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Physicochemical and microbiological characterization of sunflower oil extracted from seeds grown in the Camama experimental field in Luanda

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ABSTRACT: *Sunflower seeds (Helianthus annuus L.) are rich in lipids, particularly fatty acids such as linoleic acid, and in vitamins, notably tocopherol (vitamin E), a potent antioxidant. This study aimed to evaluate the oil yield of sunflower seeds cultivated in non-traditional soils and to characterize their physical, chemical, and microbiological properties. Oil extraction was performed using a Soxhlet apparatus with diethyl ether and petroleum ether as solvents. Microbiological analyses were conducted using two reference microorganisms, Candida albicans and Escherichia coli, following the methodologies outlined in the Clinical and Laboratory Standards Institute (CLSI) guideline M7-A6. The average oil yield from dehulled seeds was approximately 50%, with accession 2286 exhibiting the highest lipid content (34.68%). To assess oil quality, several parameters were measured, including acidity index, peroxide value, pH, density, free fatty acid content, ash content, moisture content, volatile matter, and infrared (IR) spectral characteristics. Statistical significance of the results was determined using analysis of variance (ANOVA). The antimicrobial assay showed no inhibitory activity of sunflower oil against either E. coli or C. albicans, indicating a lack of antimicrobial properties under the tested conditions.*

KEYWORDS: *Sunflower, Antimicrobial activity, Extraction, Physicochemical properties, Oil yield*

I. Introduction

Sunflower (Helianthus annuus L.) is a highly adaptable crop capable of thriving in a wide range of soil and climatic conditions. Compared to most cultivated species, it exhibits greater resistance to drought, cold, and high temperatures [1]. Its cultivation is both technically and economically viable, owing to its relative resistance to pests and diseases. Sunflower oil is primarily extracted from the seeds for food applications, but also has broad industrial utility, including in the pharmaceutical, cosmetic, paint, and cleaning product sectors. The seeds are consumed in various forms—roasted as snacks, and incorporated into cereal bars, cookies, baby food, and pet food (including for birds, dogs, and cats). Beyond oil production, sunflower plants offer additional applications: they can be used as green manure, and their stems are suitable for use in construction, particularly for thermal and acoustic insulation. In floriculture, the cultivation of sunflowers with diverse colors has increased their ornamental value. Nutritionally, sunflower oil is among the most nutrient-dense plant-derived oils, rich in lipids, minerals (such as magnesium and iron), and vitamins, particularly B1 and E—tocopherol being a well-known antioxidant. The seeds, technically classified as achenes, contain a high oil content characterized by low levels of saturated fatty acids and a high proportion of unsaturated fatty acids, notably linoleic acid (omega-6) and lecithin. Typically, sunflower seeds contain no less than 30% oil, and in some hybrid cultivars, this content may exceed 50%. Sunflower is currently the fourth most widely consumed oilseed globally, following soybean, palm oil, and canola. The Russian Federation, Ukraine, and Argentina are the largest global producers of sunflower seeds and their derivatives, with Russia and Ukraine together accounting for approximately 52% of global production and 40% of total seed exports [2]. Major consumers of sunflower oil include India, Turkey, and Egypt, which significantly contribute to global market demand. The global sunflower oil trade represents around 30% of the total vegetable oil market. The European Union is the primary destination for exports from Ukraine and Argentina, while Turkey, Egypt, India, and China are among the key importers [3]. In Angola, large-scale oilseed production for industrial use began in 1970. Sunflower cultivation gained prominence during this period, expanding to approximately 38,000 hectares by the 1971/72 harvest season, with an estimated yield of 25,000 tons [2]. The provinces of Malanje, Cuanza-Norte, Cuanza-Sul, and Benguela were major centers of cultivation. However, following the country's independence, sunflower production declined sharply and remains minimal today. This study aims to evaluate the physical and chemical

properties of sunflower seeds cultivated in non-traditional agricultural zones and assess their potential for oil production.

II. Methodology

2.1 Sunflower Seed Treatment Process Diagram

The flowchart in Fig 2.1 illustrates the sequential steps involved in the sunflower seed treatment process, encompassing the characterization of the raw material through to the quantification of the final product.

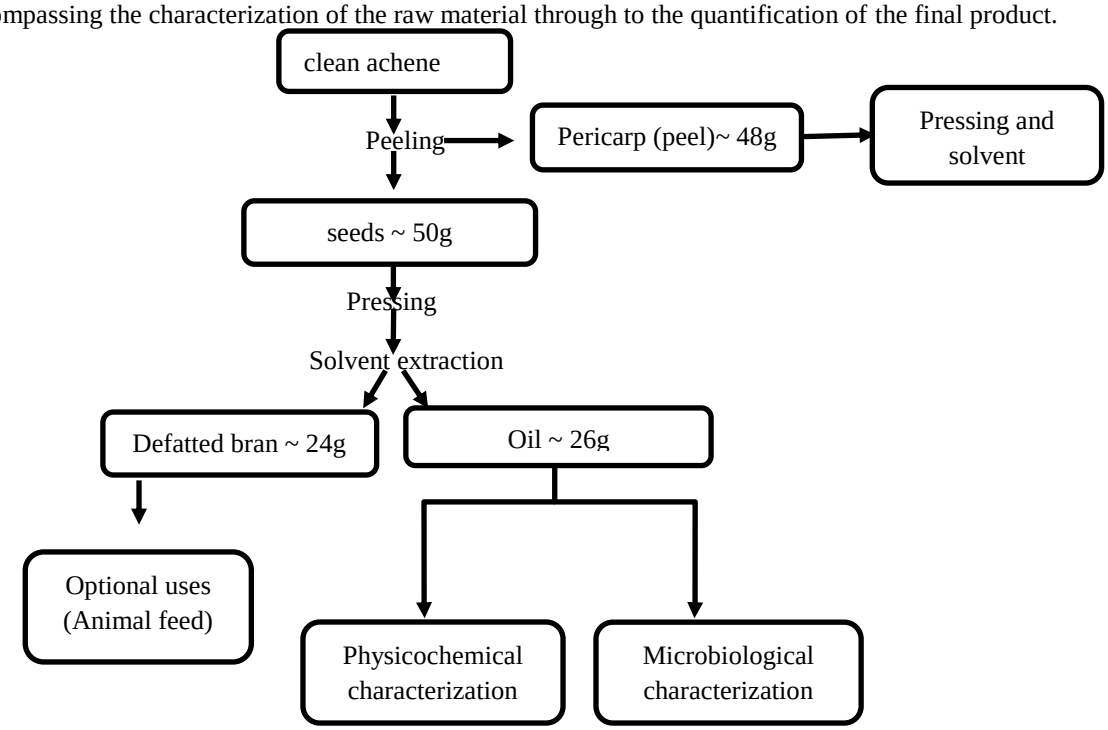

Figure 2.1. Flowchart outlining the sunflower seed preparation process, including subsequent physicochemical and microbiological analyses.

Sunflower seeds were collected from the Experimental Field of the Plant Genetic Resources Center, located on the Agostinho Neto University (UAN) campus in the Camama district of Luanda. This site is characterized by non-traditional soils, not typically used for experimental sunflower cultivation. Seeds were obtained from both fertilized and unfertilized plots. Following collection, the seeds were mechanically ground using a press, resulting in a seed cake, which was then oven-dried at 100 °C to eliminate residual moisture. Oil extraction was subsequently performed.

2.2 Solvent Extraction

For exhaustive oil extraction from sunflower seeds, the procedure recommended by the AOCS Official Method Bc 3-49 [4] was followed, which involves continuous extraction using a Soxhlet apparatus. Petroleum ether and diethyl ether were used as solvents.

2.3 Microbiological evaluation of antimicrobial activity

The antimicrobial activity of the extracted sunflower oil was evaluated using the procedure described by Madigan [5], which involves determining the minimum inhibitory concentration (MIC) required to inhibit the growth of selected pathogens. The susceptibility of potential pathogenic microorganisms present in the oil was assessed using the dilution method, as outlined by Brooks [6]. The test organisms used in this study were *Escherichia coli* and *Candida albicans*. Antimicrobial susceptibility testing was conducted using the dilution technique recommended by the Clinical and Laboratory Standards Institute (CLSI), following guidelines M7-A6 [7]. Additional susceptibility tests for antibacterial and antifungal activity were carried out according to CLSI standards M100 and M27 [7]. Prior to testing, isolated strains of *E. coli* and *C. albicans* were subcultured and plated twice on Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA), respectively. All culture media were prepared following the manufacturer's instructions.

Suspensions of *E. coli* and *C. albicans* were inoculated onto MHA and SDA plates, respectively. Wells of 5 mm diameter were aseptically bored into the agar, and 0.1 mL of diluted sunflower oil was introduced into each well using a micropipette. Paper discs impregnated or aggregated with antimicrobial agents were also placed into the wells as controls. The plates were incubated at 25 °C and 37 °C for 24 hours, depending on the

organism. After incubation, the plates were examined for the presence of diffusion zones and inhibition halos around the wells. All tests were performed in triplicate to ensure reproducibility.

III. Physicochemical Characterization of the Oil

The physicochemical characterization of oil extracted from sunflower seeds cultivated with and without fertilizer was conducted in triplicate. Analyses followed established methodologies, including AOCS protocols for determining acid value and free fatty acid content, and ISO standards for peroxide value. Protein content was assessed according to Version 001.2020, reference PNTSL012. Additional parameters analyzed included density [8], viscosity (ASTM D446-12) [9], pH, ash content, and moisture content. Reference standards from the Codex Alimentarius (Codex Standard 210-1999) [10] and the Adolfo Lutz Institute [11] were used for quality benchmarking. Functional group identification in the extracted oil was performed via Fourier Transform Infrared Spectroscopy (FTIR), using a Nicolet IS10 spectrophotometer, with a wavenumber range of 4000 cm^{-1} to 400 cm^{-1}. Statistical analysis was performed using analysis of variance (ANOVA), with fertilizer application as the independent variable and oil content per unit seed mass as the dependent variable. The efficiency of the two solvents used for oil extraction—petroleum ether and diethyl ether—was also compared to evaluate their influence on oil yield.

IV. Results and Discussion

4.1 Oil Content Obtained by Each Sample

To determine the oil content of each sunflower seed sample, eight samples from plants grown with fertilizer application (SCF) and eight from plants grown without fertilizer application (SSF) were analyzed. Oil extraction was performed using two different solvents: diethyl ether and petroleum ether. The results are presented in Figures 2 and 3. As shown in Fig 4.1.1, diethyl ether proved to be more effective for oil extraction from fertilized seeds, yielding an average oil content of approximately 31%, compared to 29.3% with petroleum ether. Among the fertilized samples, accession 2286 exhibited the highest oil yield using diethyl ether (32.21%), whereas the best result with petroleum ether was observed in accession 2287, with an oil content of approximately 31.20%.

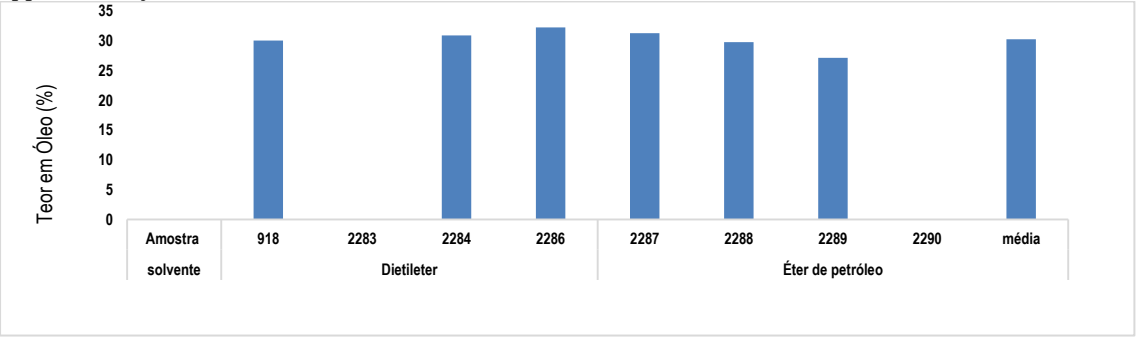

Figure 4.1.1 Oil content of sunflower seeds grown with fertilizer application (SCF), extracted using diethyl ether and petroleum ether as solvents.

For the samples grown without fertilizer application (SSF), the results shown in Figure 3 indicate a slight, though statistically non-significant, increase in average oil content extracted using diethyl ether (31.4%) compared to the fertilized samples. Accession 2286 again exhibited the highest oil yield at 34.68%. When petroleum ether was used as the solvent, the average oil content per sample was 28.47%, with accession 2288 yielding the highest extraction (Fig 4.1.2).

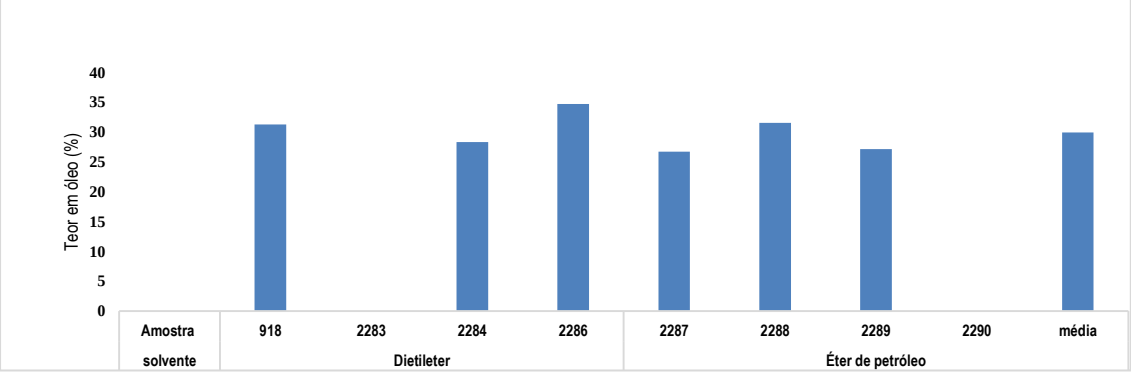

Figure 4.1.2. Oil content of sunflower seeds grown without fertilizer application (SSF), extracted using diethyl ether and petroleum ether as solvents.

Data from the extraction of 100 g of achenes containing the pericarp (unpeeled seeds), cultivated both without fertilizer application (S.S.F.) and with fertilizer application (S.C.F.), indicate that under identical experimental conditions, the average oil extraction yield for seeds grown without fertilizer was 32.97%, while for seeds grown with fertilizer it was slightly lower at 32.53%. Although the difference of 0.44% is minimal, this may be attributed to the influence of NPK fertilizers promoting protein synthesis over lipid accumulation, as nitrogen is a key element in amino acids and thus essential for protein formation. When petroleum ether was used as the extraction solvent, the average oil content per 100 g of achenes (S.S.F. and S.C.F.) was significantly lower—approximately 2.6% and 2.78% less, respectively—compared to values obtained using diethyl ether. This discrepancy in extraction efficiency can be explained by the chemical properties of the solvents: diethyl ether is an organic compound containing an ether bond (-O-) flanked by alkyl groups (R-O-R'), enabling it to better solubilize certain lipid fractions. In contrast, petroleum ether is a hydrocarbon mixture devoid of ether bonds and incapable of forming hydrogen bonds, potentially limiting its solvent capacity for some oil components. Overall, the highest oil yields were obtained from seeds grown without fertilizer, suggesting these seeds contain a greater proportion of oil trapped within their tissues. Additionally, diethyl ether proved to be the superior solvent for oil extraction under the conditions tested. These results indicate that fertilizer application does not enhance the oil yield extractable from sunflower seeds.

4.2. Physicochemical Characterization

The results of the physicochemical characterization are summarized in Table 4.2.1

Table 4.2.1. Physicochemical characterization results of sunflower seed oil extracted under varying cultivation conditions.

Parameter	Value obtained	Limit	Method	Standard
Acid Value (KOH/g)	4,2	5,95	AOCS Cd 3d-63	
Ash content (%)	0,12	3	Gravimetry at 550 ^{o}C	Codex
Free fatty acid (%)	3,23	0,5	AOCS Ca 5a-40	
Peroxide value (meq(O_2)/Kg)	8,8	10	ISO 660:2009	Cx-Standard 210-1999
% wet protein (%)	0,087			
pH	4,43			
Moisture (%)	5,8	4,8		
Density (g/cm^3)	0,914	0,914	AOAC 982.10	
Viscosity	62,815		ASTM D446 – 12(2017)	

The results of the physicochemical characterization indicate that both fatty acid and moisture contents exceed the recommended values. The iodine value suggests that the sunflower seed oil should undergo refining processes—such as degumming and bleaching—prior to food or industrial use. This is necessary to remove impurities, including phosphatides, lecithin, and residual fertilizer components, which negatively affect oil quality. The measured viscosity of the oil is slightly higher than that of commercial refined sunflower oil (41.3 mPa·s at 30 °C), likely due to the absence of refining in the samples analyzed [6]. The iodine value reflects the oil's unsaturation level and its oxidative stability during storage, whereas the free fatty acid content is an indicator of chemical and oxidative degradation occurring over time. According to Perkins (1992) [12], these two parameters are inversely related. The relatively high fatty acid content observed in the samples may be attributed to suboptimal storage conditions. Notably, the peroxide and acidity indices fall within the limits recommended by FAO and WHO standards for vegetable cooking oils (i.e., below 10 meq O_2/kg). However, due to the elevated free fatty acid content, refining the oil is advised to improve its quality and shelf life [13].

4.3 FTIR Analysis

Fig 4.3.1 and 4.3.2 present the Fourier Transform Infrared (FTIR) spectra of sunflower oil. Spectral measurements were performed using a Nicolet FTIR spectrometer within the wavenumber range of 4000 to 400 cm^{-1}, employing a sodium chloride (NaCl) cell. Each spectrum was acquired with 32 scans at a resolution of 2 cm^{-1}. Prior to each measurement, a baseline correction was conducted to eliminate any spectral interference. The oil samples were analyzed directly in the cell without any prior sample preparation.

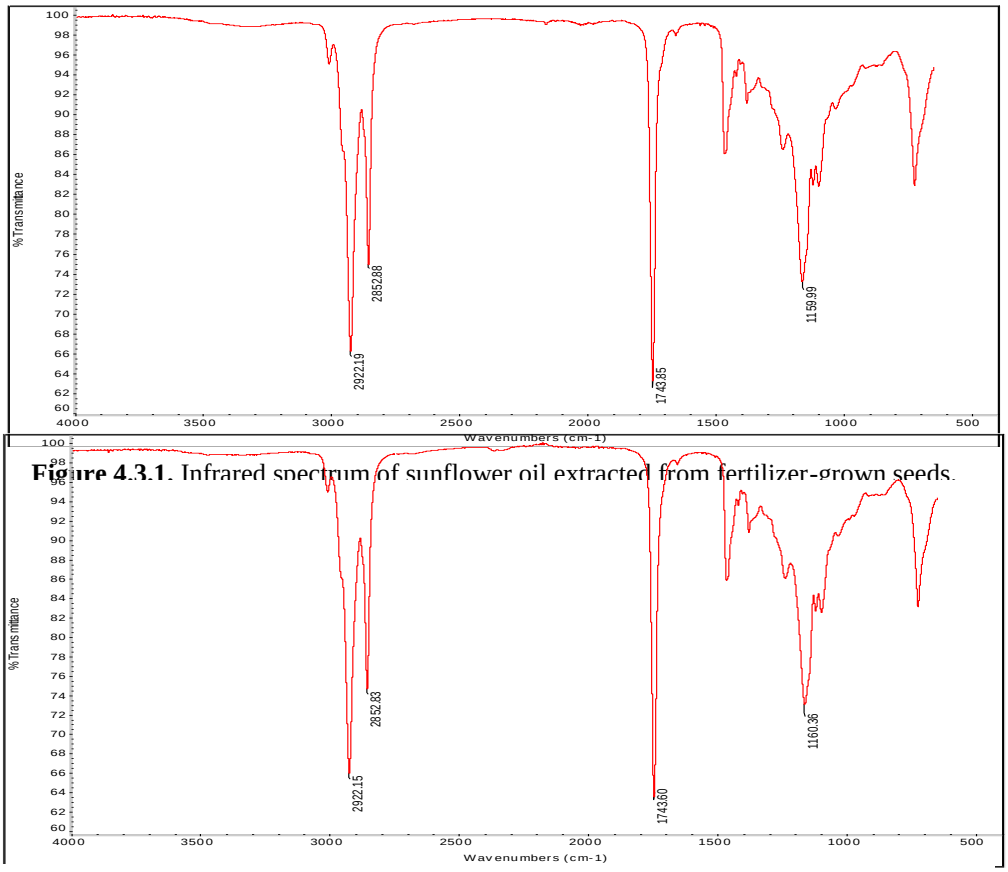

Figure 4.3.1. Infrared spectrum of sunflower oil extracted from fertilizer-grown seeds.

Figure 4.3.2. Infrared spectrum of sunflower oil extracted from seeds grown without

The FTIR spectra presented in Figures 4 and 5 show no significant structural differences between oils extracted from seeds grown with and without fertilizer application. Both spectra exhibit a weak absorption band between 3010 and 3004 cm^{-1}, corresponding to the stretching vibrations of =CH groups in unsaturated hydrocarbons. Intense bands observed around 2922.19 cm^{-1} (Fig 4.3.1) and 2922.15 cm^{-1} (Fig 4.3.2) are attributed to the asymmetric C–H stretching vibrations of methylene (–CH_2–) groups present in the oil matrix. Symmetric methylene C–H vibrations appear as bands near 2852.88 and 2852.83 cm^{-1} in both spectra. Strong absorption bands at 1743.85 cm^{-1} and 1743.60 cm^{-1} correspond to carbonyl (C=O) stretching vibrations typical of esters and carboxylic acids, key functional groups in triglycerides. Medium-intensity bands at approximately 1159.99 cm^{-1} and 1160.36 cm^{-1} are likely due to C–O stretching vibrations of ester linkages. In the region below 900 cm^{-1}, a low-intensity band near 720 cm^{-1} is attributed to rocking vibrations of methylene groups, suggesting the presence of long carbon chains (four or more methylene groups) characteristic of triglycerides in sunflower oil.

4.4 Significance Test

The results of the significance tests are presented in the tables below.

Table 4.4.1. Summary of significance test comparing measured parameters between sunflower seed samples cultivated with fertilizer (ACF) and without fertilizer (ASF).

Grups	Count	Sum	Mean	Variance
ACF	6	181,06	30,18	3,06
ASF	6	179,67	29,95	9,54

The analysis presented in Table 4.4.1 focuses on the standard deviation of oil yield. For samples grown with fertilizer application, the standard deviation was 1.74, indicating low variability around the mean oil content of 30.17%, as depicted in Fig 4.3.1. Conversely, samples grown without fertilizer exhibited a higher standard deviation of 3.08, reflecting greater variability around a mean of 29.94%, as shown in Fig 4.3.2. This suggests that, within the fertilized group, oil yield per seed is relatively consistent, with minimal differences between samples—making selection among these samples unlikely to yield significant improvements. In contrast, the higher variability among non-fertilized samples implies less uniformity in oil content; therefore, selecting specific samples could lead to improved yields. From this perspective, accession 2286 stands out as the most promising candidate and is recommended for further breeding studies.

Table 4.4.2. Analysis of variance (ANOVA) results for testing the rejection or acceptance of the null hypothesis.

Source of variation	SQ	df	MQ	F	P value	F crítical
Between groups	0,161008	1	0,161008	0,025559	0,876165	4,964603
Within groups	62,99448	10	6,299448			
Total	65,5549	11				

Since the calculated F value (0.025) is less than the critical F value (4.96), the difference between the means is not statistically significant. Therefore, the null hypothesis cannot be rejected. These results indicate that fertilizer application does not have a significant effect on the oil yield per seed unit (Table 4.4.2).

Table 4.4.3 Summary of analysis of variance (ANOVA) for oil extraction efficiency using diethyl ether (ED) and petroleum ether (EP) as solvents.

Groups	Count	Sum	Mean	Variance
ED	6	187,29	31,215	4,61411
EP	6	173,44	28,90667	4,819947

The average oil content yields exhibit notable differences between the two solvents. Extraction with diethyl ether resulted in a higher average yield (31.21%) compared to petroleum ether (28.90%). However, the standard deviations of 2.19 and 2.14, respectively, indicate considerable variability around these mean values (Table 4.4.3).

Table 4.4.4. Analysis of variance (ANOVA) testing the effect of solvent type on oil extraction yield and the decision to reject or accept the null hypothesis.

Source of variation	SQ	gl	MQ	F	P value	F critical
Between groups	15,98521	1	15,98521	3,38883	0,095461	4,964603
Within groups	47,17028	10	4,717028			
Total	63,15549	11				

Although the calculated F value (3.38) is less than the critical F value (4.96) and the p-value (0.09) exceed the 0.05 significance threshold, the null hypothesis cannot be rejected. Nonetheless, the observed differences in oil yield between the two solvents should not be overlooked. The ANOVA results (Table 4.4.4) suggest a trend toward significance, indicating that diethyl ether performs better than petroleum ether for oil extraction from sunflower seeds, even if this difference is not statistically conclusive at the 95% confidence level. Therefore, diethyl ether can be considered the more effective solvent for this application.

4.5 Antimicrobial susceptibility test (ast) analysis

The antimicrobial susceptibility tests (AST) conducted with crude sunflower oil yielded negative results. These findings are consistent with those reported by Menzel [14], who, using a colorimetric assay via the well microdilution method, also observed no antimicrobial activity. Despite the uniform growth of *Candida albicans* across the entire medium surface, no inhibition zones were detected around the wells containing oil dilutions. Repeated tests confirmed these results. Subsequently, AST was performed using five different samples of crude sunflower oil in triplicate, all of which again showed no inhibitory effect against *Candida albicans*, confirming its resistance to the oil. Similarly, antimicrobial susceptibility testing against *Escherichia coli* was conducted with oil dilutions ranging from 80 mg/mL to 2.5 mg/mL. Multiple replicates demonstrated the absence of inhibition zones, indicating no antibacterial activity. Additional testing using Tryptic Soy Agar (TSA) in triplicate with five different crude oil samples corroborated these findings, confirming the resistance of *E. coli* to sunflower oil.

V. Conclusion

Following oil extraction and the physicochemical and microbiological characterization (against *Candida albicans* and *Escherichia coli*), the following conclusions were drawn:

Diethyl ether was identified as the most effective solvent for oil extraction from sunflower seeds, regardless of fertilizer application. The sunflower plant demonstrates adaptability to climates similar to that of Luanda Province and soils comparable to those of the Camama Experimental Field, as approximately 50% of the seed mass consists of lipids. The extracted oil is suitable for human consumption, provided it undergoes refining to reduce its elevated free fatty acid content.

It should be noted that the measured oil yields may not be entirely intrinsic to each individual sample due to potential cross-pollination among plants. Nevertheless, sample 2286 exhibited the highest oil content per seed

unit and represents a promising candidate for genetic improvement programs. Variations in physicochemical properties observed among samples do not appear to be influenced by fertilizer application. Instead, factors such as storage duration and the choice of extraction solvent likely contributed to these differences.

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