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Research Article

Preparation Of a Hand Cleansing Emulsion Mix from The Agrowaste Residues of *Tamarindus Indica* L. & *Citrus Limon* (L.) Burm. F

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ABSTRACT

Herbal compounds, especially polyphenols have traditionally been used in skin care for centuries. Many measures have been adopted to incorporate polyphenols in natural cosmetics, with the aim to suppress the damages caused by UV exposure. Among the polyphenol sources, agrowastes remains one of the prominent sources. Agrowastes are the post-harvest waste materials from agricultural sector and many innovative measures, technical approaches have been adopted for the eco- friendly treatment and disposal of these waste materials. Among the different strategies adopted, the utilization of these wastes into cosmetical products is the new trend and was chosen as the subject of present study. The objective of the current study was the extraction of potent bioactive from Tamarind shell (TS) and Lemon peel (LP) and using them as additives in cosmeceuticals. Both conventional (cold extraction - CE) and non-conventional methods (microwave assisted extraction- MAE) methods were adopted for extraction. Further, the extracts were utilized for the formulation of nanoemulsions, and subjected to physical and chemical characterization. Characterized emulsion formulations (TSE, LPE) were identified to be stable, active and efficient for subsequent applications. Herein, mixtures of the two emulsions were used as the basal medium for the preparation of skin cleansers. Physical characteristics such as homogeneity, irritability, washability, foamability and pH were evaluated for the different cleanser formulations. Also subjected to antibacterial, cleansing action along with antioxidant efficacy, particle size, zetapotential and viscosity analysis. The formulated cleansers displayed desirable physical characteristics and biological activities, including ambient particle size and surface charge.

INTRODUCTION

Our skin, being the body's largest organ, plays a vital role in shielding us from harsh environmental

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conditions, therefore it requires efficient caring and protection. Since, maintaining a healthy skin barrier is vital for our existence, a proper skin care routine is essential [1]. Skin care is the practice of maintaining and improving the health and appearance of skin through cleansing, moisturizing and protecting it from extreme environments, using personal care products. Therefore, personal care products have become an essential component for the upkeep of our health and hygiene. The skin care products may be classified based on their functions, specific ingredients and formulation-type. Among the skincare routines, cleansing occupies the primary position. For this reason, the present report deals with the development of a cleanser – 'an emulsion mix' for cleaning off hardy dirt and grime from the skin surface. The clientele-base likely to be benefitted include people who get exposed to different types of harmful chemicals and dyes while working for their livelihood, especially people working in automobile repair and servicing centers. They get exposed to grease, oil and other contaminants released from the mechanical parts of vehicles. Workplace hygiene and worker health require repeated washing to remove the sticky, staining materials from the skin. However, it is known that repeated scrubbing and cleansing with harsh chemicals in soaps and detergents could destroy the skin surface and make it dry and rough. One option would be the use of strong cleansers but this is usually discouraged since they have many limitations. Inhalation of spray mists from strong cleansers may cause irritation to the eyes, mucous membranes of the throat, nose and the upper respiratory tract leading to nasal and respiratory irritation. This may also affect the central nervous system causing headache, dizziness and nausea. Prolonged and/or repeated contact with the skin may produce irritation and dryness of skin and possible occurrence of dermatitis. Intentional abuse may be harmful or even fatal. On the other hand, milder versions such as skin wipes are available but may not be effective in all cases. Mixtures of two or more commonly available ingredients are being used as home-remedies. For example, mixtures of (i) sugar and liquid dish soap, (ii) hand cleaner loaded with pumice, (iii) citrus-based cleansers, (iv) instant coffee, powdered laundry detergent and olive oil. Obviously, some of these mixtures work better for some people and not for others. Nowadays, people prefer natural (plant-based) materials for skin care, mainly cleansers with phytochemicals as additive. There are many reports, show that phytochemicals are very gentle and mild in their action but are strong and can protect against many degenerative skin conditions [2]. Extracts could provide the necessary vitamins, antioxidants, essential oils, polyphenols and other bioactive compounds for the necessary action. Phenolic antioxidants with high free radical scavenging capacity, are widely used for blocking the harmful effects of UV radiations [3]. Phytochemicals such as saponins and tannins, can also be used for cleansing. Accordingly, Among the existing natural phytochemical sources, agrowastes stand out as prominent. Research has shown that agrowastes need not be considered entirely as waste. They may be secondary sources of many organic and inorganic compounds. In fact, agriculture is a primary source of income and employment for the agrarian communities in developing countries and a substantial amount of revenue generated by them is contributed to the country's Gross domestic product (GDP). However, this vital sector also generates a significant volume of agricultural waste, and its unregulated disposal patterns would cause considerable threat to ecosystems [4,5]. Therefore, it is possible to devise many innovative pre-treatment technologies and cost-effective conversion processes to transform agrowaste biomass into functional bioactive molecules [6]. Even, global research is currently focused on

finding innovative and practical solutions to convert waste into valuable products [7]. This present study focused on utilizing crude extracts from the agrowaste residues of two acidic fruits, *Tamarindus indica* and *Citrus limon*, and their conversion into an emulsion formulation to serve as a base for a skincare product. This approach aims to valorize agrowastes, efficiently transform these residues, and produce natural skincare products. Emulsions are effective vehicles for incorporating bioactive compounds and are known to enhance the quality of the final product, making them suitable for this purpose.

MATERIALS AND METHODS

Collection: Shells of *Tamarindus indica* L. and Peels of *Citrus limon* L. were used for study. Shells of *Tamarindus indica* were collected from a few households in Kollam and Peels of *Citrus limon* were collected from local juice shops at Kariavattom, Trivandrum, Kerala.

Processing of collected materials: Shells of *T. indica* and peels of *C. limon* were washed in running tap water and cut into small pieces to facilitate drying. Shells of *T. indica* could be powdered easily while the shredded pieces of citrus peels had to be dried in lyophilizer (stored at –80°C for two days) to facilitate powdering. Both materials were ground using an electric blender and the powdered samples were stored in air-tight glass containers until needed for analysis.

Solvent extraction: Cold extraction (CE) and Microwave assisted extraction (MAE) was used to obtain Tamarind shell extract (TS) & Lemon peel extract (LP). For CE, about 10g of sample was immersed in 100ml of 50% and 70% ethanol separately in an incubator shaker for continuous three days. For MAE, 1g of sample was immersed in 10ml of 50% and 70% ethanol separately and subjected to extraction for about a few minutes (1-3 minutes). Results are expressed in yield percentage.

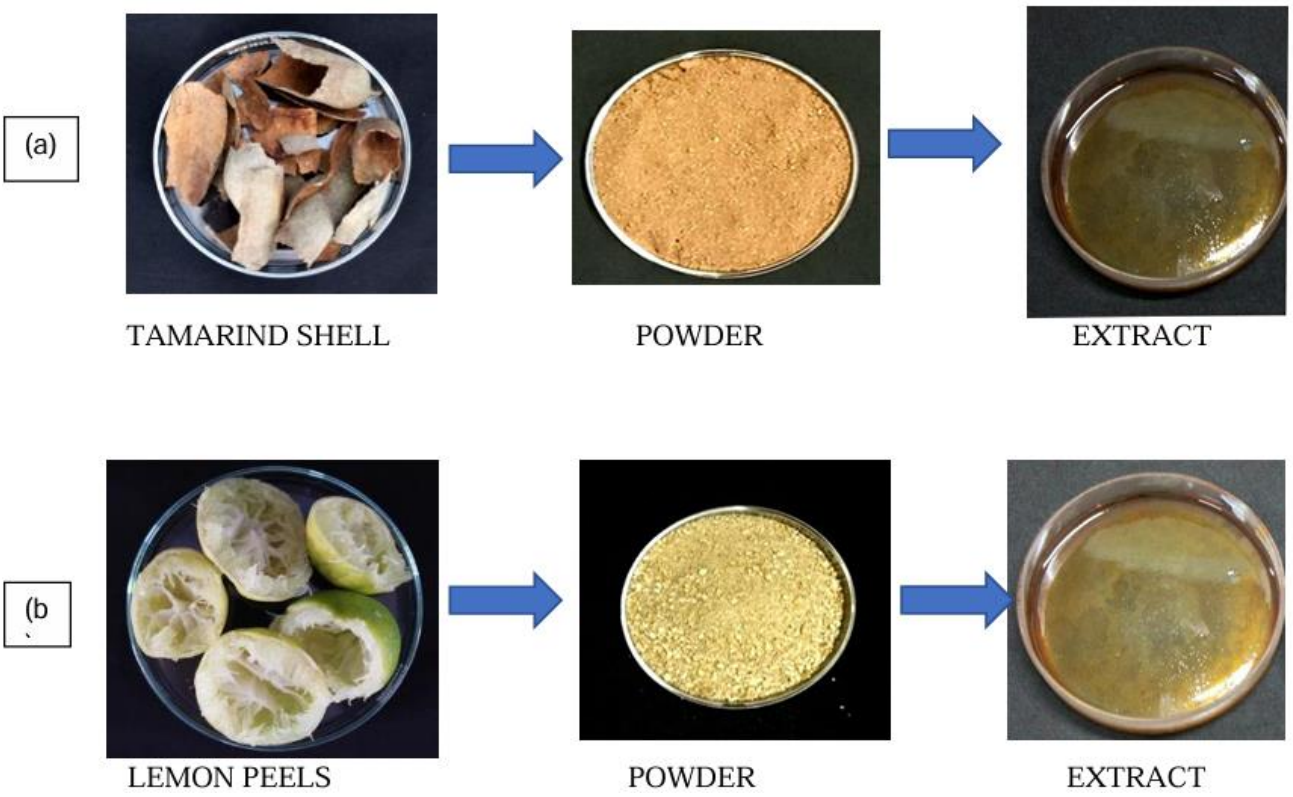


Fig.1

Schematic representation of collected samples, powdered samples & extract of (a) *T. indica* (b) *C. limon*

Preparation of emulsions from TS & LP

Oil in water emulsion was prepared for both TS & LP, using the modified procedure from [8]. For the preparation, distilled water and Tween 80 (3.5ml) were stirred together in a magnetic stirrer for about 15minutes at 1000rpm followed by addition of PEG 400 (1.5ml). The mixture was stirred for another 15minutes. To this, 1ml of gingelly oil, containing the tamarind/ lemon extract of different concentrations (1mg/ml; 5mg/ml and 10mg/ml dissolved in DMSO) was added. The solution mixture was again stirred at 1500rpm for about half an hour. The mixture was then transferred to the Ultrasound probe sonicator (Sonic vibra cell VCX 130) (for 3hr with 30% amplitude) for uniform dispersion. The prepared emulsions were named as TSE (Emulsion with Tamarind shell extract) & LPE (Emulsion with lemon peel extract). Emulsions were subjected to physico-chemical characterization using parameters such as - Visual properties, pH analyses, Stability, Identification of Emulsion type, Dilution test, and Filter paper test [8,9,10].

Formulation of Skin cleansers with TSE & LPE mixtures

Cleansers were prepared by mixing together TSE & LPE in different combinations, adopting the standard protocols [11,12]. For the preparation, the ingredients such as citric acid -Preservative (0.025mg/ml), Propylene glycol- Moisturizer (3.000), Sodium lauryl sulphate - Foaming agent (0.500), were taken and dissolved together with a small quantity of water. To the above solution, carbopol940- gelling agent (1mg/ml), was added slowly and stirred until a dispersion was obtained. To this, emulsions of both extracts (TSE, LPE) were added in varying concentrations to get a complete gel-like consistency. Finally, to adjust the pH, a few drops of triethanolamine (2mg/ml) was added to the mixture.

Primary characterisation of prepared Cleanser formulations.

Visual evaluation: The emulsion formulations were visually examined to assess their uniformity, colour, texture, and stability.

Homogeneity: All developed emulsions were tested for homogeneity by visual inspection (presence or formation of aggregates) after the gels were set in the container.

Irritability: A small amount of the sample was applied on the skin and kept for few minutes and irritability tested.

Washability: A small quantity of sample was applied over the skin and was washed off with water to determine its washability

Foamability: Small amount of the sample was shaken with water in a graduated measuring cylinder and foam generated was noted.

pH: The pH of various gel formulations was determined using the digital pH meter. About 2.5 g of gel was accurately weighed, dispersed in 25ml of distilled water and stored in a glass beaker for 30minutes.Triplicate values were taken.

Antibacterial activity: Both ethanolic extracts, and prepared cleanser samples were subjected to antibacterial analysis according to the standard procedures [13]. *Staphylococcus aureus* (MTCC3160), was used as a reference for the antibacterial assay. The standard bacterial strains were acquired from the clinical isolates of the microbial culture collection of Microbiology laboratory, Department of Botany, University of

Kerala, Kariavattom. The tested strains were cultured in nutrient broth (Hi-media) at 37°C and stored in nutrient agar slants at 4°C. Antibacterial assay was carried out by well diffusion method. Mueller-Hinton agar (15-20ml) was poured onto petri plates of same size and allowed to solidify. Each agar plate was punched to make 4 wells of 6mm size (20mm apart from one another) with a well cutter. Standardized inoculums of the test microorganism were uniformly spread on agar surface of each plate using sterile cotton swab. Samples of ethanolic extracts and prepared cleansers (concentration 80mg/ml) were poured into the wells. Streptomycin (10µg/ml) was used as positive control and was placed at the centre of the plate. DMSO was considered as negative control. The plates were allowed to standby for about 30minutes. The plates were incubated at 37°C for 24h. After incubation, plates were observed for antibacterial activity by measuring the diameter (in mm) of the zone of inhibition formed around the well.

Cleansing action and efficacy: The cleansing effectiveness of the prepared samples was evaluated using a standardized hand-washing test. Engine oil was applied to the skin on the back of the hand (of a particular individual) and allowed to dry. The dried oily patch was subsequently washed with a fixed amount of sample. The cleansing result was visually assessed on a hedonic scale of 0 (clean) to 4 (no cleansing action) and are provided in the reference figures (**Fig.2** a-e). The scores are given on the top right corner of each figure. The test was repeated at least three times with varying amounts of cleanser (1-4ml) to compare the remaining dirt and determine the amount needed to achieve equal cleansing results. Due to individual variations in skin structure and the inability to fully standardize the washing process, only comparative assessments were made, as described by [14].

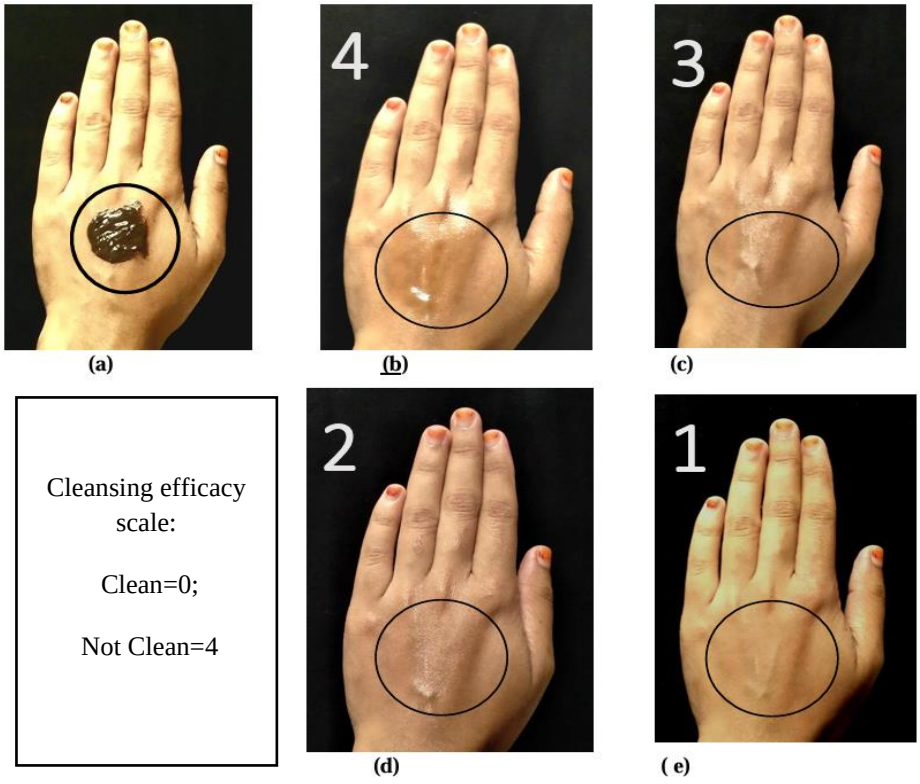


Fig. 2

Illustration of testing cleansing action and the degree of efficacy of prepared formulations on the human skin surface.

Cleanser samples showing better cleansing actions were selected for further characterization

Secondary characterization of the emulsion samples and selected cleanser samples

Antioxidant activity of prepared cleanser samples

Antioxidant activity was determined by conducting DPPH Radical scavenging assay [15]. Different volumes (220µl) of plant extracts were made up to 40µl with DMSO and 2.96ml of DPPH (0.1mM) solution was added [0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol]. The reaction mixture was incubated in the dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. About 3ml of DPPH was taken as control. Ascorbic acid was used as reference. The % radical scavenging activity of the plant extracts was calculated using the following formula,

$$\%RSA = \frac{\text{Abs Control} - \text{Abs sample}}{\text{Abs Control}} \times 100$$

RSA = Radical Scavenging Assay, Abs Control = Absorbance of DPPH + Methanol, Abs Sample = Absorbance of DPPH +Plant extract

Particle size analysis: Particle size analysis of selected cleanser sample and corresponding emulsion was measured by the diffraction method using a light scattering particle size analyser counter (Horiba SZ-100 DLS instrument) at 25ºCelsius at the instrumentation facility of Department of Chemistry, University of Kerala.

Zeta potential analysis: The Zeta potential & Conductivity of the selected Cleanser sample and corresponding emulsion were measured using the particle size analyser counter Horiba SZ-100 DLS instrument. This parameter describes the stability, charge and type of the prepared sample.

Viscosity: Viscosity of selected cleanser sample was determined using Brookfield rotational viscometer (rpm 20) at the instrumentation facility of Department of Chemistry, University of Kerala.

Statistical analyses

All the experiments were done in triplicate and the data was statistically reported as mean ± standard error (SE). One-way & Two-way Analysis of Variance (ANOVA) was done using SPSS 22.0 software to determine the statistical significance ($p < 0.05$) of the observations with multiple comparison test of Duncan.

RESULTS AND DISCUSSION

Tamarind crude aqueous extracts are known to contain a variety of secondary metabolites alkaloids, saponins, tannins, flavonoids, terpenoids, quinones, glycosides and phenols [16]. GC–MS results suggest that the seeds and peel extracts (chloroform and ethyl acetate) contains a wide range of compounds (including flavonoids, isovanillic acid, fatty acids and phenolic compounds) which can be utilized for therapeutic purpose [17]. The aqueous peel extracts showed the presence of carbohydrates, alkaloids, tannins, fixed oils, proteins, cardiac glycosides, steroids, phenols and flavonoids and amino acids, whereas the ethanolic peel extracts revealed that they contained carbohydrates, saponins, tannins, fixed oils, cardiac glycosides, steroids, phytosterols, phenols and flavonoids [18, 19] could also observe that the major phytochemicals in the citrus peels were phenolic acids, flavonoids, furanocoumarins

with the key phytochemicals being hesperidin, naringin, and limonene. Chemical profiling of the non-polar extract using gas chromatography-mass spectrometry by [20] revealed the presence of volatile terpenic compounds (limonene, valencene, nootkatones, etc.), long-chain alkanes (tricosane, nonacosane, and triacontane), triterpenes, phytosterols, fatty acids (linoleic and palmitic acids), and polylmethoxylated flavones (nobiletin, 3-methoxynobiletin, tangeretin and tetramethyl-O-scutellarin). Thus, perusal of literature showed that the crude extracts of agrowaste materials such as Tamarind shells and Lemon peels could be exploited for the extraction of useful biomolecules with proven bioactivity. Abundance of polyphenols in these samples was evident [1,21,22].

Yield assessment and quantitative estimation of polyphenols of Tamarind shells and Lemon peels

The selected materials were subjected to two methods of extraction (CE and MAE) with ethanol (50% and 70%) as solvent (**Fig.3**). The yield was noted to be higher for LP in both extraction methods (CE and MAE). In the case of LP, CE (50% ethanol - 15%; 70% ethanol -18.04%) showed relatively better results compared to MAE (13.4 - 17.51%). A comparable yield percentage was exhibited by TS only in MAE (12.5-15.5%).

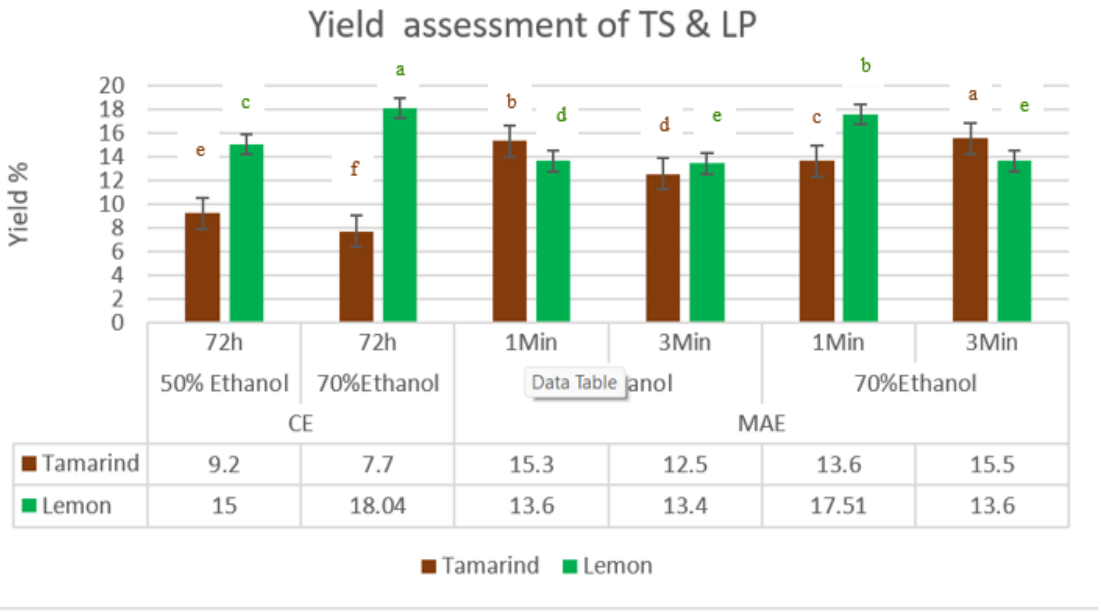


Fig.3

Yield assessment of TS & LP in both extraction methods (CE & MAE)

Each value is expressed as mean ± standard error ($n = 3$). Different superscripts ($^{a, b, c}$) indicate significant differences ($p < 0.05$) between groups using Two-way ANOVA by Duncan`s multiple range test

The extracted materials were subsequently utilized for Emulsion preparation

Preparation of oil in water emulsion from Tamarind shell and Lemon peel extract

The emulsions (TSE & LPE) were prepared according to the method and ratios mentioned in methodology section (Emulsion 1- 1mg/ml, Emulsion 2- 5mg/ml, Emulsion 3-10mg/ml).

Ultrasound probe sonicator (Sonic vibra cell VCX 130) was used for dispersing the finite solute molecules in the solution. Within the first few minutes (6-10 minutes), an opaque off-white solution was formed, which after about an hour, became a clear and transparent solution. After 3hr of continuous sonication, the emulsions became clear and were then stored in sterile sample vials

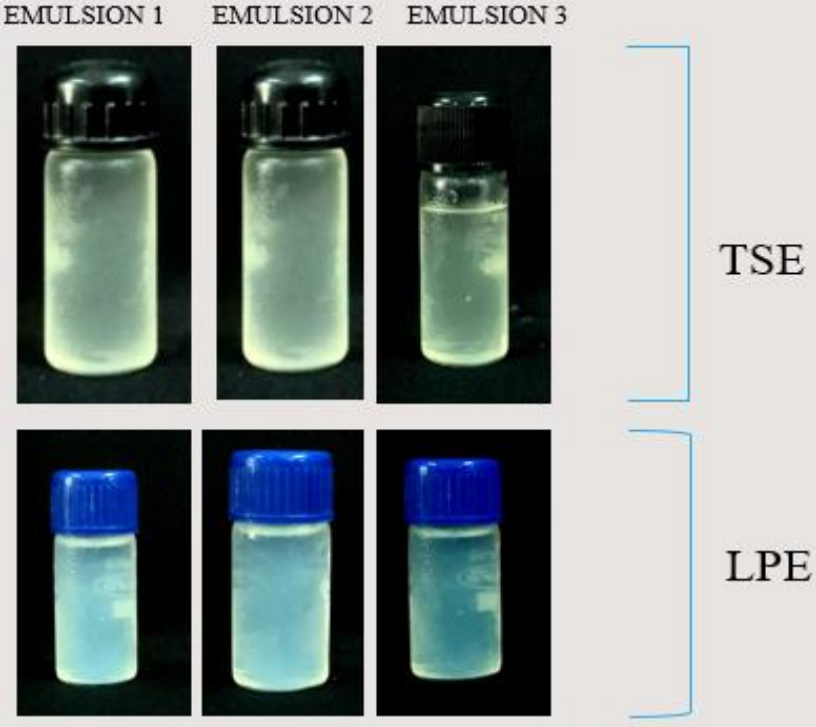


Fig.4

Prepared emulsions (a) Emulsion 1mg/ml (b) Emulsion 5mg/ml (c) Emulsion 10mg/ml

Physico-chemical characterisation of prepared emulsions

Physico chemical properties of the prepared emulsions were evaluated by analysing six different parameters.

Visual properties: When exposed to light, the prepared emulsions (TSE & LPE) exhibited a nearly transparent nature with yellowish-white colour and a watery texture.

pH: Both TSE & LPE emulsions showed almost similar pH values ranging from 6.1 (emulsion A&B), 6.2 (Emulsion C) for TSE and 4.6 (Emulsion A), 5.2 (Emulsion B), 5.9 (Emulsion B) for LPE. Since emulsion systems are usually used in topical cosmetic products, the pH must be suitable for human skin (pH 4.5–6.0), which is slightly acidic to near neutral.

Stability: The prepared emulsions were stored at 4°C in the refrigerator for six months and continuously monitored at intervals for visual changes in colour, and texture. The emulsions (TSE, LPE) didn't exhibit any noticeable changes. A total absence of creaming, flocculation, and cracking was noted manifesting its stability. An emulsion is considered stable when it lacks separation of its internal phase, shows no creaming, remains unaffected by microbial degradation, and maintains its appealing attributes including appearance, colour, fragrance, and texture. The primary forms of instabilities are droplet clustering in emulsions such as

flocculation and coalescence [23]. Flocculation involves the assembly of multiple droplets into an aggregate whereas coalescence indicates the fusion of droplets, resulting in the creation of a single, larger droplet. The predominant forms of colloidal interactions among droplets in emulsions encompass van der Waals forces, electrostatic interactions, steric effects, hydrophobic interactions, depletion interactions, and bridging interactions, these interactions typically manifest as attractive forces, whereas steric and electrostatic interactions typically result in repulsion. Generally, as droplet size increases, both attractive and repulsive colloidal interactions tend to intensify. Droplets within an emulsion can exhibit diverse forms of aggregation states depending on the nature of the interaction potential profile.

Identification of emulsion type: Types of the prepared emulsions was identified by conducting dilution test and filter paper test

Dilution test (Dispersity test): The result of dilution test showed that upon the addition of water (Continuous phase), the prepared emulsion was clear in less than 10 seconds without any cracking or precipitation. The appearance of clear emulsion in less than 1 minute indicates that the emulsion formed was a stable Oil in water (O/W) emulsion. The continuous phase (distilled water) could be easily incorporated into emulsion without affecting its stability revealing its type. The principle underlying the test relies on the observation that an additional continuous phase can be introduced into an emulsion without compromising its stability Consequently, an oil-in-water (o/w) emulsion can be diluted using water, while a water in-oil (w/o) emulsion can be diluted with oil [9,10].

Filter paper test: The emulsion upon dropping into a filter paper spread rapidly which is an indication that the formed emulsion was oil in water(o/w) type. This test relies on the principle that an oil in water emulsion upon contact with a filter paper disperse rapidly due to the presence of water as the continuous phase while a water in oil emulsion spread out gradually as the continuous phase (oil) resists the motion [10].

Table.1 Physicochemical characterization of Formulated Emulsions

S. No	Characters	Remarks
1	Colour	TSE: Slightly yellow colour LPE: Transparent
2	Homogeneity	Fluid consistency in all samples
3	pH	TSE: 6.1 – 6.2 LPE: 5.9 – 4.6
4	Stability	All the samples of TSE & LPE are stable
i. Flocculation ii. Creaming iii. Coagulation iv. Phase inversion		Absent in all samples of TSE & LPE
5	Dilution test	All the samples of TSE & LPE were easily diluted with water
6	Filter paper test	All the samples of TSE & LPE spreads rapidly

Preparation of Cleanser formulation using TSE & LPE

Cleansers with varying concentrations (1mg/ml, 5mg/ml, and 10mg/ml) of the base emulsions (TSE and LPE) were formulated. Then, 1ml, 2ml, and 3ml each of both emulsions (TSE, LPE) were combined in a 1:1 ratio to create six different cleanser concentrations (F1A-F3C) as given in the figure (**Fig. 5**). Similarly, a control sample (F0) was prepared without adding any emulsion. This setup allowed for the evaluation of the effects of different emulsion concentrations on the cleanser's properties.

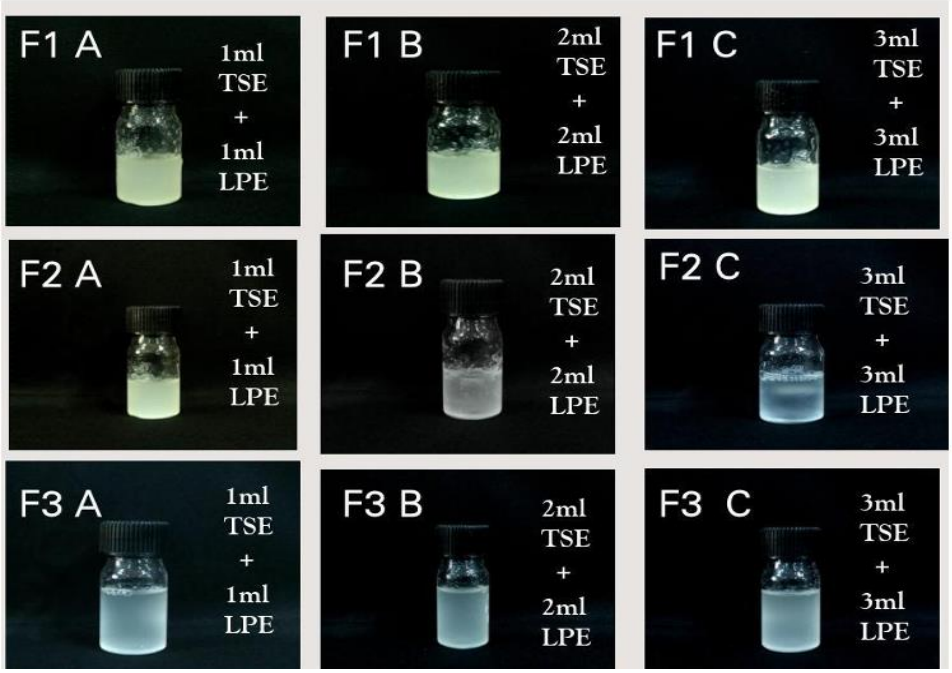


Fig.5

Different formulations of cleansers prepared.

Earlier studies have shown that tamarind shell powder & lemon peel powder are natural exfoliating agents. These powders act as skin scrubs that helps to improve the blood circulation, remove oil, sebum and other secretions and lighten the skin tone [24]. Exfoliating agents within cleansers keep the skin surface clean by promoting removal of dead skin cells and boosting blood circulation giving renewed and glowing skin. Here different cleanser samples were prepared with increasing concentrations of both emulsions (1, 2,3 ml of TSE and LPE each) to judge the effect on their cleansing capability. Cosmeceutical emulsions have a great demand as a consequence of their ability for controlled delivery and optimized dispersion of active ingredients into the desired layers of skin. In the current study, the active ingredients of both TS and LP were uniformly dispersed in the corresponding emulsions (TSE and LPE) to heighten their effect. It is assumed that the nanoemulsion mixtures of TSE, LPE could enhance the cleansing property. It is reported that the active ingredients in the form of nanoparticles have a higher surface-to-volume ratio and lower surface tension which promotes dispersibility in the emulsion, better penetration efficacy of the ingredients and better adaptation for multiple functions [25].

Characterisation of formulated cleansers

Evaluation of physical parameters

The prepared cleansers were evaluated and compared with the control (F0) with respect to various physical parameters like colour, odour, consistency, irritability, washability, foamability, pH. The parameters tested for all the samples were comparable except for their consistency.

Table.2 Physicochemical characterization of formulated cleansers

S. No.	Parameters	Remarks
1	Colour	All the samples were white in colour
2	Odour	No characteristic odour

3	Consistency	Varies from solid to fluid Solid - F0 Semi solid - F1A, F1B, F1C, F2A Fluid - F2B, F3A, F3B, F3C
4	Irritability	None of the samples are irritant
5	Washability	All the samples are washable
6	Foamability	Good
7	pH	7.3- 5.9 (acidic to near neutral)

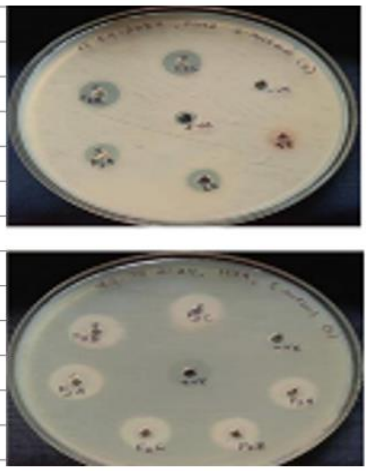
It is generally believed that the colour of a cleanser is not a primary factor for determining its cleansing efficiency. However warmer colours are preferred and is purely based on aesthetics, branding, consumer perceptions, suitability for skin type, needs and preferences. Also, cleanser formulations with semisolid to solid consistency is most preferred for its ease in handling and stability. Likewise, inert non-irritant products are also valued more by customers. Cleansers should remove grease and grime from almost all skin types. However, currently cleansers have come a long way from simply removing dirt, oil, dead skin cells, and microbes, they had to adapt to meet rising consumer expectations. Modern cleansers, with technological advancements, are now designed to be gentler, more moisturizing, and easier to be washed off [26]. The pH of the skin is also essential parameter since the pH decides the possible adhesion of resident skin microflora (*Staphylococcus epidermis*). Research has shown that certain syndets (combination of surfactants) have pH values in the range of 5.0-5.5 (close to the skin's native pH) and these have friendly mildness implying less damaging effect to the skin [27].

Antibacterial activity

The antibacterial properties of Tamarind Shell Extract (TS) and Lemon Peel Extract (LP), and the prepared cleanser samples (F1A-F3C) were evaluated against the gram-positive bacterium *Staphylococcus aureus.* Streptomycin served as the positive control, while DMSO and the blank cleanser sample (F0) acted as negative controls.

Table.3 Antibacterial activity of prepared cleansers (F1A-F3C) & extracts (TS & LP).

S. No.	Samples		Zone of inhibition(mm)
1	Positive	Streptomycin	12
2	Negative	DMSO	0
3		Control F0	0
4	Extract	TS	5
5		LP	10
6	F 1	A	10
7		B	9
8		C	10
9	F 2	A	8
10		B	9
11		C	9
12	F 3	A	8
13		B	7
14		C	7



The results (**Table.3**), showed that both LP and LP-based emulsion formulations exhibited highest antibacterial activity (LP, F1A, F1C-10mm) compared to the positive control (12mm) and TS

samples. All the prepared cleansers (F1, F2, F3) showed more or less moderate zone of inhibition (>5mm). Since the prepared emulsions had an acidic to near neutral pH, they were considered suitable candidates for cleanser preparation. It is said that an acidic pH of the skin keeps the resident bacterial flora attached to the skin, whereas an alkaline pH promotes its dispersion from the skin. This may be because, swelling of the skin under alkaline conditions potentially weakens the corneocytes, simultaneously allowing the resident bacteria to diffuse across the skin's surface as well as diminishing their beneficial attributes. This might permit the transient bacteria (*Staphylococcus aureus)* to colonize the skin and thrive under favourable conditions and is therefore referred as coagulase positive [12]. Since *Staphylococcus aureus* is considered as the main causative agent of many of the skin infections, it was chosen for the present study also. The antimicrobial activities of several -herbal based skin products composed of extracts of the plant parts of *Tamarindus indica*, *Citrus Limon* and *Tridax procumbens* have been documented earlier [12,28]. Phytochemical constituents such as tannins, alkaloids, flavonoids, phenolic compounds and several other aromatic compounds have been implicated in the antimicrobial potential of most of these herbal products [29].

Testing cleansing action and efficacy

The ability of the prepared cleanser samples to remove medium to heavy-duty dirt (engine oil) was evaluated through a simple hand wash test. The test measured the amount of cleanser sample required to achieve noticeable cleansing, with results scored on a hedonic scale of 0 (clean) to 4 (no cleansing action). Score values 1-2 (almost clean) and 3-4 (moderately clean) were also considered. The standardized washing test was conducted with varying sample quantities (1-4ml).

Table.4 Testing of Cleansing actions of formulated cleanser samples (F1A-F3C)

Cleansing actions of samples		Quantity of samples taken (ml)			
		1ml	2ml	3ml	4ml
F0		4	4	4	3
F1	A	4	4	2	2
	B	4	3	2	2
	C	3	3	2	2
F2	A	4	4	4	3
	B	4	4	4	3
	C	4	4	4	2
F3	A	3	2	2	2
	B	3	2	3	1
	C	2	2	1	1

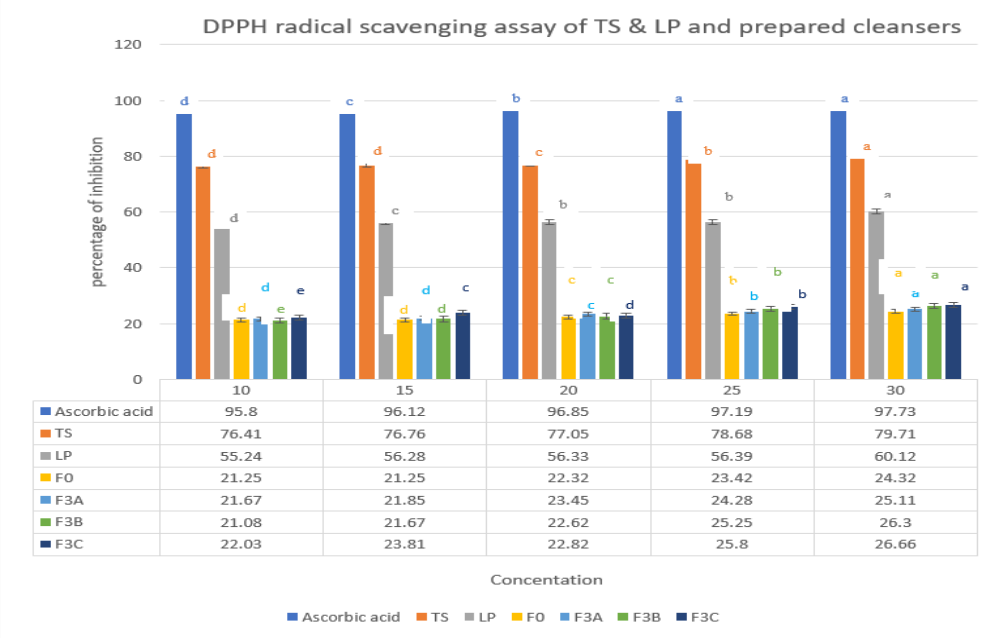
Cleansing efficacy scale: Clean=0; Not Clean=5

Visual assessment of cleansing efficacy was compared with the hedonic scale value ranges from 0-4 (Refer **Fig.2 a-e** of methodology section). Visual assessment revealed that F1 and F2 exhibited relatively weaker cleansing capabilities compared to F3. Even at higher quantities (4ml), F1 and F2 showed limited improvement (scoring 3-4), whereas F3 demonstrated superior cleansing action (scoring 1-2). Increasing the sample quantity did not enhance the cleansing effectiveness of F1 and F2, whereas F3 consistently showed remarkable dirt removing capabilities, outperforming the other samples. Therefore, F3 C sample was used for further characterization

Secondary characterization of selected cleanser samples

Antioxidant activity- DPPH radical scavenging assay

The free radical scavenging activity of both extracts (TS & LP) and the selected cleanser sample (F3A, B, C) was evaluated using the DPPH radical assay shown in **Fig.6**



	10	15	20	25	30
Ascorbic acid	95.8	96.12	96.85	97.19	97.73
TS	76.41	76.76	77.05	78.68	79.71
LP	55.24	56.28	56.33	56.39	60.12
F0	21.25	21.25	22.32	23.42	24.32
F3A	21.67	21.85	23.45	24.28	25.11
F3B	21.08	21.67	22.62	25.25	26.3
F3C	22.03	23.81	22.82	25.8	26.66

Fig.6

DPPH radical scavenging activity of extracts (TS & LP) & selected cleanser sample (F3A, B, C). Each value is expressed as mean ± standard error ($n = 3$). Different superscripts (^{a} ,^{b} ,^{c}) indicate significant differences ($p < 0.05$) between groups, using One-way ANOVA by Duncan`s multiple range test The DPPH free radical scavenging activity of plant extracts is due to neutralization of DPPH radical by transfer of hydrogen (or an electron) relative to the standard, ascorbic acid [30]. It was observed that in the present study TS showed an increase in free radical inhibition (%) in a concentration dependent manner compared to ascorbic acid. It was also observed that the free radical scavenging capacity of F3C is more than the rest of the samples and is almost comparable to ascorbic acid. This is probably because, the concentration of extracts (10mg/ml TSE and 10 mg/ml LPE) is highest in F3 C. Higher concentration also indicates the higher content of polyphenols in the emulsions. Many phenolic compounds have been reported to possess potent antioxidant activity, which might vary according to the number and position of hydroxyl groups included in the constituent, especially tannins [31]. Also, TS exhibited higher antioxidant activity than LP with an IC_{50} value of 54.05 ± 0.01µg/ml. Similarly, all the selected cleansers (F3A-C) demonstrated moderate antioxidant activity (51.23±0.02 to 52.30±0.27 µg/ml) in a dose-dependent manner. Among the samples, F3C showed the highest antioxidant activity, with an IC_{50} value of 51.84033 ± 0.012 µg/ml, indicating its conceivable free radical scavenging ability in comparison to the IC_{50} value of the standard (18.90± 0.01). Particle size analysis, Zeta potential & Viscosity of selected cleanser sample (F3 C) and its emulsion (10mg/ml) was determined.

Particle size analysis: The droplet size analysis is the best tool to identify the stability & visibility of prepared samples. In the present study, particle size of both emulsion (TSE & LPE) and selected cleanser sample (F3C) were studied using the Horiba SZ-100 DLS at 25 ºC. The droplet size was found to vary [8.6nm (TSE), 100.5nm (LPE) and 569.2nm (F3 Cleanser)] (**Fig.7**). The working of the instrument is based on diffusion-based approach and light scattering technology which represents the relationship between light intensity

and scattering angle and it is determined by the interplay between droplet size and light wavelength (ranging from 380 to 780 nanometres). As per the previous reports, the size range of the particulates in the medium can be as low as 20nm to as high as 1000nm [32,33]. Though the particle size of TSE was less, the F3C cleanser showed larger sized particles but relatively less than that in similar products. In skin care products, particle size is crucial as it impacts its penetration ability, gentleness, exfoliation, skin feel, stability, transdermal delivery, irritation and cleansing efficiency.

Zeta potential analysis: The zeta potential analysis revealed that both the emulsion (TSE & LPE) and F3C cleanser sample exhibited a slightly negative charge, with values of -12.7, -0.8, and -3.5, respectively, at a temperature range of 25-25.1 °C and electrode voltage of 3.4-3.9V shown in **Fig.7**. Zeta potential analysis offers a valuable tool for understanding the surface charge properties of particles, which is closely tied to the formulation and preparation methods used. This value serves as a key indicator of the stability of the colloidal system, providing insight into the likelihood of particle aggregation, sedimentation, or flocculation. A high positive or negative zeta potential indicates strong electrostatic repulsion, preventing particles from aggregating. In contrast, a low zeta potential suggests a higher likelihood of particle aggregation due to attractive Van der Waals forces. Zeta potential values outside the range of -30 mV to +30 mV generally indicate sufficient repulsion between particles, ensuring stability in the colloidal system [34]. However, here the lower zeta potential values, likely attributed to the use of Tween 80 as a surfactant, may be due to its large polymer head groups providing steric hindrance and maintenance of stability. Additionally, as a non-ionic surfactant, Tween 80 is said to carry minimal to no surface charge, contributing to lower zeta potential values [35].

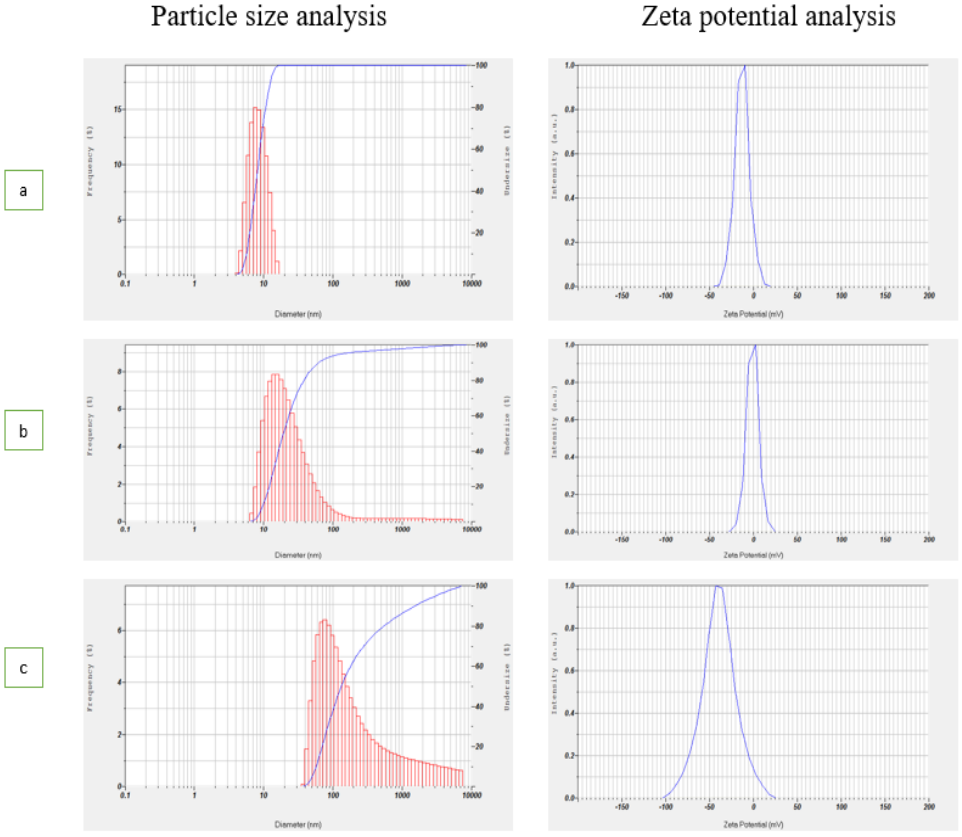


Fig.7

Particle size analysis and Zeta potential analysis of both emulsions [(a) TSE (b) LPE] & cleanser formulation [(c) F3 C Cleanser].

Viscosity: The viscosity of the cleanser sample was measured at 1762 centipoise (cP), which falls within the range of low-viscosity cleansers (1,000 to 5,000 cP). This indicates that the prepared cleanser has a thin, watery consistency, making it suitable for cleansing. The viscosity of the prepared cleanser suggests a mild and non-irritating formula, ideal for delicate skin types. It has been opined that ready spreading, quick absorption, nongreasy feel, particularly for sensitive skin or baby care applications may be considered desirable properties. Thus, it appears that all the prepared emulsions, especially F3C appears to be a suitable formulation for skin cleansing.

CONCLUSION

The current study adopted a novel strategy for the formulation of bioactive skin cleansers from mixtures of two emulsions (TSE+LPE) of two selected agrowaste materials (Tamarind shell and Lemon peel), strengthening the path towards sustainability. Also, the prepared cleanser formulations were identified to possess all the ideal physical and chemical characteristics of a potent cleanser. Therefore, the results of this study endow the accessibility of a novel approach for agrowaste management and development of green cosmetics

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