a mediator. The results obtained from the Mn(III) –malonate-mediated polymerization showed that the yield was almost 100% [6]. In 2017, Ganguly et al. proposed the work on starch functionalized biodegradable semi-IPN as a pH tunable controlled release platform for memantine Thermal stability and degradation of the hydrogel was investigated through thermo-gravimetric analysis [7].

The vast study of starch and some different starch-based blends is also reported by multiple research workers. Starch is a highly hydrophilic macromolecule. It is often used

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as the degradable additive in the preparation of biodegradable polyethylene film. Polyethylene is resistant to microbial breakdown [8]. The challenge posed by polyethylene's non-degradability is a significant global issue. Consequently, scientists have explored various combinations involving starch and polyester/PVA/PCL compounds, which have been identified as biodegradable and mechanically compatible. PCL, a prominent biodegradable polyester widely used in biomedical applications, faces limitations in tissue regeneration due to its hydrophobic nature, potentially hindering cell adhesion and degradation rate. Blending PCL with starch, considered an optimal solution, can mitigate its hydrophobicity issue. Although naturally incompatible due to thermodynamic reasons, incorporating MDI as a compatibilizer in initial studies has shown improved interfacial interaction, thus enhancing the mechanical properties of PCL and starch blends [9].

Starch is a highly hydrophilic macromolecule which is used as the degradable additive in multiple blends. Starch and polyester blends are found to be biodegradable as both components are also biodegradable and compostable. When we use the starch filler with polyester, then it improves the rate of degradation of polymers [10,11]. In the study of degradation, scientists mentioned that enzymes like poly-(hydroxybutyrate) deploymerases and lipases are capable of cleaving the ester bonds [12,13]. As polyvinyl alcohol is hydrophilic in nature which makes it suitable to blend with starch. Investigation found that the PVA and starch blends have better mechanical properties [8].

From the vast body of literature of 20 years, it has been observed that coniferyl alcohol-based polymers were scarce. Therefore, the present article depicts the synthesis of starch and coniferyl alcohol-based polymer can prove to be a green polymer.

2. Experimental

Materials

Coniferyl alcohol (98%, Merck), starch (Emparta, Merck), $MnSO_4$ (Emplura, Merck), mushroom powder (laboratory grade, Urban Platter), Hydrogen peroxide (laboratory grade, Merck), sodium malonate (for microbiology, Research Lab Fine Chem Industries), 1,4- dioxane (Emplura, Merck).

Synthesis of Starch and Coniferyl Alcohol-based Polymer

Coniferyl alcohol (98%, Merck), mushroom powder (laboratory grade, Urban Platter), and starch (Emparta, Merck) were used as received. 0.03 mole of coniferyl alcohol was dissolved in a 20% mixture of 1,4-dioxane and sodium malonate buffer (pH 6.5) to a total volume of 6 ml and placed in a syringe. Another syringe contained 6 ml of a mixture of H_2O_2 (33.3 mM) and $MnSO_4$ (66.6 mM). Both solutions were added dropwise over 3 hours into a 250 ml conical flask containing 10 ml of an aqueous mushroom powder solution. Starch was dissolved in distilled water and heated to 80°C for 30 minutes to form a slurry, which was then added to the conical flask. The entire mixture was stirred using a magnetic stirrer for 24 hours. The resulting compound, which had a vanilla-like

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scent, was then oven-dried at 50°C for 24 hours, yielding a polymer film. This process synthesizes a polymer of coniferyl alcohol and starch using a mixture of 1,4-dioxane, sodium malonate, hydrogen peroxide, and manganese sulphate. The mushroom powder facilitates the enzymatic polymerization of coniferyl alcohol with starch. After 24 hours of stirring at room temperature, the mixture was centrifuged, and the resulting polymer was collected and oven dried.

3. Results and Discussion

Characterization of Starch and Coniferyl Alcohol-based Polymer

The IR spectrum of formed polymer was recorded on a Vertex 70 (Bruker) instrument using a KBr pellet over a spectral range of 8,300—350 cm^{-1} and a maximum resolution of 0.5 cm^{-1} equipped with detector $LaTiO_3$. Thermo-gravimetric analysis was carried on using the TGA V5/A Dupont 2100 at a heating range of 10°C/min under a nitrogen atmosphere in an alumina pan. Measurements of glass transition temperature were carried out with a differential scanning calorimeter general V2.2A Dupont 9900 model. DSC curves were recorded under a nitrogen atmosphere at a flow rate of 10°C/min. Scanning electron microscopy is an excellent tool for determining the morphology of the polymers. It was carried out in a CARL ZEISS EVO 50 TUNGSTEN SEM with the resolution of 2.0 nm at 30 kV and the magnification capacity was in between 5.00X and 10.00KX. Samples were mounted on a SEM coating unit for scanning.

IR Analysis of Starch Film

In Figure 1, the narrow band was shown to be at 3507.15 cm^{-1}, which shows the presence of group OH stretching.

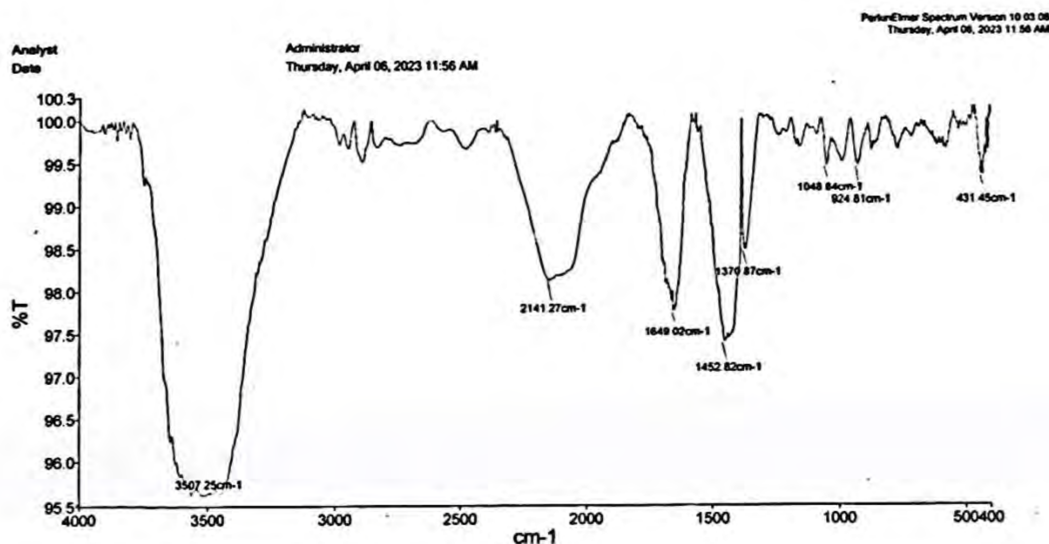

Figure 1. Starch Film IR Analysis

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Then, a band was obtained at 2141.27 cm^{-1} due to presence of group C-C triple bond stretching. At 1649.02 cm^{-1}, a band was obtained which showed the presence of C=O stretching (Ȣ-lactum). At fingerprint region, in between 1500-667 cm^{-1}, we obtain the bands of 1452.8 cm^{-1}, 1370.87 cm^{-1}, 1048.84 cm^{-1} and 924.8 cm^{-1}, which are present due to C-H bending of alkane, C-H bending of aldehyde O=C-O-C=O anhydride stretching group and trans=C-H out of plane bending group.

IR Analysis of *Pleurotus ostreatus* Enzyme

In the IR analysis of the mushroom powder, we found a band at 3382 cm^{-1} which shows the presence of a H-bonded alcohol stretching group. Further, the obtained bands at 2926 cm^{-1}, 2128 cm^{-1} and 1650 cm^{-1} depict the presence of aldehyde group having C-H stretching, C=C stretching in ring, and C=C stretching in alkenes, respectively. The bands situated at 1373 cm^{-1} and 1414 cm^{-1} show the presence of nitro stretching and sulphate stretching. We also found the bands at 1250 cm^{-1}, 1203 cm^{-1}, 1152 cm^{-1} and 574 cm^{-1} that show the presence of C-O stretching, tertiary saturated alcohol, C-O stretching and C-H bending in aromatic compounds, respect-tively. Some bands were also obtained at 1079 cm^{-1} and 1043 cm^{-1}, which shows the presence of secondary alicyclic five or six membered ring. The IR analysis of the enzyme is shown in Figure 2.

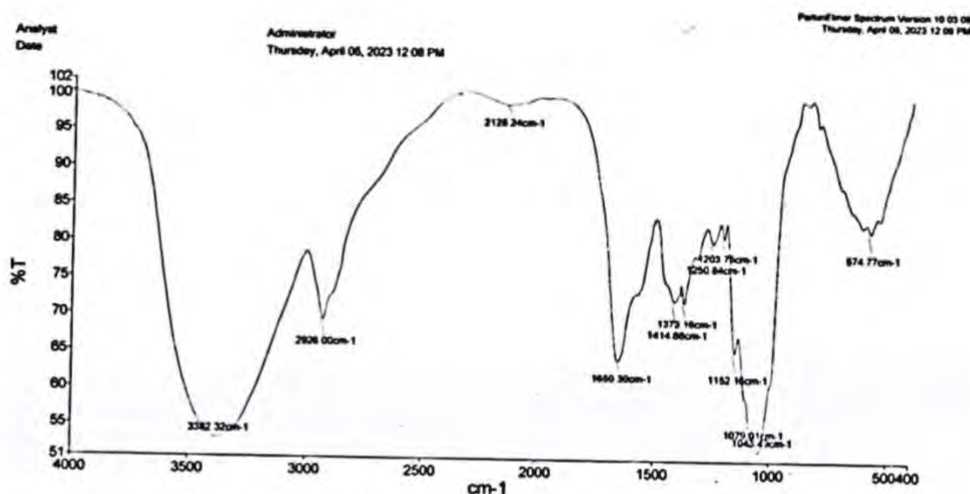

Figure 2. Enzyme IR Analysis

IR Analysis of Starch and Coniferyl Alcohol Based Polymer

Observing the sample, we found signals in the 80% to 100% transmittance region. The first band was obtained at 3416.97 cm^{-1} which is also broader than the band of starch film. This band shows the presence of alcohol group OH stretching (intermolecular bonded). Another band was obtained at 2933.37 cm^{-1} which is completely invisible in the starch film, so it shows some combination in our polymer and shows the presence of the group C-H stretching of alkane. The band situated at 2154.48 cm^{-1} and this band are

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also broader than the starch film which is due to C=C=O stretching of ketene. A band was obtained at 1655.08 cm^{-1} which is narrower than the starch film band approximately near to this region which shows C=C stretching of vinylidene. In the finger print region, 1500-667 cm^{-1}, the polymer sample shows the band at 1458.98 cm^{-1}, 1364.53 cm^{-1}, 1016.45 cm^{-1}, 860.49 cm^{-1} and 709.93 cm^{-1}, which shows the presence of C-H bending of methyl group, OH bending of phenol, OH bending of phenol, C-H bending of 1,4- di substituted or 1,2,3,4- tetra substituted alkane and benzene derivative. The bands obtained at 860.49 cm^{-1} and 709.93 cm^{-1} are absent in starch film, so these are new bands. The IR analysis of the polymer is shown in Figure 3.

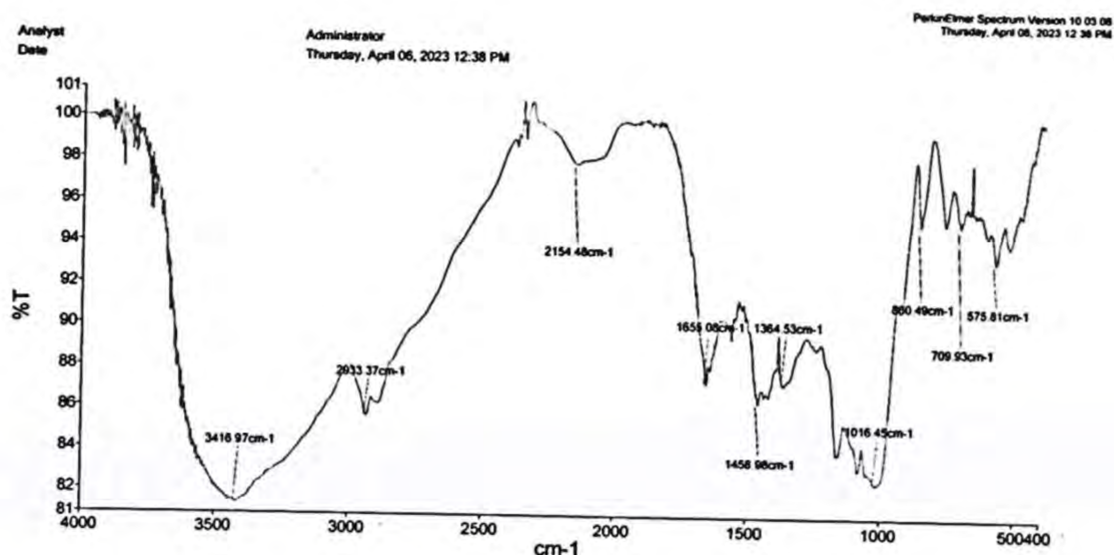

Figure 3. Polymer IR Analysis

Thermo-gravimetric Analysis

TGA analysis was carried on the V5/A Dupont 2100 at a heating range of 10°C/min under a nitrogen atmosphere in an alumina pan. The thermos-gravimetric results depict a loss in the mass on increasing the temperature. The polymer was stable up to 40°C and started losing weight above this temperature. We noted a continuous decomposition until 160°C (Figure 4).

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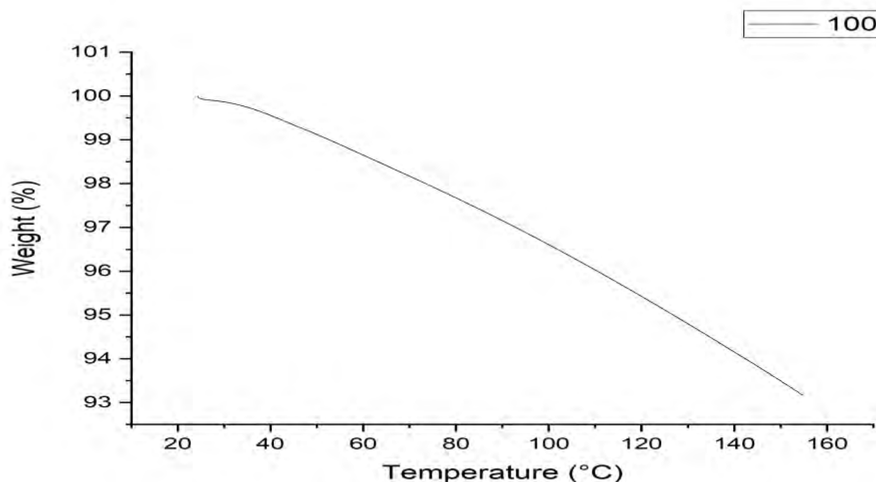

Figure 4. TGA Curve of Polymer

Differential Scanning Colorimeter (DSC)

On studying the curve obtained by the data, the phase change in the polymer sample started from 60°C and showed increasing heat flow from 100°C, so the glass transition temperature will occur at 80°C (Figure 5).

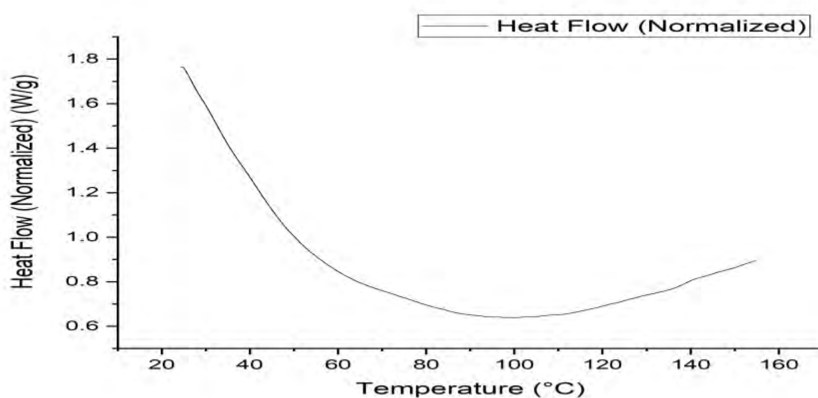

Figure 5. DSC Curve of Polymer

SEM Analysis of Polymer

From the analysis of Figure 6, it can be seen that the surface of starch films without any additive was smooth, but as the content of additives were added to film, it changed its morphological pattern, which is shown in Figure 7.

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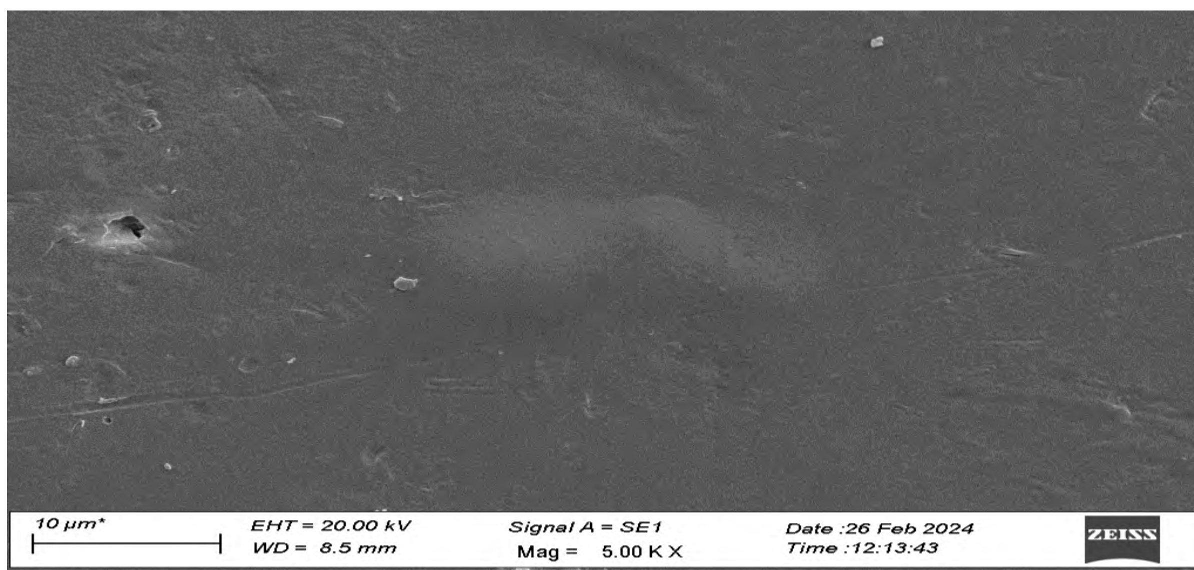

Figure 6. SEM Image of Starch Film

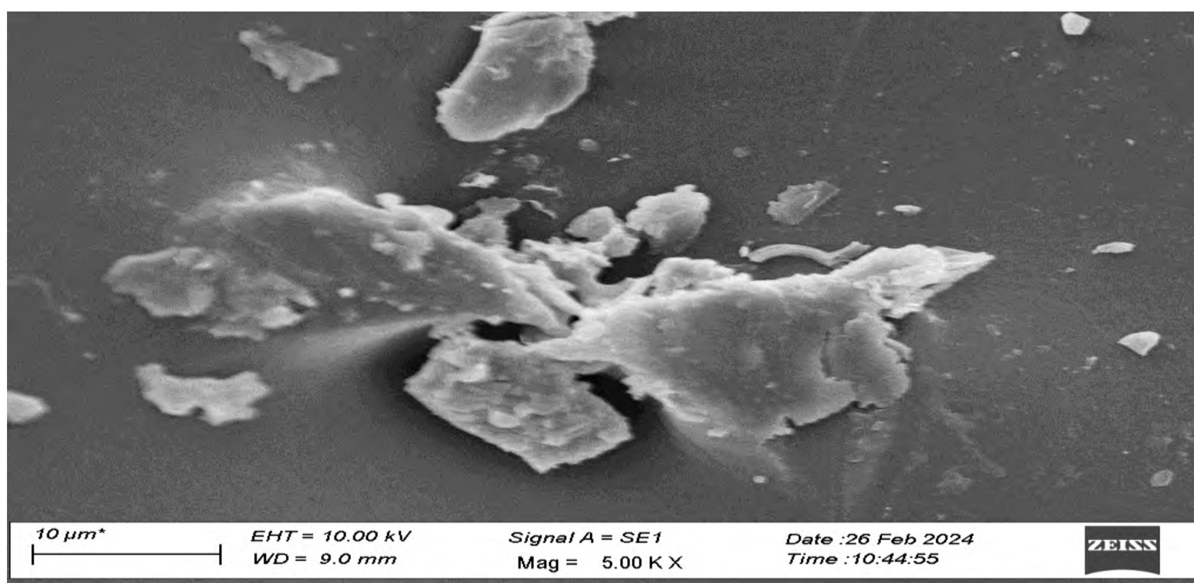

Figure 7. SEM Image of Polymer

4. Conclusion

Starch is an easily available, renewable and cheap material. It can be easily physically and chemically modified. Coniferyl alcohol is the precursor of lignin, which can be bonded to starch, and it can enhance the mechanical properties of polymer. The IR spectra of obtained film and starch film show the difference in their transmittance region, some new bands, and changes in the broadness of bands which represents the change in bonding patterns. In thermo-gravimetric analysis of the polymer, we found the

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continuous degradation of the polymer with the increase in temperature. DSC study of the graph shows the glass transition temperature at 80°C. SEM study showed the new morphological pattern found in the polymer film previously not reported in starch film.

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6. Ethical Approval

This is an observational study. The CSJMU Research Ethics Committee has confirmed that no ethical approval is required.

7. Funding

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8. Competing Interests

The authors have no financial interest.

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The Chemist
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Peptide Synthesis Methods and Protocols

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The examined publication, "Peptide Synthesis Methods and Protocols," stands out in the scientific landscape, especially for chemists and researchers in the field of peptide chemistry. It's the 2103rd installment in the esteemed "Methods in Molecular Biology" series from Springer Protocol, under Humana's publication.

The book, divided into 22 sections, takes a thorough look at various aspects of peptide synthesis. It covers everything from basic tests like the Kaiser test, checking if deprotection processes are done right, to advanced Microbioreactor Techniques for making and studying microbial peptides.

Starting with fundamental peptide synthesis, it explores Biphalin as a case study in solution phase peptide synthesis. In the following chapters then dive into different methods like *In Situ* Neutralization Protocols, Protecting Groups in Peptide Synthesis, and Synthesis of Branched Peptides. These cater to a wide audience of peptide scientists. Meanwhile, specialized insights are offered with chapters on Microwave-Assisted Solid-Phase Synthesis and New Developments in Microwave-Assisted Solid Phase Peptide Synthesis, promising better results in less time.

The book takes on a significant challenge in peptide synthesis – modifying peptides after synthesis, discussed in chapters like Synthesis of Amide Backbone-Modified Peptides and Decarboxylative Couplings for Late-Stage Peptide Modifications.

Notable are chapters focusing on peptides as tools for studying diseases, like Peptidomimetic Synthesis: Drug Discovery for Alzheimer's Disease, and Metal–Peptide Complexes to Study Neurodegenerative Diseases.

In summary, the book, written with precision, serves as an excellent guide for laboratory practitioners. It's recommended for scientists with basic knowledge in peptides, looking to tackle modern challenges in this field. Whether you're exploring foundational concepts or advanced techniques, this publication is a valuable asset for researchers navigating the complexities of peptide synthesis.

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Approved by the AIC Board of Directors, April 29, 1983

The profession of chemistry is increasingly important to the progress and the welfare of the community. The Chemist is frequently responsible for decisions affecting the lives and fortunes of others. To protect the public and maintain the honor of the profession, the American Institute of Chemists has established the following rules of conduct. It is the Duty of the Chemist:

1. To uphold the law; not to engage in illegal work nor cooperate with anyone so engaged;

2. To avoid associating or being identified with any enterprise of questionable character;

3. To be diligent in exposing and opposing such errors and frauds as the Chemist's special knowledge brings to light;

4. To sustain the institute and burdens of the community as a responsible citizen;

5. To work and act in a strict spirit of fairness to employers, clients, contractors, employees, and in a spirit of personal helpfulness and fraternity toward other members of the chemical profession;

6. To use only honorable means of competition for professional employment; to advertise only in a dignified and factual manner; to refrain from unfairly injuring, directly or indirectly, the professional reputation, prospects, or business of a fellow Chemist, or attempting to supplant a fellow chemist already selected for employment; to perform services for a client only at rates that fairly reflect costs of equipment, supplies, and overhead expenses as well as fair personal compensation;

7. To accept employment from more than one employer or client only when there is no conflict of interest; to accept commission or compensation in any form from more than one interested party only with the full knowledge and consent of all parties concerned;

8. To perform all professional work in a manner that merits full confidence and trust; to be conservative in estimates, reports, and testimony, especially if these are related to the promotion of a business enterprise or the protection of the public interest, and to state explicitly any known bias embodied therein; to advise client or employer of the probability of success before undertaking a project;

9. To review the professional work of other chemists, when requested, fairly and in confidence, whether they are:
    a. subordinates or employees
    b. authors of proposals for grants or contracts
    c. authors of technical papers, patents, or other publications
    d. involved in litigation;

10. To advance the profession by exchanging general information and experience with fellow Chemists and by contributing to the work of technical societies and to the technical press when such contribution does

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11. To work for any client or employer under a clear agreement, preferable in writing, as to the ownership of data, plans, improvements, inventions, designs, or other intellectual property developed or discovered while so employed, understanding that in the absence of a written agreement:
    a. results based on information from the client or employer, not obtainable elsewhere, are the property of the client or employer
    b. results based on knowledge or information belonging to the Chemist, or publicly available, are the property of the Chemist, the client or employer being entitled to their use only in the case or project for which the Chemist was retained
    c. all work and results outside of the field for which the Chemist was retained or employed, and not using time or facilities belonging to a client or employer, are the property of the Chemist;

12. Special data or information provided by a client or employer, or created by the Chemist and belonging to the client or employer, must be treated as confidential, used only in general as a part of the Chemist's professional experience, and published only after release by the client or employer;

13. To report any infractions of these principles of professional conduct to the authorities responsible for enforcement of applicable laws or regulations, or to the Ethics Committee of The American Institute of Chemists, as appropriate.

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Manuscript Style Guide

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Categories of Submissions

RESEARCH PAPERS

Research Papers (up to ~5000 words) that are original will only be accepted. Research Papers are peer-reviewed and include an abstract, an introduction, up to 5 figures or tables, sections with brief subheadings and a maximum of approximately 30 references.

REPORTS

Reports (up to ~3000 words) present new research results of broad interest to the chemistry community. Reports are peer- reviewed and include an abstract, an introductory paragraph, up to 3 figures or tables, and a maximum of approximately 15 references.

BRIEF REPORTS

Brief Reports (up to ~1500 words) are short papers that are peer-reviewed and present novel techniques or results of interest to the chemistry community.

REVIEW ARTICLES

Review Articles (up to ~6000 words) describe new or existing areas of interest to the chemistry community. Review Articles are peer-reviewed and include an abstract, an introduction that outlines the main point, brief subheadings for each section and up to 80 references.

LETTERS

Letters (up to ~500 words) discuss material published in The Chemist in the last 8 months or issues of general interest to the chemistry community.

BOOK REVIEWS

Book Reviews (up to ~ 500 words) will be accepted.

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Manuscript Preparation

RESEARCH PAPERS, REPORTS, BRIEF REPORTS & REVIEW ARTICLES

- **The first page** should contain the title, authors and their respective institutions/affiliations and the corresponding author. The general area of chemistry the article represents should also be indicated, i.e. General Chemistry, Organic Chemistry, Physical Chemistry, Chemical Education, etc.

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- **Abstracts** explain to the reader why the research was conducted and why it is important to the field. The abstract should be 100-150 words and convey the main point of the paper along with an outline of the results and conclusions.

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- **References and notes** should be numbered in the order in which they are cited, starting with the text and then through the table and figure legends. Each reference should have a unique number and any references to unpublished data should be given a number in the text and referred to in the references. References should follow the standards presented in the AIC Reference Style Guidelines below.

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References should be cited as numbers within square brackets [] at the appropriate place in the text. The reference numbers should be cited in the correct order throughout the text (including those in tables and figure captions, numbered according to where the table or figure is designated to appear). The references themselves are listed in numerical order at the end of the final printed text along with any Notes. Journal abbreviations should be consistent with those presented in Chemical Abstracts Service Source Index (CASSI) (http://www.cas.org) guide available at most academic libraries.

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- **Tables** should be in numerical order as they appear in the text and they should not duplicate the text. Tables should be completely understandable without reading the text. Every table should have a title. Table titles should be placed above the respective tables.

  Table 1. Bond Lengths (Å) of 2-aminophenol

- **Figure legends** should be in numerical order as they appear in the text. Legends should be limited to 250 words.

  Figure 1. PVC Melt Flow Characterized by Analytical Structural Method

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  Booth DE, Isenhour TL. *The Chemist*, 2000, 77(6), 7-14.

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  McCapra F, Tutt D, Topping RM, UK Patent Number 1 461 877, 1973.

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  Smith AB, Jones CD, *Environmental Impact Report for the US*, final report to the National Science Foundation on Grant AAA-999999, Any University, Philadelphia, PA, 2006.

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  Smith AB. Presented at the Pittsburgh Conference, Atlantic City, NJ, March 1983, paper 101.

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  Jones AB, Ph.D. Thesis, Columbia University, 2004.

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