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Optimizing the Formulation of Face Cream using the Shells of *Myristica fragrans, Juglans regia* and Carboxymethyl Cellulose and its Cytotoxicity Evaluation

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Abstract

The medicinal plants *Myristica fragrans* and *Juglans regia* has been used in distinct fields especially in pharmacological and cosmetic industries. Both were rich in anti-oxidants and has high anti-microbial and anti-inflammatory properties. The entire structure of *Myristica fragrans* and *Juglans regia* composed of its shell and only the minimum part of it is edible. Due to the useful properties of shell wastes, it can be used in cosmetics to brighten, moisturize and prevent ageing of the skin. In order to homogenize solutions, a thickener is needed. Carboxymethyl cellulose has thickening, bonding, dispersing, homogenizing, emulsifying and stabilizing properties. Hence, carboxymethyl cellulose was used as a thickening agent in the formation of face cream. The objective of the current study was to formulate an inexpensive face cream to produce a moisturizing effect and skin glow and its organoleptic properties, physical parameters, stability testing, qualitative and quantitative analysis, and cytotoxicity of face cream on normal human dermal fibroblast cells were evaluated. The formulations of the face cream developed were totally novel, and there were no published works based on the same cream. F1 and F2 formulations of the cream were developed and investigated. F1 formulation with a high amount of emulsifier had shown good stability, no discernible changes in other parameters were seen and it exhibited strong anti-microbial activity and the cream was found to be non-toxic on normal human dermal fibroblast cells. As a consequence, it became obvious that the F1 formulation was stable. Hence, it can be safely used on the skin as a moisturizer and skin-glow enhancer.

***Key words*:** Emulsifier, Moisturize, Non-toxic, Novel cream, Skin glow

The chemicals utilized on the body to improve appearance are termed as cosmetics. Chemical components that are synthesized or obtained from natural sources are mixed together to compose cosmetics. It is used in the enhancement and maintenance of skin. It can be sticks or liquids that are anhydrous, powders that are pressed or loose, cream emulsions or dispersions. Cream is applied cosmetically to have a softening and purifying effect [1]. There are numerous kinds of creams, including ones for the hands, body, massage, sleep, cleaning, cold, and foundation. Products made from herbs have been touted for their inherent acceptance, effectiveness, and lack of adverse effects, which are frequently associated with synthetic products. In contemporary times, extracts are frequently incorporated in formulations since customers are concerned about artificial additives and chemicals [2]. Herbal compounds in skin care formulations help to bog down the generation of free radicals [3]. Free radicals generally break down collagen and accelerate ageing.

The evergreen nutmeg tree, *Myristica fragrans*, is a member of the Myristicaceae family of flowering plants that are native to Asia, Africa, and the Pacific islands [4]. It is found to have antibacterial, anti-inflammatory, and antioxidant qualities that help to enhance the general health and look of the skin [5]. Although it is widely used, its shells are typically thrown away, leaving behind waste that is just as beneficial as the seed. Therefore, using the shells can help avoid waste while optimizing its use and resulting in a valuable cream with low cost. *Juglans regia* is a commonly found plant native to South East Asia and it belongs to the family Juglandaceae. The *Juglans regia* shell is a hard, biodegradable, naturally occurring substance that makes up over 70% of the fruit's overall weight. As a result, the shells are the most plentiful byproduct, rich in polyunsaturated fatty acids, proteins, B vitamins, and minerals like potassium, magnesium, manganese and omega-3 fatty acids. These components in the *Juglans regia* supports youthful healthy skin. Antioxidants can shield the skin from damaging

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UV rays, dust, and pollution, while proteins aid in the quicker healing of wounds [6].

Emulsifiers play a critical role in stabilizing cosmetic products by lowering surface tension between the molecules of the two substances. One of the most prevalent bioactive, low-cost materials in nature, polysaccharides have a long history of usage in the food, cosmetic, and pharmaceutical sectors. The most common water-soluble cellulose derivative made and used in industry is carboxymethyl cellulose [7-8]. It is a common thickening, emulsifying, and stabilizing ingredient. It contributes to improve product viscosity, texture, and overall performance in cosmetics. Because of its superior ability to bind water, it helps in skin care and hair care products to retain moisture and provide creamy, smooth compositions while supplying uniformity and stability. Jasmine oil has been included in folk remedies in numerous nations because of its multiple uses. Jasmine oil also improves skin tone, rejuvenates, treat acne. In aromatherapy, it is used to elevate people's moods and alleviate despair [9]. This study was conducted to formulate a multipurpose cream that gives a skin glow and can be used as a moisturizer.

MATERIALS AND METHODS

Extraction process

The shells of *Myristica fragrans* and *Juglans regia* were extracted using the decoction method. Decoction is perhaps the most pertinent and easiest extraction method. The shells were air- dried and roughly grounded. The minced mixture was steeped in water and then boiled in separate beakers. The solution was subsequently filtered. The resulting *Myristica fragrans* and *Juglans regia* filtrate were utilized as extracts for cream formulation [10].

Cream formulation

The face cream was designed as an oil-in-water (O/W) emulsion where the dispersed phase was oil and the continuous phase was water. The cream evolved by merely combining a couple of phases (the oil phase and the water phase). Aqueous phase had been produced by combining hydro extracts of both shells with benzoic acid. Carboxymethyl cellulose was blended with jasmine oil to form the oil phase. The aqueous and oil phases were taken in separate beakers. Both phases had been boiled solely in a water bath at 75°C [11]. When both phases underwent heating, the aqueous phase was infused with the oil phase while stirring. The continual swirling of both phases produced a cream-like consistency. After attaining a sufficient quantity of thickening, the cream was stored in a sterilized, airtight container for further testing [5], [12]. The F1 and F2 formulas for the face cream are shown in (Table 1).

Table 1 Composition of the face cream

Ingredients	Role	F1	F2
Juglans regia shell extract	Improves skin tone	2 ml	2 ml
Myristica fragrans shell extract	Antimicrobial activity	2 ml	2 ml
Carboxymethyl cellulose	Emulsifier	4 g	2 g
Jasmine oil	Fragrance	10 ml	10 ml
Benzoic acid	Preservative	0.02 g	0.02 g

Organoleptic evaluation

The prepared face cream's organoleptic characters (colour, odour, homogeneity, and state) and various other parameters such as pH, irritancy, emollience, washability, phase division were evaluated [13-14].

Physiochemical evaluation

Spreadability

The spreadability is defined as the number of seconds required for two slides separated by a cream layer to separate under a certain stress. Glass slides were kept in two sets. Following that, a slide with the appropriate dimensions was selected, and the cream formulations were placed on it. It was then covered with another slide. The cream between the two slides were then uniformly compressed to form a thin layer by pressing a weight or other force on the upper slide. Finally, the weight was removed, and any excess cream formulations that had clung to the slides was scraped away. The weight linked to the upper slide provided sufficient power for it to move freely. It was observed how long it took for the upper slide to drop off [15].

$$\text{Spreadability} = m \times l/t$$

Where;
m = standard weight, which is tied or placed over the upper slide (30 g),
l = length of a glass slide (5 cm),
t = time taken in seconds.

Stability testing

Stability testing was carried out as per ICH guidelines. Cycle testing and centrifuge testing was conducted to check the stability.

Cycle testing

Three temperature testing cycles, ranging from 10 °C to 25 °C, must be tolerated by the product. The face cream was first kept at 10°C for 24 hours, and then it was kept at normal temperature (25°C) for an additional 24 hours. With this, one cycle finishes. Following the successful completion of three cycles, the cream's stability was examined [16].

Centrifuge testing

In an oil-in-water emulsion, the dispersed phase leads to separation and ascending to the top, generating a coating of oil droplets. We refer to this phenomenon as "creaming.". One of the earliest indicators of approaching emulsion instability is creaming, which needs to be treated seriously. Centrifugation is an effective test technique to predict creaming. The emulsion was centrifuged for thirty minutes at 3000 rpm after heating to 50°C. The final product for indications of creaming was examined. Any product that contains powder of any type, including liquid or cream makeup, must undergo this test. After 10, 20, and 30 days, the physical evaluation tests were conducted once more. Following the identification of the most stable formulation, assessments for total viable count, microbiological stability and cytotoxicity evaluation were conducted.

Qualitative analysis

Anti-microbial activity evaluation

The efficacy of our components in the cream formulations was examined using an antimicrobial assay. Fungal strain *Candida albicans* and bacteria strains, namely *Klebsiella pneumonia* (Gram-positive), *Pseudomonas aeruginosa* (Gram- negative), and *Bacillus subtilis* (Gram - negative), were used in this study. Antimicrobial activity was measured using the Kirby-Bauer test. In the Kirby-Bauer test, antibiotic wafers (white discs) were kept on a plate containing bacteria grown on solid growth medium. The antibiotic slowed bacterial growth, as evidenced by patches of clear medium surrounding the discs when the bacteria were allowed to proliferate for 16 to 18 hours at 35 to 37 °C. Zones shows how tested materials inhibit pathogen growth on the cultured plates. To determine the antimicrobial viability, zones of inhibition were measured using a ruler and compared to the control. As the distance from the source increases, the concentration of antibiotics that diffuse into the media drops. Therefore, the clear bacterium-free zone that forms around the antibiotic-containing disc grows larger, making the bacteria more susceptible to that particular antibiotic. Therefore, assessing the effectiveness of the cream formulation with an ideal microorganism was facile. Amikacin and Nystatin were used as controls [17].

Quantitative analysis

Total viable count

Measuring the total count of aerobic mesophilic bacteria, along with yeast and fungi are necessary to ascertain the microbiological specification of the final cosmetic product. Blood agar plates, Macconkey agar plates, and Sabouraud agar plates (fungal) were used to determine the total viable count. For the detection, the medium was prepared and sterilized. The pH of the medium should be checked at the time of preparation and should be 7.2 to 7.4. Plates were inoculated within 15 minutes of preparation of suspension of the sample. A sterile cotton-wool swab was dipped into the suspension and surplus removed by rotation of the swab against the side of the tube above fluid level. The medium was inoculated by even streaking of the swab over the entire surface of the plates in three directions. Plates were incubated for 16 to 18 hours at 35 to 37°C aerobically or in CO_2 atmosphere for fastidious organisms [18].

Cell line and cell culuture

The Human Dermal Fibroblast (HDF) cell line was obtained from Hi Media Laboratories, India. The cells were kept at 37°C in a CO_2 incubator with DMEM-high glucose media supplemented with 10% FBS and 1% antibiotic-antimycotic solution. They were also sub cultured every two days in an environment of 5% CO_2, 18–20% O_2, and temperature.

Evaluation of cytotoxicity using MTT

A tetrazolium salt called 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) is used to measure the viability and proliferation of cells depending on mitochondrial efficiency. With an appropriate cell density of 20,000 cells per well using 200µl of cell suspension and no test agent, the cells were allowed to proliferate in a 96-well plate. The plates were then incubated for 24 hours at 37°C with 5% CO_2 in an atmosphere, after which the proper concentrations of the specified test agent were added. The MTT reagent was added to the plates at a final concentration of 0.5 mg/mL of total volume after the incubation period, during which the plates were taken out of the incubator and the spent media was separated. Again, the plates were incubated for 3 hours. Subsequently, 100 µL of dimethyl sulfoxide (DMSO) was added to replace the solution. With the use of an ELISA reader, the absorbance was measured at 570 nm. Cell viability was calculated using the below formula:

$$\% \text{ cell viability} = \frac{\text{Mean abs of treated cells}}{\text{Mean abs of untreated cells}} \times 100$$

RESULTS AND DISCUSSION

Pre- formulation study

Organoleptic characters

In this test colour, odour, homogeneity and state were observed until 30 days (Table 2). No change in organoleptic properties was seen [19]. Figure 1 represents the F1 and F2 formulations of the face cream.

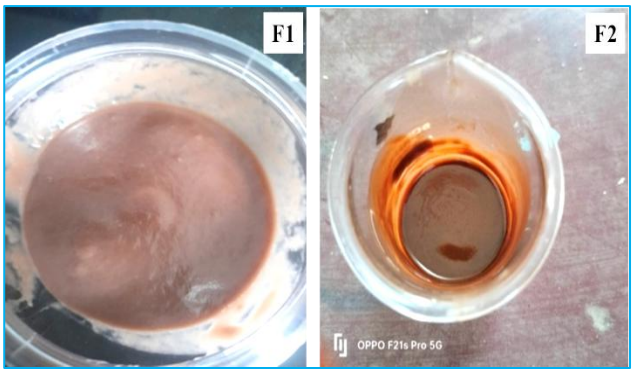

Fig 1 Formulation of the face cream F1 and F2

Table 2 Organoleptic characters of F1 and F2 formulations of the face cream

Organoleptic character	Day 1		Day 10		Day 20		Day 30	
	F1	F2	F1	F2	F1	F2	F1	F2
Colour	DB	DB	DB	DB	DB	DB	DB	DB
Odour	P	P	P	P	P	P	P	P
Homogeneity	S	S	S	S	S	S	S	S
State	SS	L	SS	L	SS	L	SS	L

DB - Dark Brown; P – Pleasant; S – Smooth; SS - Semi solid; L – Liquid

Study of pH: The pH was assessed using a pH meter, and the results are tabulated in (Table 3). The pH got altered in both formulations. This could be related to changes in the environmental condition [20].

Table 3 pH of F1 and F2 formulations of the face cream

Parameter	Day 1		Day 10		Day 20		Day 30	
pH	F1	F2	F1	F2	F1	F2	F1	F2
	5	6.5	5.15	6.5	5.2	6.56	5.5	6.4

Irritancy test

A 1 cm^2 zone was identified on the dorsal left surface. The time was recorded, and the cream was applied to the specified area. Erythema, swelling, and irritation were observed for up to 24 hours (Table 4). No irritancy, erythema and edema were found [21].

Table 4 Irritancy test of F1 and F2 formulations of the face cream

Parameter	Day 1		Day 10		Day 20		Day 30	
	F1	F2	F1	F2	F1	F2	F1	F2
Irritancy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Eythema	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Edema	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 5 Emollience of F1 and F2 formulations of the face cream

Parameter	Day 1		Day 10		Day 20		Day 30	
Greasiness	F1	F2	F1	F2	F1	F2	F1	F2
	Non-greasy	Non- greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy

Effect of emollience: Greasiness was checked and tabulated (Table 5). F1 and F2 formulations were found to be non- greasy.

Washability: Cream was applied on the skin and washed. Both the formulation of the cream was found to be easily washable (Table 6).

Table 6 Washability of F1 and F2 formulations of the face cream

Parameter	Day 1		Day 10		Day 20		Day 30	
Washability	F1	F2	F1	F2	F1	F2	F1	F2
	Easily washable	Easily washable	Easily washable	Easily washable	Easily washable	Easily washable	Easily washable	Easily washable

Phase separation: The prepared cream was stored at room temperature for 30 days in a covered container. Phase separation had been examined. The formulation F1 was found to be stable and F2 was found to be unstable (Table 7).

Table 7 Phase division of F1 and F2 formulations of the face cream

Parameter	Day 1		Day 10		Day 20		Day 30	
Phase division	F1	F2	F1	F2	F1	F2	F1	F2
	No separation	No separation	No separation	Separated	No separation	Separated	No separation	Separated

Spreadability

Spreadability of the face cream was checked (Table 8). The F2 formulation was found to have liquid like consistency it started to flow faster when placed between slides. But the spreadability of F1 formulation was found to be lower because of its solid or creamy consistency.

Table 8 Spreadability of F1 and F2 formulations of the face cream

Parameter	Day 1		Day 10		Day 20		Day 30	
Spreadability	F1	F2	F1	F2	F1	F2	F1	F2
	18	19.5	17	19	16	19	16.6	19.2

Stability testing

Cycle testing and centrifuge testing

Throughout the first centrifuge test, it was noted that the two formulations held their stability throughout a 30-minute centrifugation at 3000 rpm. The stability of the F1 formulation lasted until 30 days in both centrifuge and cycle testing. However, after 20 days of centrifuge testing, it was discovered that the F2 formulation separated into oil and water (i.e., it became a miscible solution). During the first cycle of the cycle testing, the F2 formulation remained stable. But during the second cycle on day 20, the F2 formulation of the face cream began to slightly separate. However, after the third cycle, the F2 formulation of the face cream became fully miscible. (Table 9) shows the results of the stability tests of the face cream. Thus, it was evident from the physical assessment of the F1 and F2 formulations of the manufactured face cream that the F1 formulation had passed every test that were carried out. Therefore, microbial analysis, total viable count of bacteria and fungi and cytotoxicity evaluation were performed for F1 formulation [22-24].

Table 9 Cycle test and centrifuge tests of F1 and F2 formulations of the face cream

Parameter	Day 1		Day 10		Day 20		Day 30	
Cycle test	F1	F2	F1	F2	F1	F2	F1	F2
	Stable	Stable	Stable	Stable	Stable	Unstable	Stable	Unstable
Centrifuge test	F1	F2	F1	F2	F1	F2	F1	F2
	Stable	Stable	Stable	Stable	Stable	Unstable	Stable	Unstable

Qualitative analysis

Microbial analysis

The susceptibility and resistance of our prepared face cream to bacteria and fungus were investigated in this study and

juxtaposed with the typical zone of inhibition of the control. (Fig 2, Table 10) shows the zone of inhibition of F1 formulation of the cream against *Candida albicans, Bacillus subtilis, Klebsiella pneumonia, and Pseudomonas aeroginosa.* Comparison revealed a high zone of inhibition value for our developed cream against *Pseudomonas aeroginosa* than *Klebsiella pneumonia* and *Bacillus subtilis*. Additionally, the results for *Klebsiella pneumonia* and *Bacillus subtilis* were in the moderate range. *Pseudomonas aeroginosa*has the potential for triggering infections in people's eyes and skin. It is undesirable in cosmetic products due to its potential pathogenicity and ability to alter the physiochemical properties of the cosmetic formula. The prepared F1 formulation of the face cream had been found to be more effective against *Pseudomonas aeroginosa*. Due to the high activity of the cream against *Pseudomonas aeroginosa*, it can effectively inhibit the proliferation of this bacteria. It also turned out that *Candida albicans* was sensitive to the face cream [25-26].

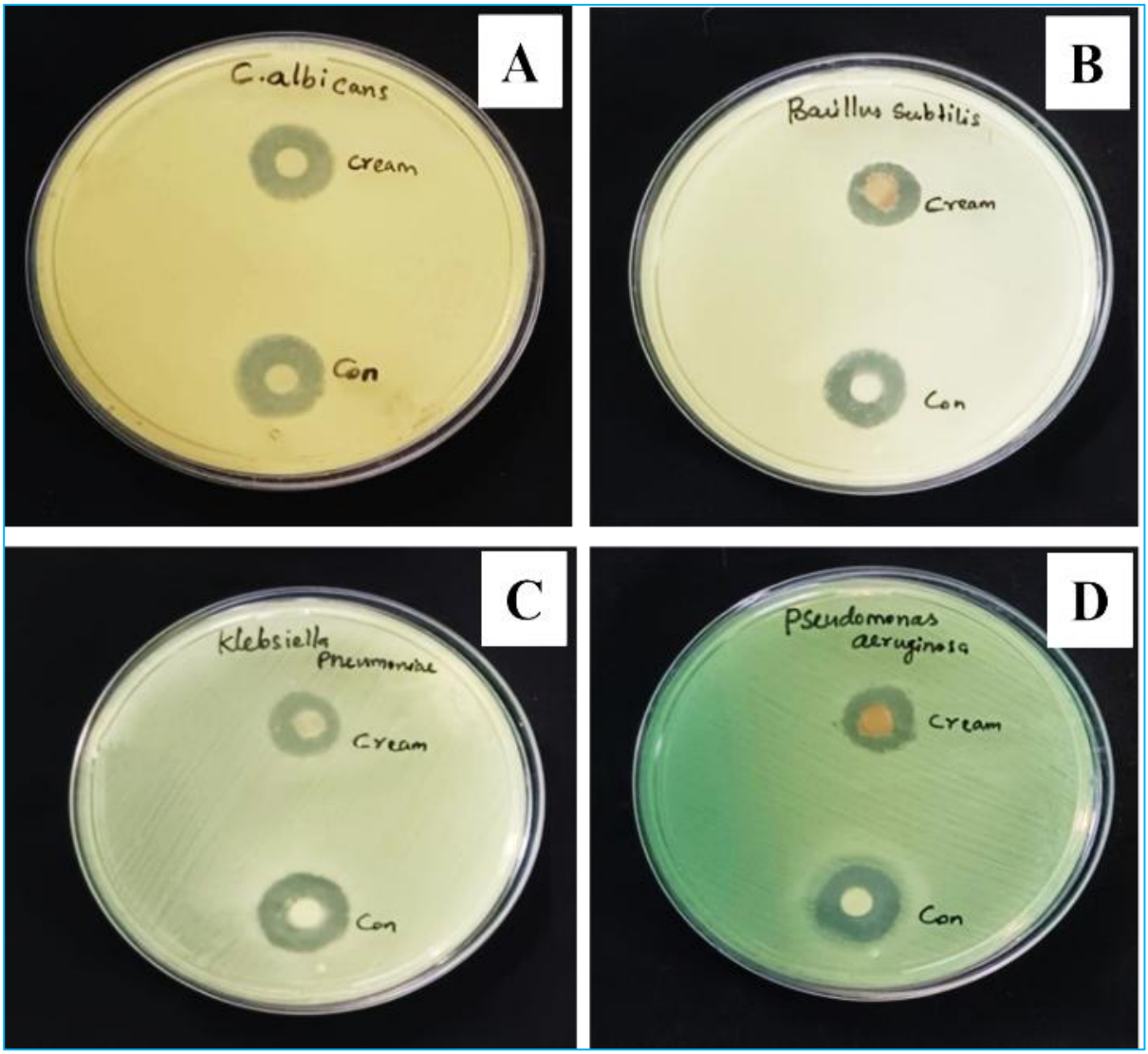

Fig 2 Antimicrobial zone of inhibition activity of F1 formulation against (A) *Candida albican* (B) *Bacillus subtilis* (C) *Klebsiella pneumonia* and (D) *Pseudomonas aeroginosa*

Table 10 Microbial study of F2 formulation of the face cream

Fungi	Cream	Control (Nystatin)
Candida albicans	16 mm	17mm
Bacteria		Control (Amikacin)
Klebsiella pneumonia	14 mm	18 mm
Bacillus subtilis	14 mm	16 mm
Pseudomonas aeruginosa	17 mm	17 mm

Quantitative analysis

Total viable count

Total viable count commonly known as standard plate count or aerobic plate count is a laboratory technique used to investigate the total amount of bacterial or yeast growth in the given sample (Fig 3). Using this technique, colonies were counted (plate count) on a non-selective agar medium. It became necessitate to neutralise the sample's potential to hinder microbial growth inorder to detect live microorganisms. The smear report shows no pus-cells and no bacteria [27].

During the fungus smear, there was no budding yeast like organisms and no fungal filaments were seen. At the final detection the sample showed no bacterial and fungal growth. For the cosmetic cream it is compulsory to ensure that the prepared formulation is free from harmful pathogenic growth. Hence, the results of the total viable count stands as a testimony to the credibility of the prepared formulation. There was negligible bacterial or yeast growth in the formulation [28].

Cytotoxicity effects of face cream on HDF cells

The MTT test was used to assess the toxicity of face cream on normal human dermal fibroblast cells (NHDF) in

vitro. The purpose of the cytotoxicity assay was to evaluate the toxicity of face cream and determine the ideal concentrations required for the correct usage of the face cream. When yellow MTT is converted to purple formazan in the mitochondria of living cells, the MTT cell survival experiment was used to calculate the relative survival cells. According to the results of the cytotoxicity study conducted using the MTT assay, the test substance, cream was shown to be non-toxic to NHDF following a 24-hour incubation period. (Fig 4) shows the % cell viability values of normal human dermal fibroblasts (NHDF) cells treated with different concentrations of cream after the incubation period of 24 hours [29-30].

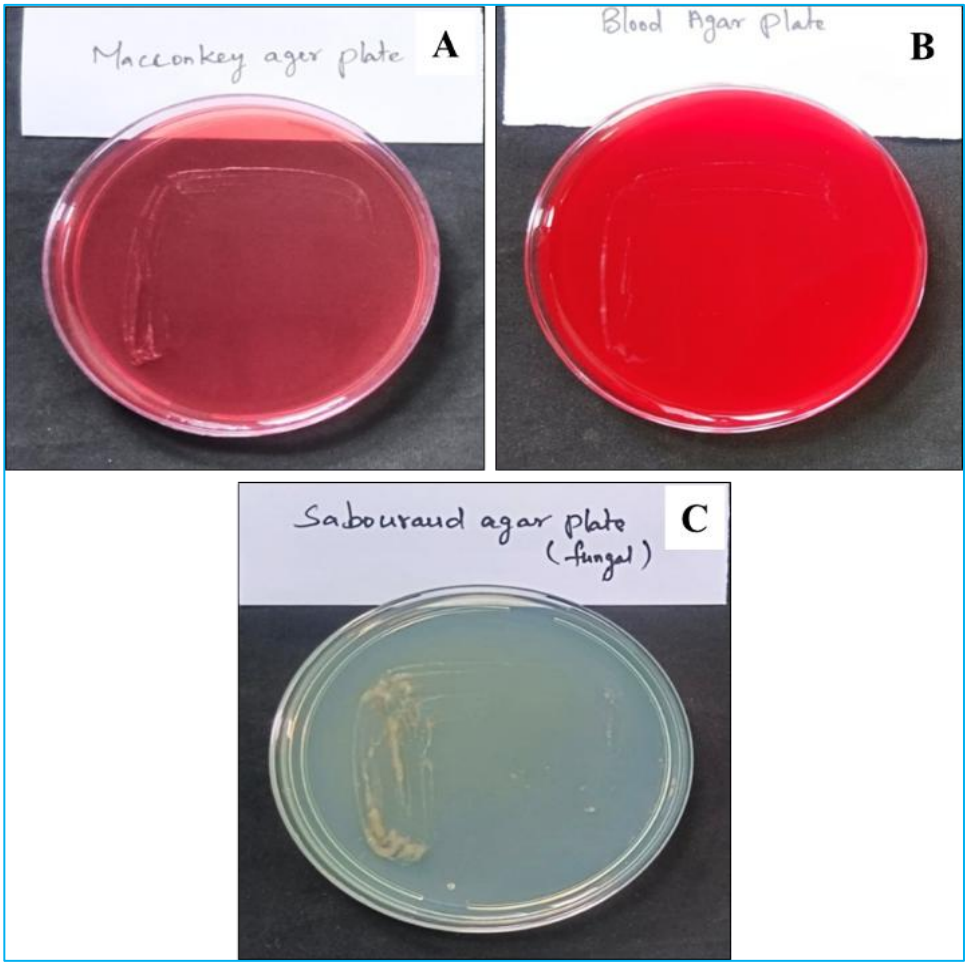

Fig 3 Total viable count of F1 formulation in (A) macconkey agar plate (B) blood agar plate and (C) Sabouraud agar plate

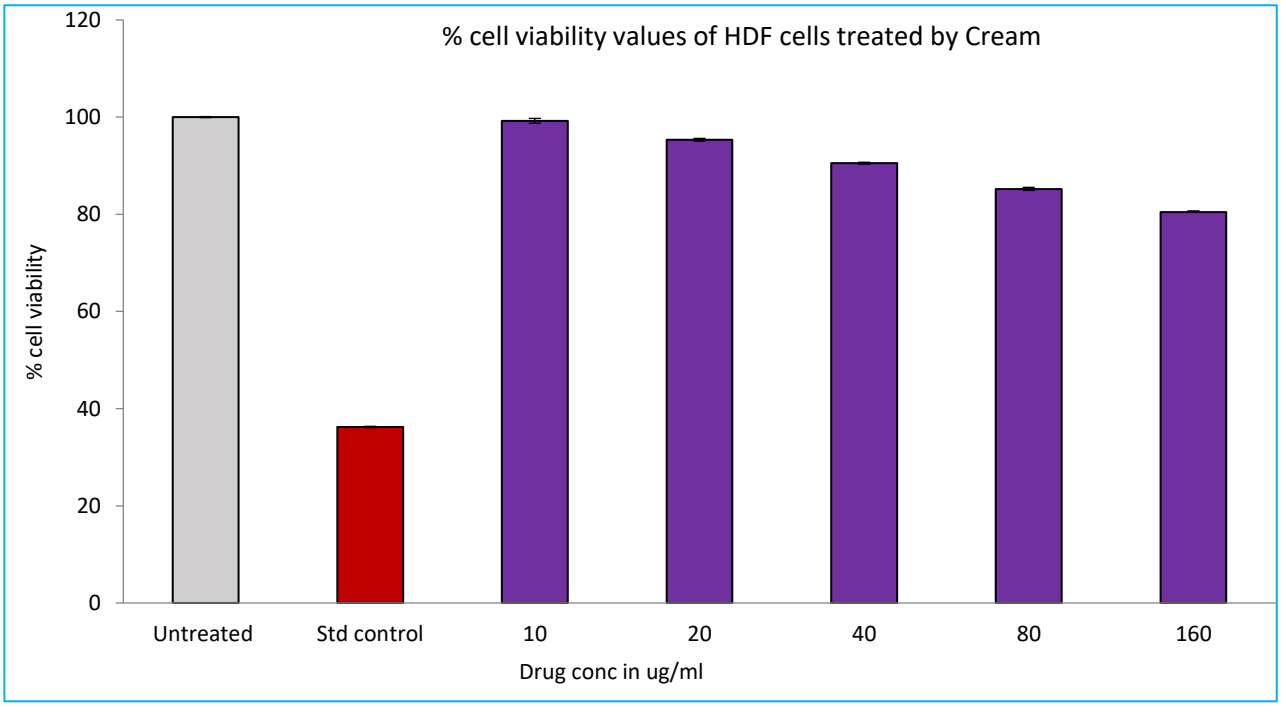

Fig 4 % cell viability values of HDF cells treated with different concentrations of cream after the incubation period of 24 hrs. Doxorubicin with 1ug/ml was used as toxic control and cell without treatment was considered as control

CONCLUSION

Myristica fragrans shells, *Juglans regia* shells, carboxymethyl cellulose and other ingredients were blended to make a face cream. Since oil droplets were distributed in the water phase, an oil- in-water (O/W) emulsion was ensured for the face cream. The two face cream formulations (F1 and F2) were assessed. It emerged that the F2 formulation was inadequate for use as a face cream owing to its state of liquidity, phase separation into an oil and water layer, and fragility during stability inquiries. When the parameters under examination persisted within dominant ranges and did not considerably change, the F1 formulation implied formidable physical stability aside from spreadability, which favoured the F2 formulation. The F2 formulation's liquid fluidity resulted from the emulsifier (carboxymethyl cellulose) being used in a reduced quantity. Emulsifiers were used to blend water and oils into a homogeneous mixture for cream. It lessens the tension that exists between two immiscible liquids. Increased emulsifier content produced a creamy consistency and good stability. There were no tweaks to the formulation. In contrast to the F2 formulation, F1 had superior quality. Owing to its exceptional qualities, the F1 formulation had been selected as the best face cream formula. For the optimal formulation of F1, the total viable count, cytotoxicity evaluation, microbiological tests for bacterial and fungal growth were conducted. According to the results, face cream exhibited high action against *Pseudomonas aeroginosa*, and there was no bacterial, mold and yeast growth were seen. Based on the results of the MTT assay, the face cream was found to be non-toxic to human dermal fibroblast cells following a 24-hour incubation period. A maximum dose of 160 ug/ml was found to be a safer level, with 80% cell viability. Finally, from the results obtained, it was concluded that *Myristica fragrans* and *Juglans regia* shells worked well as face cream since they have strong antibacterial qualities, stability and non-toxicity. Once applied, the cream left the skin feeling really pleasant with a noticeable radiance and moisturizing effect. This study revealed the face cream's potential for moisturizing, with ongoing tests the long-term effectiveness of the cream can be proved in future studies.

Conflicts of interest

The authors declare that they have no potential conflicts of interest.

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