COMPARATIVE PURIFICATION AND PHYSICOCHEMICAL CHARACTERIZATION OF WASTE COOKING OIL FOR DEVELOPMENT OF VALUE-ADDED CONSUMER PRODUCTS

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ABSTRACT:

Waste edible cooking oil (WCO) is produced in substantial quantities worldwide and poses significant environmental and public health concerns when improperly managed. The present study investigates the purification, physicochemical characterization, and value-added utilization of WCO for the development of surface disinfectant, handwash, and candle formulations. Three purification approaches—activated charcoal adsorption, sulfuric acid treatment, and sedimentation—were comparatively evaluated based on colour, odour, free fatty acid content, saponification value, peroxide value, moisture content, and viscosity. Cytotoxicity evaluation using the lactate dehydrogenase (LDH) release assay indicated markedly reduced toxicity in purified oil compared to untreated WCO, emphasizing the necessity of purification prior to reuse. The refined oil was successfully employed for the formulation of household and personal care products. Antibacterial assessment demonstrated effective inhibition of Escherichia coli, Salmonella typhi, and Listeria monocytogenes. The findings highlight the feasibility of converting WCO into functional, eco-friendly products, supporting sustainable waste management and circular economy practices.

Keywords: *Waste cooking oil (WCO), purification, activated charcoal, saponification, LDH assay, cytotoxicity, surface disinfectant, handwash, candle, circular economy.*

1.INTRODUCTION:

Used edible cooking oil (UECO), commonly termed waste cooking oil (WCO), is generated extensively from domestic kitchens, restaurants, hotels, and food processing establishments due to repeated frying operations. Continuous thermal exposure leads to oil degradation, resulting in the formation of undesirable compounds such as free fatty acids, peroxides, aldehydes, and polymerized triglycerides. Consumption or improper reuse of such degraded oils has been associated with adverse health effects, while indiscriminate disposal contributes to drainage blockage, water contamination, and ecological imbalance. Consequently, effective management and reuse of WCO have gained increasing attention worldwide.

One of the major challenges in reusing waste cooking oil is the presence of impurities, oxidation products, moisture, and unpleasant odours resulting from repeated heating. Effective purification techniques are therefore essential to improve oil quality and enable its reuse. Various physical, chemical, and adsorption-based purification methods have been reported, including sedimentation, acid refining, and adsorption using activated carbon. Among these, adsorption using activated carbon is considered environmentally friendly and effective in removing colour, odour, free fatty acids, and oxidative degradation products without excessive oil loss. Sulfuric acid treatment, although effective in impurity removal, may lead to reduced yield and residual odour, highlighting the need for comparative evaluation of purification methods.

Following purification, chemical characterization of the recovered oil is crucial to assess its quality and suitability for downstream applications. Parameters such as iodine value, peroxide value, acid value, and insoluble impurities provide insights into the degree of saturation, oxidation status, and overall stability of the oil. Oils with lower peroxide and acid values are particularly suitable for formulating skin-contact and household products.

The present study focuses on the **valorisation of used edible cooking oil collected from the RUCO initiative** through systematic purification, chemical characterization, and conversion into **value-added by-products**, namely **surface disinfectant, handwash, and candle**. The antimicrobial efficacy and cytotoxic safety of the formulated products were evaluated using standard microbiological assays and LDH release assays, respectively, to assess their functional performance and safety. By integrating waste management, chemical processing, and product development, this work demonstrates a sustainable approach for transforming waste cooking oil into useful, eco-friendly consumer products, thereby contributing to waste reduction, environmental protection, and circular economy principles.

2. SAMPLE COLLECTION:

The waste cooking oil sample used in the present study was collected from the **RUCO (Repurpose Used Cooking Oil)** initiative located in **Tiruchengode District, Tamil Nadu**. RUCO is a government-supported program established with the objective of creating a structured and sustainable ecosystem for the systematic collection, handling, and conversion of used cooking oil (UCO) into value-added products such as biodiesel, soap, and other industrial applications. The primary goal of this initiative is to prevent the repeated use and improper disposal of waste cooking oil, which can lead to serious health risks and environmental pollution. By promoting the safe collection and responsible utilization of UCO, RUCO plays a vital role in supporting public health, environmental protection, and sustainable waste management practices.

In addition, the RUCO initiative contributes significantly to the development of the biofuel sector by facilitating the conversion of used cooking oil into biodiesel. This not only reduces dependence on fossil fuels but also creates new economic opportunities for local communities, waste collectors, and small-scale industries, thereby strengthening the circular economy model. Through this approach, waste is transformed into a valuable resource, aligning with national sustainability and renewable energy goals.

For the present study, **five litres of waste cooking oil** were collected from the RUCO collection centre. The collected sample was initially subjected to **primary filtration** using a clean cotton cloth to remove large food particles, suspended solids, and visible impurities. This preliminary treatment step ensured the removal of coarse contaminants before further processing. Following primary filtration, the oil sample was subjected to **different purification processes** for enhanced filtration and quality improvement, making it suitable for characterization and subsequent conversion into value-added by-products.

Fig.1. *WUCO Collected from RUCO*

2.2. Reagents and chemicals:

2.2.1. Activated Carbon:

Fig.2. *Charcoal Sample*

Activated carbon (AC) is a porous carbonaceous material derived from carbon-rich precursors through physical or chemical activation processes. Due to its high surface area and well-developed pore structure, AC is widely used as an adsorbent for the removal of organic contaminants, colour bodies, and oxidation products in liquid systems. In the context of waste cooking oil purification, activated carbon adsorption has been reported as an effective method for improving oil clarity and reducing odour and free fatty acid content.

2.2.2. Sulfuric Acid (H_2SO_4):

In the chemical activation of charcoal powder, sulfuric acid (H_2SO_4) plays a dual role as both a dehydrating agent and an oxidizing agent, which significantly enhances the surface characteristics of the carbon material. Upon contact, concentrated H_2SO_4 removes bound water from the biomass structure and promotes the breakdown of hemicellulose, cellulose, and lignin residues, leaving behind a more carbon-rich matrix. The strong acidic environment causes protonation of oxygen-containing surface groups, which increases the number of active sites.

Simultaneously, H_2SO_4 facilitates partial oxidation and etching of the carbon surface, leading to the formation of a well-developed porous network with higher surface area. This porosity improvement directly enhances the adsorption capacity for organic and inorganic pollutants. Additionally, sulfonic functional groups (SO_3H) may be introduced onto the carbon surface, improving hydrophilicity and acid base interaction potential.

2.2.3. Making Activated Carbon (S Miskah *et al.*,2019):

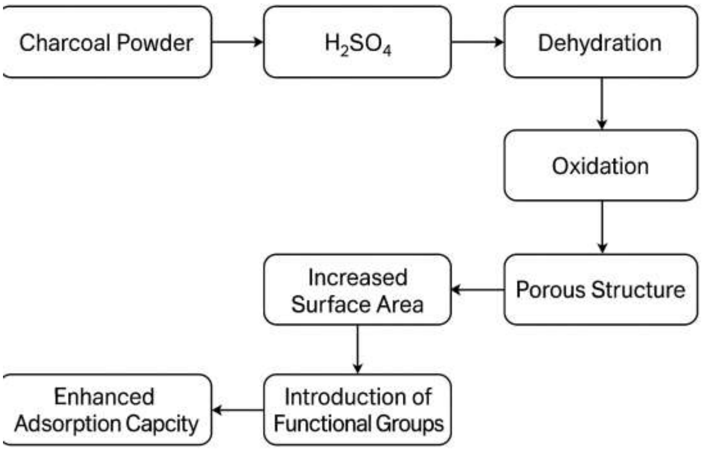


Fig.3. *Activation of Carbon using Sulfuric Acid*

Sulfuric acid activated carbon has been reported to significantly enhance adsorption capacity, leading to effective impurity removal and improved oil clarity (S. Miskah et al., 2019). In the preparation process, charcoal powder is initially washed with deionized water to eliminate surface impurities, cut into smaller fragments, and sun-dried for three days. The durian peel is subsequently carbonized in a furnace at 500 °C for 2 hours. The resulting carbon is ground to achieve a finer particle size and then chemically activated using a 1 N H_2SO_4 solution for 24 hours. After activation, the carbon is repeatedly washed with deionized water until a neutral pH (7) is attained and then filtered. The activated carbon is dried at 110 °C for 2 hours to remove residual moisture, followed by grinding and sieving through a 0.328 mm mesh to obtain uniform particle size.

3.MATERIALS AND METHODS:

The purification and characterization of waste cooking oil were carried out following standard analytical and processing procedures with minor modifications. Initially, the collected oil samples were filtered to remove suspended food residues and moisture. Purification was performed using adsorption, chemical refining, and sedimentation techniques. The refined oil samples were subsequently analysed for key physicochemical properties, including iodine value, peroxide value, acid value, moisture content, and insoluble impurities, using established analytical methods. All experiments were conducted in triplicate to ensure reproducibility.

Finally, the produced by-products were subjected to detailed analysis to evaluate their functional, chemical, and performance characteristics. This included standard quality tests to compare the products with existing commercial standards. The overall workflow ensures effective waste utilization, environmental sustainability, and economic value generation from used edible oil.

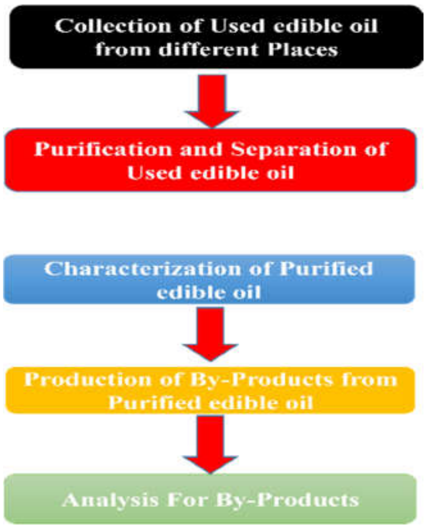


Fig.4. *Outlines of methodology*

4. PURIFICATION OF COLLECTED SAMPLE:

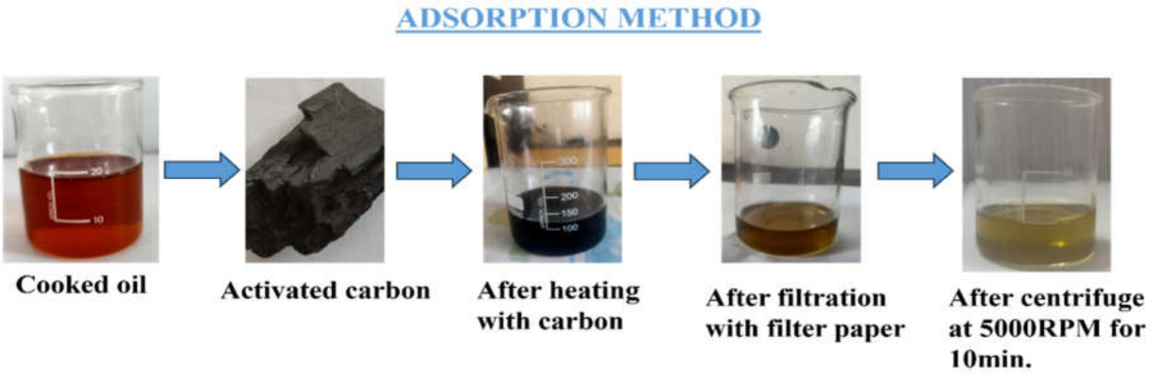


Fig.5. *Adsorption Method*

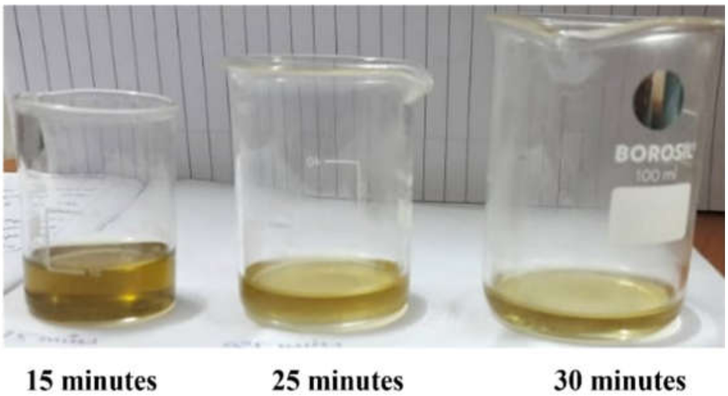
Absorption by Activated charcoal:

Initially, 250 g of used cooking oil was measured and mixed with deionized water in a 1:1 ratio. The oil–water mixture was heated until its volume was reduced to approximately half of the original volume. The oil phase was then separated using a separating funnel and allowed to stand for 1 hour to facilitate phase separation. Subsequently, the oil layer was filtered to remove residual impurities. A portion of the used cooking oil (150 g) was heated to 35 °C to reduce odor, after which 8 mL of 16% NaOH solution was added and the mixture was stirred for 10 minutes at 40 °C. The mixture was then allowed to cool for 10 minutes, during which soap formed as a result of the reaction between oil and NaOH; the soap was removed from the oil.

For the adsorption step, 100 mL of the treated oil was mixed with 6 g of activated carbon and stirred at room temperature. The contact time between the oil and activated carbon was varied at 30, 60, 90, 120, and 150 minutes for different experimental trials. Finally, the purified cooking oil was filtered through filter paper and collected for subsequent analysis.

Different Trials Result:

Fig. 6. *Visual appearance of oil after adsorption purification*



15 minutes **25 minutes** **30 minutes**

The contact time between the used cooking oil and activated carbon was varied at 30, 60, 90, 120, and 150 minutes for different experimental trials, and the corresponding results are presented in the following figures.

Inference:

The adsorption method using activated charcoal produced an **odourless** oil, indicating effective removal of volatile compounds and oxidative degradation products responsible for unpleasant smells in waste cooking oil. The resulting product exhibited a **rich golden colour**, reflecting both the retention of desirable natural pigments and the substantial reduction of suspended and colloidal impurities through the adsorption process. The **yield was approximately 190 mL**, representing a relatively high recovery compared to other purification methods, with minimal loss of usable oil during processing. The high surface area and porosity of the activated charcoal facilitated efficient binding of polar compounds, free fatty acids, and trace pigments, leading to significant improvements in both sensory quality and visual clarity. Overall, the process proved to be a balanced method, offering good recovery rates, improved aesthetic properties, and complete odor elimination, making it a promising approach for both edible and non-edible reuse of recovered cooking oil.

4.2 Chemical Purification:

Sulfuric acid refining is among the oldest oil purification techniques and remains one of the most efficient methods in use today. In the oil industry, it is widely applied to eliminate asphaltic and resinous materials, acid-containing and sulphur-containing compounds, as well as other harmful impurities. During sulfuric acid refining, the treated oil separates into two distinct liquid phases: an upper layer known as acid oil and a lower layer referred to as acid sludge. Most hazardous contaminants, excluding organic acids, are removed from used oils along with the acid sludge, while most of the oil's hydrocarbon structure remains largely unaffected. **(Aneu Aneu *et al.*, 2022).**

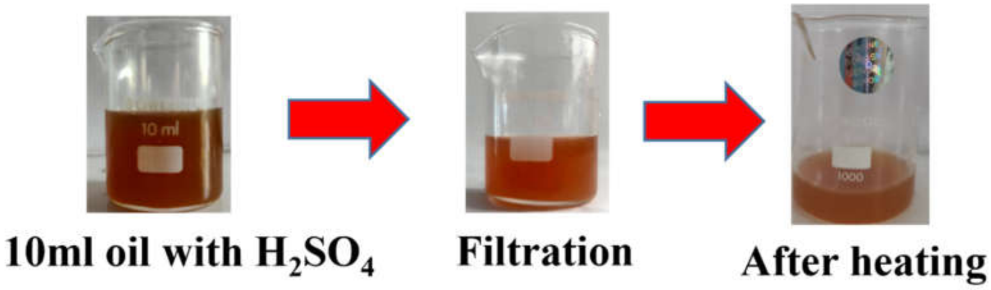

10ml oil with H_2SO_4 Filtration After heating

Fig.7. Purification by Sulfuric Acid

250 g of waste cooking oil is weighed and mixed with deionized water in a 1:1 ratio. The mixture is gently heated until its volume is reduced to approximately half of the initial total. The oil phase is then separated using a separating funnel and allowed to settle for 1 hour. The clarified oil layer is filtered to remove suspended solid impurities. A measured 150 g portion of the pretreated oil is transferred into a three-neck flask equipped with a mechanical stirrer, thermometer, and condenser. The oil is heated to 60 °C, and concentrated sulfuric acid (H_2SO_4) at 1% w/w of oil mass is slowly added while stirring continuously to ensure uniform dispersion. The mixture is maintained at the reaction temperature for 30 minutes to promote coagulation of gums, phospholipids, and free fatty acid esterification. After treatment, the mixture is allowed to cool to room temperature, and the precipitated sludge is removed by decantation and vacuum filtration. The purified oil is then washed with hot distilled water (70–80 °C) until the wash water reaches neutral pH. The oil is dried at 105 °C for 30 minutes to remove residual moisture before further quality analysis.

Inference:

The sulfuric acid purification method resulted in an oil product with an **extra light amber colour**, indicating substantial removal of suspended solids, gums, and oxidized pigments through acid-induced coagulation and precipitation. However, the treated oil retained a noticeable **sulphurous odor**, likely due to residual acid traces or sulphur-containing reaction byproducts, which would require additional washing or deodorization to improve sensory quality. The process achieved a **very low yield of only 120 mL**, reflecting the aggressive nature of the treatment, which led to significant loss of oil volume through sludge formation and separation of degraded fractions. While the visual clarity was markedly improved compared to untreated oil, the low recovery rate and residual odor highlight the method's limitations for edible oil regeneration without further post-treatment. Nevertheless, the high impurity removal efficiency makes sulfuric acid treatment valuable for applications where maximum cleanliness is required, especially for non-food industrial purposes.

4.3 Sedimentation method:

Sedimentation represents the initial and essential step in the oil purification process, involving the quiescent settling of suspended mechanical impurities and water. In this process, gravitational forces play a critical role in facilitating particle separation. According to Stokes' law, particles with larger sizes and higher densities settle more rapidly than smaller or lighter ones. Consequently, metallic particles, resinous matter, and coke-like substances tend to settle more readily than other contaminants. However, sedimentation of used oil does not always yield satisfactory results. In certain cases, even prolonged settling times fail to remove a significant portion of impurities, which remain suspended. This limitation is particularly evident in the purification of used diesel and automotive oils containing dispersing additives or oils contaminated with finely divided additive particles.

Procedure:

250 g of waste cooking oil is weighed and poured into a clean container. The oil is first preheated to 60 °C for 10 minutes to reduce viscosity, facilitating the release of suspended particles and water. The heated oil is then transferred into a tall, narrow settling vessel and allowed to stand undisturbed at ambient temperature for an extended sedimentation period of 24–48 hours, as suggested by Yan Wang (2021). During this period, heavier impurities such as food residues, carbonized particles, and coagulated proteins settle at the bottom due to gravity, while lighter materials float to the surface. After settling, the top clear oil layer is carefully siphoned or decanted to avoid disturbing the sediment layer. The recovered oil is filtered through fine mesh or filter paper to remove any remaining fine particles. In cases where higher clarity is required, a second sedimentation step of 12 hours is performed. The resulting oil is collected for further drying or analysis. This method requires no chemical reagents, making it cost-effective and environmentally friendly, though it is time-intensive and may not completely remove dissolved impurities.

Inference:

The sedimentation method produced a clarified oil with a **distinct golden-orange color**, indicating partial removal of suspended solids and burnt residues while retaining some natural pigments from the original cooking oil. The recovered oil yield was approximately **180 mL** from the initial processed volume, demonstrating a moderate recovery efficiency for a low-intervention purification approach. Although the visual clarity improved compared to the raw waste oil, the product retained a **slight fishy odor**, suggesting that sedimentation alone is insufficient to fully eliminate volatile odor compounds or degradation byproducts. The method effectively removed heavier particulate matter and moisture through gravity settling, resulting in a cleaner oil phase suitable for non-edible applications or further refining. However, dissolved impurities, free fatty acids, and trace odor compounds remained, indicating the need for supplementary purification steps such as adsorption or chemical treatment to enhance sensory quality and stability.

Fig.8. *Purification by Sedimentation Method*

5. CHEMICAL CHARACTERIZATIONS:

Chemical characterization was carried out to assess the quality and stability of purified waste cooking oil and to evaluate its suitability for conversion into value-added products. Standard physicochemical parameters, including iodine value, peroxide value, and acid value, were determined using established analytical methods. All analyses were performed in triplicate, and mean values were reported to ensure accuracy and reproducibility.

I) Iodine Value Test

The iodine value is an important parameter used to determine the degree of unsaturation present in oils and fats. It represents the amount of iodine absorbed by the double bonds of unsaturated fatty acids and is expressed as grams of iodine absorbed per 100 g of oil. Oils with higher iodine values contain a greater proportion of unsaturated fatty acids and are generally more prone to oxidative degradation.

Procedure:

The iodine value of the oil samples was determined using the Wij’s method. A known quantity of the oil sample was reacted with an excess of iodine monochloride solution under controlled conditions. After the reaction period, the unreacted iodine was quantified by titration with standardized sodium thiosulfate solution using starch as an indicator. A blank determination was carried out simultaneously, and the iodine value was calculated using standard equations based on titration values and sample weight.

Fig.9. *Titration end point for IV test*

Calculation:

$$\text{Iodine Value}=W12.69\times(VB - VS)\times N$$

Where:

- V_B = Volume of sodium thiosulfate for blank (mL)
- V_S = Volume of sodium thiosulfate for sample (mL)
- N = Normality of sodium thiosulfate
- W = Weight of oil sample (g)

SAMPLE	WS(g)	VB	VS	N	Iodine Value (g/100g)
Absorption oil	2.72	39.1	13.6	0.1	10.62
Cooking oil (peanut oil)	2.67	39.7	1.5	0.1	84
Waste cooking oil	2.06	38.9	1.2	0.1	168

Table 1: *Comparison of Trials for Iodine value results*

Inference:

The purified absorption oil exhibited a low iodine value (10.62 g/100 g), indicating a lower degree of unsaturation compared to fresh cooking oil and waste cooking oil. This reduced unsaturation enhances oxidative stability and makes the oil suitable for the formulation of household and personal care products with minimal risk of rancidity or skin irritation.

II) Insoluble Impurities Testa

This method determines the amount of dirt, meal, and other substances that are not soluble in oil which determines its quality.

Procedure:

Place a filter paper in hot air oven at 103°C for 20 minutes and cooled in desiccator. Weigh the empty filter paper(W1). Weigh 20ml of oil sample (Ws) and mixed with ethanol. Filtered with sterilized filter paper. The wet filter paper is placed in the hot air oven at 103 °C for 20 minutes and cooled in desiccator. Weigh the filter with impurities (W2).

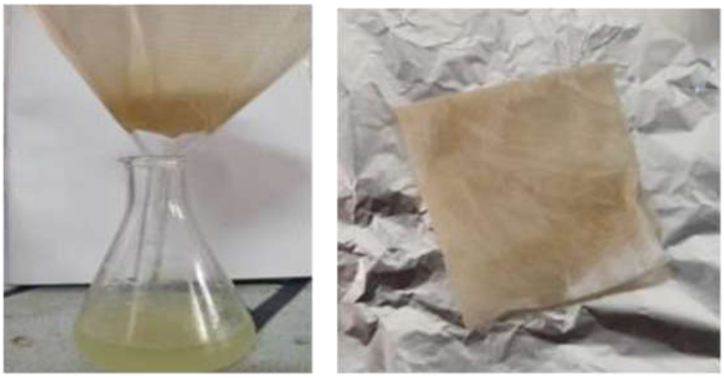


Fig.10. *Filtration process and Filter paper with impurities*

Fig.11. *Filter paper*

Calculation:

Insoluble impurities = W2 – W1 % WS

OIL SAMPLE	W1	W2	WS	Insoluble Impurities(g)
Cooking oil	2.9g	3.7g	20g	0.04
Waste cooking oil	2.9g	10.5g	20g	0.38
Absorption oil	2.9g	3.9 g	19g	0.04

Table 2: *Insoluble Impurities Test*

III) Peroxide Value Test

Peroxide value is a measure of the extent of primary oxidation in oils and fats. It quantifies the concentration of peroxide compounds formed during the early stages of lipid oxidation and is expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg). Lower peroxide values indicate fresher oils with better oxidative stability.

Procedure:

The peroxide value of oil samples was determined by iodometric titration. The oil sample was dissolved in an acetic acid–chloroform mixture, followed by the addition of potassium iodide, which reacts with peroxides to liberate iodine. The released iodine was titrated against standardized sodium thiosulfate solution using starch as an indicator. Blank determinations were performed under identical conditions.

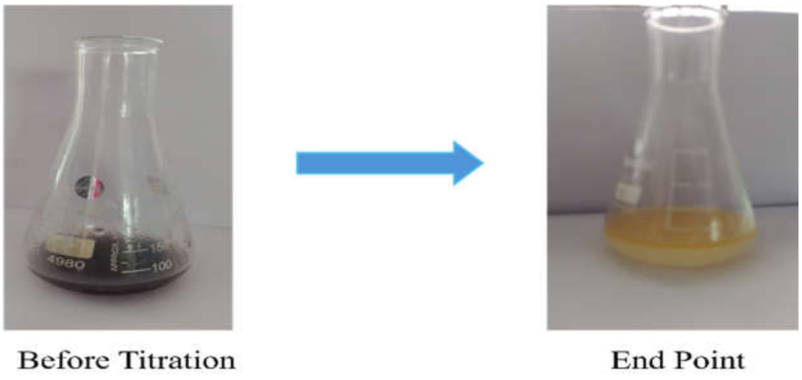


Fig.12. *Peroxide Value Test*

Calculation:

$$\text{Peroxide Value (meq/kg)}=W(S-B)\times N\times 1000$$

Where:

- S = Volume of sodium thiosulfate for sample (mL)
- B = Volume of sodium thiosulfate for blank (mL)
- N = Normality of sodium thiosulfate
- W = Weight of oil sample (g)

To ensure the stability of results, three replicates of every sample were analysed.

Sample	Weight of the sample	Trail 1 (mEq/kg)	Trail 2 (mEq/kg)	Trail 3 (mEq/kg)
Absorption oil	5.02	4.47	5.68	6.01
Waste cooking oil	5.1	58.23	59.04	58.35
Cooking oil (peanut oil)	5.04	3.90	3.79	3.45

Table 3: *Comparison of Trials for Peroxide value Test*

Inference:

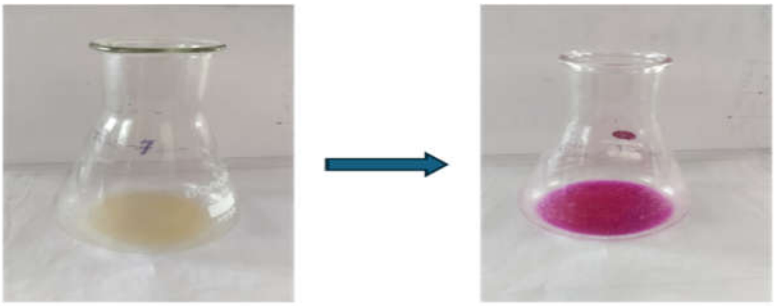
The peroxide values of absorption oil and peanut oil were well below 10 meq/kg, indicating good oxidative stability and freshness. In contrast, waste cooking oil exhibited extremely high peroxide values, confirming severe oxidative deterioration caused by repeated heating. These results highlight the importance of purification before reuse of waste cooking oil.

IV) Acid Value Test

Acid value represents the free fatty acid (FFA) content of oils and is defined as the amount of potassium hydroxide (KOH) required to neutralize free fatty acids present in one gram of oil. A high acid value indicates hydrolytic degradation of triglycerides, which negatively affects oil quality, shelf life, and suitability for further applications.

Procedure:

The acid value of oil samples was determined by neutralization titration. A known quantity of oil was dissolved in ethanol and titrated with standardized potassium hydroxide solution using phenolphthalein as an indicator. The endpoint was identified by the appearance of a persistent pale pink colour.



Before Titration

Titration End Point

Fig.13. *Acid Value Test*

Calculation:

$$\text{Acid Value (mg KOH/g)}=WM\times V\times 56.1$$

Where:

- M= Molarity of KOH solution
- V= Volume of KOH used (mL)
- W= Weight of oil sample (g)

Sample	Weight of the sample	Trail 1 (mEq/g)	Trail 2 (mEq/g)	Trail 3 (mEq/g)
Absorption oil	5.02	1.46	1.68	1.01
Waste cooking oil	5.1	4.76	7.04	5.15
Cooking oil (peanut oil)	5.04	0.89	0.77	0.65

Table 4: *Comparison of Trials for Acid value Test*

Inference:

The acid values of absorption oil and peanut oil were comparatively low, indicating limited hydrolytic degradation and good oil stability. Waste cooking oil showed significantly higher acid values, confirming extensive triglyceride breakdown due to repeated thermal exposure. This reinforces the necessity of purification prior to reutilization.

6. BY-PRODUCTS

I) SURFACE DISINFECTANT

In this work, UCO was infused with citrus waste (peels/seeds of orange, lemon, key lime) at several ratios (10 %, 25 %, 50 % w/v) and tested for antibacterial/disinfectant efficacy. This demonstrates that UCO, when functionalized with bioactive waste citrus compounds, can serve as a green disinfectant formulation. This is directly relevant for your paper's by-product section (surface disinfectant). The antimicrobial performance is limited (not complete sterilization), and the mechanism is partly attributed to the bioactive compounds from citrus waste rather than UCO alone.

6.1.1 Methodology:

The synthesis process for developing a surface disinfectant from used edible cooking oil (UECO) involves several systematic stages, beginning with the collection and preliminary treatment of the waste oil. UECO are converted into more reactive compounds suitable for blending with bioactive agents. Subsequently, selected natural antimicrobial additives such as citrus peel extract, herbal distillates, or essential oils are incorporated into the modified oil matrix under controlled stirring and temperature conditions to enhance its disinfectant efficacy. Addition of rubbing alcohol, white vinegar, lecithin, and distilled water. The resulting mixture is allowed to homogenize and settle before being filtered to obtain a clear formulation. Finally, the product is evaluated for physicochemical characteristics (pH, viscosity, and stability) and subjected to microbiological testing against common pathogens such as Escherichia coli and Staphylococcus aureus to assess its antimicrobial efficiency.

Trail-1 Trail-2 Trail-2

Fig 14: *Trails of Surface Disinfectant*

This sequential process ensures the effective conversion of used edible cooking oil into a value-added by-product with potential applications as an eco-friendly surface disinfectant. Different Trials were carried out to validate the cleaning efficiency, microbial inactivation, odour are displayed in following table.

MATERIALS	TRIAL -1	TRIAL-2	TRIAL-3
Citrus infused oil	30 ml	30ml	30ml
Rubbing alcohol	5ml	10ml	10ml
lecithin	2g	5g	5g

Distilled water	5ml	10ml	15ml
Soup base	-	-	5g
White vinegar	5ml	10ml	10ml
Inference	1.Oily consistency 2.After spray on the surface, the surface become oily. 3.Citrus smell 4.oil water dispersed	1.Oily consistency 2.After spray on the surface, the surface become oily. 3.Combination of citrus and vinegar smell.	1.Liquid consistency 2.After spray on the surface, the surface become moist. 3.Citrus smell

Table 6: *Comparison of Trials of Surface Disinfectant*

6.1.2. Analysis for Surface Disinfectant:

Antimicrobial Test:

Screening of Antibacterial Activity

The antibacterial activity of the formulated samples was evaluated using the disc diffusion technique, following the method described by Fernandez et al. (1966) with minor modifications. Briefly, Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions (≈2.0×10^6 CFU/mL). Sterile paper discs impregnated with the test formulations were placed on the inoculated agar surfaces and incubated at 37 °C for 24 h. After incubation, the zones of inhibition were measured and recorded as an indicator of antimicrobial efficacy.

***Table 6**:* Screening of Antibacterial Activity

Preparation of Inoculum

Stock bacterial cultures were preserved on nutrient agar slants at 4 °C. For experimental purposes, a loopful of bacterial growth was aseptically transferred from the stock cultures into test tubes containing Mueller–Hinton Broth (MHB) and incubated statically at 37 °C for 24 h. The resulting cultures were subsequently diluted with fresh MHB to achieve a bacterial

Organism	**Control Amoxicillin (10 mcg)**	**Concentration (µl)**			
		30	**40**	**50**	**60**
Escherichia coli	21	20	22	23	25
Salmonella typhi	20	18	20	21	23
Listeria monocytogenes	22	21	23	24	26

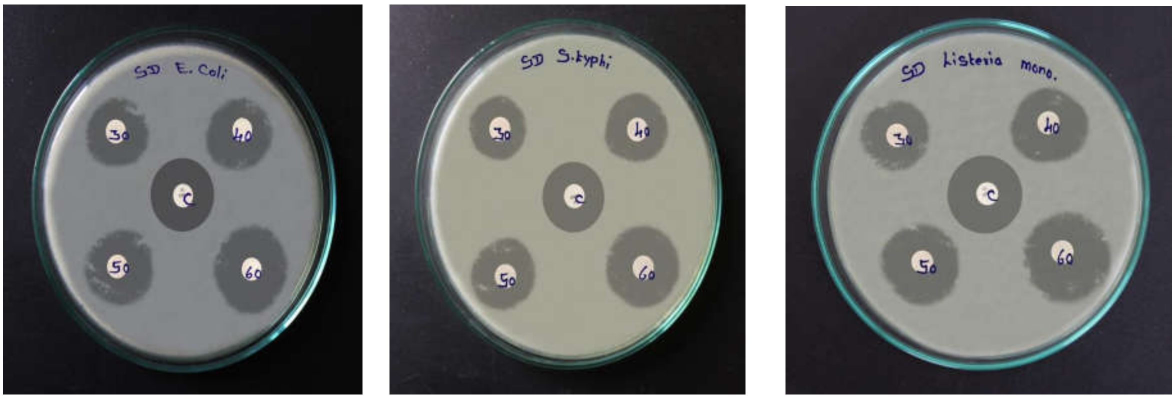
suspension with an optical density corresponding to approximately 2.0×10^6 CFU/mL, which was used as the working inoculum.

***Fig 15**: Antimicrobial Susceptibility Test*

Antimicrobial Susceptibility Test (Disc Diffusion Method)

The antibacterial activity of the formulated samples was evaluated using the disc diffusion technique following Fernandez et al. (1966) with minor modifications. Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions ($\approx 2.0 \times 10^6$ CFU/mL). Sterile paper discs impregnated with test formulations were placed on the agar surface and incubated at 37 °C for 24 h. Zones of inhibition were measured in millimetres to assess antimicrobial efficacy.

Toxicity Assessment for Surface Disinfectant (LDH Release Assay — SOP (Spectrophotometric)):



Cytotoxicity of the developed formulations was assessed using the lactate dehydrogenase (LDH) release assay according to the manufacturer's instructions. Cells were exposed to different concentrations of the test samples, and LDH activity released into the culture supernatant was quantified spectrophotometrically at 490 nm. Percentage cytotoxicity was calculated relative to spontaneous and maximum LDH release controls.

Procedure:

For cell seeding, cells were seeded into 96-well plates at a density of approximately 1×10^4 cells per well in 100 µL of complete medium and allowed to attach for 16–24 hours at 37°C in a 5% CO_2 incubator. Suspension-type cells were seeded according to standard protocols and allowed to recover. During treatment, test compound dilutions were prepared in culture medium with a final vehicle concentration not exceeding 1% (v/v), including a vehicle control. The medium was removed, and 100 µL of test compound or control solution was added to each well (or added directly on top if appropriate), followed by incubation for the desired exposure period, typically 24 hours. For time-course experiments, supernatants were collected at defined time points. After exposure, supernatant collection was carried out by gently mixing each well and transferring 50–100 µL of culture supernatant to a new 96-well plate designated for LDH measurement, taking care not to disturb the cell layer if additional assays were planned. Samples were kept on ice if the delay before analysis exceeded 30 minutes. For reagent preparation, the LDH reaction mix was freshly prepared according to the kit's instructions, typically by combining the substrate, cofactor, tetrazolium salt, and electron mediator, and protected from light. During the reaction, 50–100 µL of LDH reaction mix was added to each well containing the collected supernatant, followed by incubation at room temperature or 37°C (depending on kit specifications) for 20–30 minutes in the dark. Colour development was monitored visually, and the reaction was stopped if required by the addition of a stop solution. For reading, absorbance was measured at 490 nm with an optional reference wavelength between 600–690 nm using a plate reader. For determining maximum LDH release (M), after removing the medium for spontaneous release, 50–100 µL of lysis buffer (1% Triton X-100) was added to the original wells and incubated for 10–15 minutes to ensure complete cell lysis. The resulting supernatant was mixed and transferred to a new plate, and the LDH reaction was carried out as described above to obtain the maximum release value.

Sample Codes	**Absorbance 490 nm**		
	1	**2**	**3**
Blank	0.0532	0.0515	0.0491
Spontaneous Control	0.205	0.1986	0.2065
Maximum Control	1.0805	1.0453	1.0453
Surface disinfectant	0.8126	0.8191	0. .8065

Sample	**% Cytotoxicity (LDH)**			**% Cytotoxicity (LDH) (Mean ± SD)**
	1	**2**	**3**	
Surface disinfectant	69.40	73.28	71.53	71.41 ± 1.95

Table 7: *Absorbance of Surface Disinfectant*

INFERENCE:

The given Oil showed no detectable toxicity found protein denaturation assay. The higher concentration of 50 µl of the Disinfectant shows 68 % protect the toxicity.

pH test:

The pH test of surface disinfectants is a chemical analysis performed to assess the acidic or alkaline nature of the formulation. It determines the concentration of hydrogen ions (H^+) present in the solution and is expressed on a pH scale ranging from 0 to 14, where values below 7 indicate acidity, a value of 7 represents neutrality, and values above 7 indicate alkalinity.

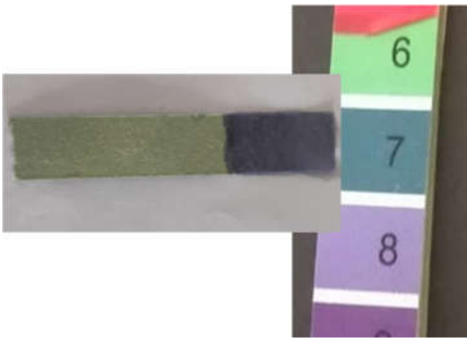


Fig 19: *pH for Surface Disinfectant*

RESULT:

A disinfectant with normal pH level typically falls within the range of 5-7, the disinfectants have pH 7 in both environment and hand which is considered as neutral. This means that it is neither too acidic nor too alkaline. So, it is safe to use.

II) HANDWASH

Antimicrobial Procedure

Screening of Antibacterial Activity:

The antibacterial activity of the formulated samples was evaluated using the disc diffusion technique, following the method described by Fernandez et al. (1966) with minor modifications. Briefly, Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions (≈2.0×10^6 CFU/mL). Sterile paper discs impregnated with the test formulations were placed on the inoculated agar surfaces and incubated at 37 °C for 24 h. After incubation, the zones of inhibition were measured and recorded as an indicator of antimicrobial efficacy.

Preparation of Inoculums:

Stock bacterial cultures were maintained on nutrient agar slants at 4 °C. For experimentation, a loopful of bacteria was aseptically transferred into test tubes containing Mueller–Hinton Broth (MHB) and incubated statically at 37 °C for 24 h to obtain actively growing cultures. The resulting suspensions were diluted with fresh MHB to reach an optical density equivalent to approximately 2.0×10^6 CFU/ml.

Antimicrobial Susceptibility Test

The antibacterial activity of the formulated samples was evaluated using the disc diffusion technique following Fernandez et al. (1966) with minor modifications. Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions (≈2.0×10^6 CFU/mL). Sterile paper discs impregnated with test formulations were placed on the agar surface and incubated at 37 °C for 24 h. Zones of inhibition were measured in millimetres to assess antimicrobial efficacy.

Organism	**Control Amoxicillin (10 mcg)**	**Concentration (µl)**			
		30	**40**	**50**	**60**

Escherichia coli	21	18	20	22	24
Salmonella typhi	19	15	17	19	21
Listeria monocytogenes	23	19	21	23	25

Table 8: *Antimicrobial Susceptibility Test*

Toxicity Assessment for Handwash (LDH Release Assay — SOP (Spectrophotometric)):

Cytotoxicity of the developed formulations was assessed using the lactate dehydrogenase (LDH) release assay according to the manufacturer's instructions. Cells were exposed to different concentrations of the test samples, and LDH activity released into the culture supernatant was quantified spectrophotometrically at 490 nm. Percentage cytotoxicity was calculated relative to spontaneous and maximum LDH release controls.

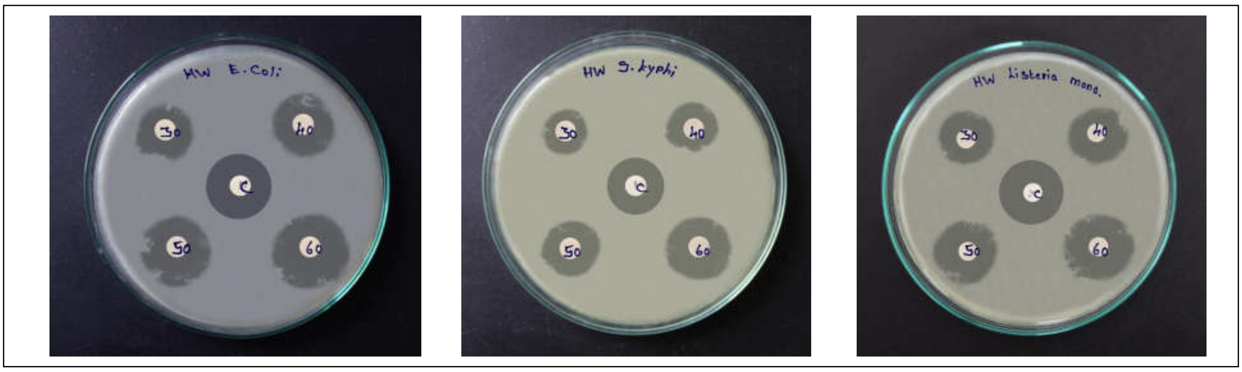


Fig 17*: LDH Assay Test*

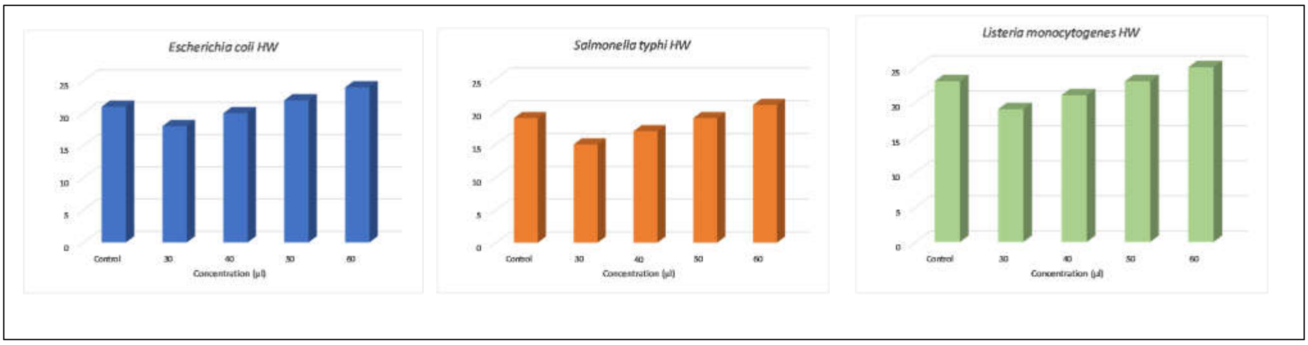


Fig 18*: Graphical for LDH Assay*

Procedure:

For cell seeding, cells were cultured in 96-well plates at a density of approximately 1 × 10^4 cells per well in 100 µL of complete medium and allowed to attach for 16–24 hours at 37°C in a 5% CO_2 incubator. Suspension-type cells, where applicable, were seeded according to standard protocols and allowed to recover before treatment. During treatment, test dilutions of the handwash sample were prepared in culture medium, ensuring that the final vehicle concentration did not exceed 1% (v/v), and a vehicle control was included. The culture medium was carefully removed, and 100 µL of either the test sample or control solution was added to each well (or directly layered if appropriate), followed by incubation for the desired exposure period, typically 24 hours. For time-course studies, culture supernatants were collected at specific intervals for analysis. After the exposure period, supernatant collection was performed by gently mixing each well and transferring 50–100 µL of the supernatant into a new 96-well plate designated for LDH measurement, ensuring the cell monolayer remained undisturbed if additional assays were required. Samples were kept on ice if analysis was delayed by more than 30 minutes. For reagent preparation, the LDH reaction mixture was freshly prepared according to the assay kit's protocol—typically by combining the substrate, cofactor, tetrazolium salt, and electron mediator—and was protected from light during handling. During the reaction step, 50–100 µL of the LDH reaction mixture was added to each well containing supernatant and incubated at room temperature or 37°C (depending on the kit instructions) for 20–30 minutes in the dark. Colour development was visually monitored, and the reaction was terminated with a stop solution when required. For absorbance measurement, readings were taken at 490 nm using a microplate reader, with an optional reference wavelength of 600–690 nm. To determine maximum LDH release (M), after removing the medium for spontaneous release, 50–100 µL of 1% Triton X-100 lysis buffer was added to the original wells to ensure complete cell lysis. The plates were incubated for 10–15 minutes, and the resulting lysate supernatant was transferred to a new plate. The LDH assay reaction was then performed as described above to determine the maximum enzyme release.

Sample Codes	**Absorbance 490 nm**		
	1	**2**	**3**
Blank	0.0532	0.0515	0.0491
Spontaneous Control	0.205	0.1986	0.2065
Maximum Control	1.0805	1.0453	1.0453
Handwash	0.5144	0.5047	0.5078

Sample	**% Cytotoxicity (LDH)**			**% Cytotoxicity (LDH) (Mean ± SD)**
	1	**2**	**3**	
Handwash	35.34	36.15	35.92	35.80 ± 0.42

Table 8: *Absorbance of Hand wash*

pH test:

The pH of surface disinfectants was determined to assess the acidity or alkalinity of the solution. This test measures the concentration of hydrogen ions (H^+) and expresses it on a scale from 0 to 14, where values below 7 indicate acidic conditions, a value of 7 represents neutrality, and values above 7 indicate alkaline conditions.

pH test for hand wash using pH strip

Fig 19: *pH for Hand wash*

Result:

A handwash with normal pH level typically falls within the range of 5-7, Our handwash has 7 which is considered as neutral. This means that it is neither too acidic nor too alkaline. So, it is gentle and safe for daily use on the skin.

III) CANDLE

Antimicrobial Evaluation of Candle Sample

Screening of Antibacterial Activity

The antibacterial activity of the formulated samples was evaluated using the disc diffusion technique, following the method described by Fernandez et al. (1966) with minor modifications. Briefly, Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions (≈2.0×10^6 CFU/mL). Sterile paper discs impregnated with the test formulations were placed on the inoculated agar surfaces and incubated at 37 °C for 24 h. After incubation, the zones of inhibition were measured and recorded as an indicator of antimicrobial efficacy.

Preparation of Inoculum

Stock cultures of the test organisms were preserved on nutrient agar slants at 4 °C. For experimentation, a loopful of each culture was aseptically transferred into test tubes containing Mueller–Hinton Broth (MHB) and incubated statically at 37 °C for 24 hours. The actively growing cultures were then diluted with sterile MHB to obtain a bacterial suspension of approximately 2.0×10^6 CFU/mL, ensuring consistent inoculation for the antimicrobial assay.

Antimicrobial Susceptibility Test

The antibacterial activity of the candle sample was evaluated using the disc diffusion method. Mueller–Hinton Agar (MHA) plates were prepared and inoculated with 0.1% bacterial suspension using a sterile swab. Sterile 6 mm discs were impregnated with varying amounts of the melted candle sample and placed onto the inoculated agar surface. Amoxicillin (10 µg) was used as a positive control. The plates were incubated at 37 °C for 24 hours, after which the zones of inhibition were measured in millimeters to assess antibacterial efficacy.

Toxicity Assessment of Candle Sample

LDH Release Assay (Spectrophotometric Method)

Cytotoxicity of the developed formulations was assessed using the lactate dehydrogenase (LDH) release assay according to the manufacturer's instructions. Cells were exposed to different concentrations of the test samples, and LDH activity released into the culture supernatant was quantified spectrophotometrically at 490 nm. Percentage cytotoxicity was calculated relative to spontaneous and maximum LDH release controls.

pH Analysis of Candle

The pH of the candle formulation was evaluated by dissolving a known quantity of the candle in distilled water and measuring using a calibrated digital pH meter.

Result

The pH of the candle sample was determined to be approximately neutral (~7), indicating chemical stability and a minimal likelihood of acidic or alkaline emissions during burning. This suggests that the candle is suitable for indoor use without posing risks of irritation or corrosive effects.

Procedure:

The candle was prepared using purified adsorbed oil as the base material. Initially, 20 mL of the adsorbed oil was measured accurately and transferred into a clean, dry glass beaker. To enhance the hardness and burning stability, 10 mL of stearin was added to the oil. The mixture was heated in a double boiler at 70 °C for 10 minutes with gentle stirring to ensure complete melting and homogeneous blending of the oil and stearin. After slight cooling, 0.5 mL of perfume was incorporated and mixed thoroughly to distribute the fragrance evenly. The molten mixture was then poured into a pre-prepared candle mold with a wick and left undisturbed at room temperature for 30 minutes to solidify. Once fully set, the candle was carefully removed from the mold and stored for subsequent analyses.

Result

The candle prepared from purified adsorbed oil exhibited good structural stability and a smooth appearance. It solidified properly within the expected time and maintained its shape at room temperature. During burning, the candle produced a steady flame with minimal smoke and no excessive dripping. The added fragrance was mild and evenly released. Overall, the results confirm that adsorbed waste oil can be effectively utilized for producing a functional and acceptable candle.

7. CONCLUSION:

The present study successfully demonstrates the effective valorisation of used edible cooking oil (UECO) collected under the RUCO initiative through systematic purification, chemical characterization, and conversion into value-added household and personal care products. Among the purification techniques evaluated, adsorption using activated carbon proved to be the most efficient, yielding odourless oil with improved clarity, acceptable recovery, and favourable physicochemical properties compared to sedimentation and sulfuric acid treatment. Chemical characterization results, including iodine value, peroxide value, acid value, and insoluble impurity analysis, confirmed significant improvement in oil quality after purification, indicating its suitability for further utilization.

The purified oil was successfully formulated into three by-products: surface disinfectant, handwash, and candle. Microbiological evaluation using the disc diffusion method revealed notable antibacterial activity of all formulations against selected pathogenic microorganisms, confirming their functional effectiveness. Cytotoxicity assessment using the LDH release assay indicated acceptable safety profiles for the developed products at tested concentrations, supporting their potential for controlled household and personal use. The neutral pH values observed further reinforced their compatibility with skin and indoor environments.

The candle prepared from purified adsorbed oil exhibited good structural stability, uniform burning behaviour, minimal smoke formation, and pleasant fragrance release, highlighting the feasibility of using waste-derived oil for solid fuel and decorative applications. Overall, this work emphasizes that used cooking oil, when properly treated, can serve as a valuable raw material for eco-friendly consumer products rather than being an environmental liability. The findings support the adoption of sustainable waste oil management strategies and promote circular economy principles by converting waste into economically and environmentally beneficial products.

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