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Research Article

Formulation of Anti-Acne Gel Containing *Citrus aurantifolia* (Christm.) and *Aloe barbadensis* (L.) Extracts and Evaluating The Impact of High-Pressure and Microwave Processing

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ABSTRACT

Acne is one of the most prevailing skin disorders caused by bacteria, dead skin cells, and oil clogging of hair follicles. In this study, a polyherbal anti-acne gel containing *C. aurantifolia* and *A. barbadensis* extracts was developed as a cosmeceutical. Major bioactive fractions in the plant extracts were evaluated by gas chromatography-mass spectrometry (GCMS) analysis. Moreover, *C. aurantifolia* depicted higher total phenolic, flavonoids, tannins, and ascorbic acid than *A. barbadensis* extract. The anti-acne gel was prepared by adding 1% of both plant extracts and evaluated for organoleptic properties (color, odor, homogeneity, consistency, washability), spreadability, viscosity, extrudability, pH, and drug contents and compared with a commercial herbal formulation (NuTeen®). The developed gels depicted greater inhibition of *Staphylococcus aureus* than the commercial formulation with a growth inhibition diameter of 2.40 mm. In-vitro permeation of plant extracts from a gel into phosphate buffer was found at 27.4% after 2.5 hr, and the release behavior was best explained by the Higuchi model (R^2=0.97). Finally, for the possible replacement of paraben (synthetic preservative) from the gel, high-pressure processing (600 MPa, 120 s), and microwave pasteurization (700 W, 80 s) were adopted and the stability of gels was evaluated after 4 weeks, and found comparable to their paraben-containing normal counterpart.

Key words: Antimicrobial, cosmeceutical, pasteurization, polyherbal gel, stability

INTRODUCTION

With the growing consciousness of safety among the population, natural cosmeceuticals are prevailing in the sector of personal care products. In this regard, novel formulations including gels, creams, lotions, and foams, etc. are developed using natural herbal-based bioactive ingredients (Hin *et al*., 2023). Herbal cosmetics besides being cheaper and equally effective to synthetic counterparts are considered much safer and possess minimum-to-null side effects (Daneluz *et al*., 2020). These herbal cosmeceuticals are proven good against mild-to-intermediate issues related to skin care (Varma *et al*., 2014; Borse *et al*., 2020). Amongst the most prevailing cosmeceuticals are the ones designed for the treatment of acne vulgaris.

Acne vulgaris, minor to severe, is one of the most prevalent issues among the population of all ages. According to Borse *et al*. (2020), bacteria like *Cutibacterium acnes, Staphylococcus epidermidis*, and *Staphylococcus aureus*, besides the dead skin cells and oils, clog hair follicles and result in acne vulgaris. According to Sharma & Dev (2018), this causes nodules, papules, pustules, blackheads, and whiteheads on the skin. One of the main contributing factors is the hormonal imbalance that occurs during puberty and is produced by the adrenal glands in both genders. However, among adolescents, its prevalence rate is assessed to vary from 35% to >90% (Wolkenstein *et al*., 2018).

Most of those affected are teenagers, as it interferes with their daily activities and causes them to lose confidence in their appearance, hence finding a solution for their acne. According to Hou *et al*. (2019), the rising frequency of using different antibiotics for skin care purposes can result in the development of antibiotic resistance as well as several mild-to-severe side effects, such as erythema, photosensitivity, allergic dermatitis, excessive skin irritation, urinary problems, joint and muscle pain, headache, and depression. Hence, medicinal plants could be considered an alternate acne vulgaris treatment.

Previous research has shown that *C. aurantifolia* juice is an efficient antimicrobial in treating two bacteria that cause acne: *S. epidermidis* and *C. acnes* (Aini *et al*., 2018). On the other hand, using *C. aurantifolia* juice topically right away is impractical due to a higher acidity (pH<3.0) and free-flowing fluid nature. To mitigate this issue, *C. aurantifolia* juice can be developed into a gel by employing a synthetic polymer called Carbopol-940, which is composed of cross-linked carbomers that create a microgel structure. Unlike lotion and cream, a gel is a semisolid preparation that is easier to apply to the skin. Due to its less

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sticky nature, the cream and lotion are rapidly washed off the skin, thus limiting the bioavailability of bioactive agents (Varma *et al*., 2014). Hence, using polymeric fractions in pharma gels can control the release of desired active ingredients and prolong the contact time with the skin (Dantas *et al*., 2016). It was previously determined that *C. aurantifolia* extract was the sole ingredient in an anti-acne gel designed to treat acne brought on by *C. acnes* and *S. epidermidis* (Kusuma *et al*., 2018). However, the pure extract showed lower antimicrobial activity compared to the gel. Nonetheless, synergistic effects have been reported when more than one bioactive components are employed in any antimicrobial formulation (Iyer *et al*., 2021; Hin *et al*., 2023). Thus, a single extract from a plant might not deploy the desired therapeutic outcomes based on the meagreness of their bioactive fractions and developed formulation (Kola-Mustapha *et al*., 2020).

The juice of *C. aurantifolia* contains bioactive substances such as tannins, flavonoids, and phenol, etc. (Adebayo-tayo *et al*., 2016). Similarly, bioactive secondary metabolites including phenols, flavonoids, and tannins are also present in *A. barbadensis* gel (Manna & Rudra, 2020). According to Nejatzadeh-Barandozi (2013), its secondary metabolites provide anti-inflammatory, antibacterial, antioxidant, immune-boosting, anticancer, anti-diabetic, anti-aging, and sunburn-relieving properties. Additionally, *A. barbadensis* contains lignin, saponin, salicylic acid, sterols, triterpenoids, vitamins, minerals, phenolic compounds, and organic acids (Benzidia *et al*., 2019).

Therefore, for a potentially greater therapeutic impact, it would be desirable to produce *C. aurantifolia* and *A. barbadensis* extracts based on gel formulation that can treat acne vulgaris. Hence, the objective of this study was to develop a natural gel formulation containing *C. aurantifolia* and *A. barbadensis* extracts. Moreover, the gels prepared without paraben (a potential preservative) were treated by high-pressure processing and microwave pasteurization treatment to investigate the possible replacement of parabens and the impact of the treatment on the gel's physical stability was investigated.

MATERIALS AND METHODS

Materials

Citrus aurantifolia fruits and *A. barbadensis* leaves were locally harvested and employed for extract preparation according to our published study (Hin *et al*., 2023). The excipients such as Carbopol-940 $(C_3H_4O_2)n$, propylene glycol-400, methylparaben, propylparaben, and triethanolamine were purchased from the online store HairDuta, Malaysia. A commercial herbal formulation, NuTeen® was purchased from Guardian Pharmacy Store, Malaysia. All the chemicals used in this study were of analytical grade.

Qualitative analysis of phytoconstituents by GCMS

A crude extract (3 mL) was mixed with 10 mL hexane and stirred using a vortex mixer for 25 sec. The hexane layer was separated from the aqueous layer and then heated in an oil bath for 10 min at 70°C. The sample was dried over anhydrous sodium sulfate and kept at 4°C (Ahmed *et al*., 2019). A gas chromatograph-mass spectrometer (Agilent 7890, USA) was used to analyze plant extracts equipped with an HP-5ms column (30 m x 0.25 mm ID, film thickness 0.25 m) using helium as a carrier gas. The temperature of the column oven was maintained from 60 to 210°C at a rate of 3°C min^{-1}; followed by 210 to 240°C at a rate of 20°C min^{-1}, held at 240°C for 8 min, with injector and detector temperatures of 280°C and 290°C, respectively. The helium gas was flowing at a rate of 1 L min^{-1}. A split ratio of 1:50 was used to inject 1.0 µL of extract and the mass spectra covering the full scan mode of m/z 40–650 were collected. Each compound was identified by matching its mass spectra to the online National Institute of Standards and Technology (NIST) library.

Quantitative analysis of phytoconstituents

All plant extracts were quantitatively evaluated for their phytoconstituents. The compounds such as phenols, tannins, flavonoids, and ascorbic acid were analyzed using published methods (Hin *et al*., 2023).

Preparation of gels

The gels were made using 1% of extracts of both plants. Formulation batches were prepared according to the composition shown in Table 1.

Table 1. The composition of the developed herbal formulation containing plant extracts

Ingredients	Amount
C. aurantifolia extract	1%
A. barbadensis extract	1%
Carbopol-940	1%
Methylparaben	0.1%
Propylparaben	0.1%
Propylene glycol- 400	2%
Triethanolamine	q.s
Water	q.s

*q.s= quantum satis (quantity sufficient for a given volume)

The gel was prepared according to a previously published method (Chandrasekar & Kumar, 2020). The plant extracts namely, *C. aurantifolia* and *A. barbadensis* were added at 1% due to our previous optimization experiment. Paraben (0.1%) as a preservative was dissolved in a separate beaker of distilled water. The plant extracts were added to the mixture and Carbopol-940 solution (1%) was also added and mixed thoroughly. Next, the polypropylene glycol-400 (2%) (as a humectant) and triethanolamine (as a stabilizer) were added and the pH of the gel was adjusted to near 7.0. The addition of distilled water

was made to make the total volume of 50 mL. To eliminate bubbles, the formulated gels were kept at room temperature for 24 hr (Chandrasekar & Kumar, 2020).

Organoleptic evaluation of gels

The formulated gel was evaluated visually for color against a white background. The odor was perceived by smelling the gels after dissolving in the distilled water (Borse *et al*., 2020). Homogeneity and consistency were checked by applying the gels to a transparent glass plate. The presence of particles was examined with the naked eye (Kumar & Eswaraiah, 2020). A gel is considered homogenous if the color is uniform and there are no lumps, whereas a gel is in good consistency if it spreads over the skin with ample force. The washability was estimated by the ease and extent of washing off from the skin under running tap water (Keshri, 2020).

pH

The pH of the gel was measured with a calibrated digital pH meter at ambient temperature.

Spreadability

The spreadability was determined by measuring the spreading diameter of the gel across two rectangular glass plates (2.54 cm x 7.62 cm). The gel was applied on a circle of 2 cm diameter marked on the glass plate and the second glass plate was placed over it. For 5 min, a 500 g weight was applied to the upper glass plate. The diameter of the gel circle was measured at ambient conditions using a Vernier caliper after removing the applied weight (Daryab *et al*., 2022).

Viscosity

A Brookfield viscometer (DV-II+, USA) with a stainless steel spindle (C S93) spinning at 5-50 rpm was used to estimate the viscosity of the gels at ambient conditions.

Extrudability

The gel was filled in an aluminum collapsible tube and sealed, and the weight of the tube was recorded. Between two glass slides, the tube was positioned and clamped at room temperature. After placing a 500 g weight over the glass the cap was opened. The amount of extruded gel was collected and weighed. The percentage of extruded formulated gel was calculated; and grades of excellent, good, and fair were assigned (Borse *et al*., 2020).

Drug content of gels

By comparing the blank, 1 g of formulated gel was dissolved in 100% methanol in a 50 mL volumetric flask. Then, 5 mL of this solution was further diluted to 25 mL with 100% methanol. The drug content (Aloin as a major polyphenolic fraction) was measured at 280 nm using a UV-Vis spectrophotometer according to Borse *et al*. (2020). The percentage of drug content in the gel was calculated by comparing the absorbance of the gel to the slope of the standard curve of the methanol extract of plants.

Antibacterial activity of gels

The antibacterial activity of the gel was evaluated according to Mate *et al*. (2021) with minor modifications. In a sterile petri dish (8.5 cm i.d.), 20 mL of molten Mueller Hinton agar was poured having *S. aureus* (1 x 10^8 CFU/mL), and allowed to harden. The wells of 6 mm were drilled in the agar with a sterile cork borer filled with 20 µL of diluted gel formulation (250 mg/mL), and finally incubated for 24 hr at 37°C. The diameter (mm) of bacterial growth inhibition was measured using a Vernier caliper.

Particle size measurement

A particle size analyzer (Litesizer 500, Anton Paar, Austria) was employed to estimate the size distribution of gel particles at ambient conditions according to Bhattacharyya & Reddy (2019). Almost 500 mg of the gel was diluted with 5 mL distilled water and analyzed in the range of 0.10 to 10000 nm with a 10 sec equilibration time for each run. The mean particle size and polydispersity index of the sample were determined.

Gel permeation and kinetic analysis

The permeation of gel formulation was carried out using a Franz glass diffusion cell according to Prabhakar (2020) with slight modifications. The receptor compartment was filled with 5.0 mL of pH 7.4 phosphate buffer, covering 0.64 cm^2 of the effective diffusional area to maintain a sink condition. The temperature of the receptor compartment was maintained at 37°C by a thermostat bath and stirred at 200 RPM continuously with a magnetic stirrer. The cellulose acetate membrane was used as the diffusion membrane. A syringe was used to inject 0.1 mL of the gel formulation onto the cellulose acetate membrane. At various time intervals i.e., 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50 hr, 0.3 mL aliquot was removed, and analyzed at 280 nm using UV-visible spectrophotometer and immediately replaced with the same volume of fresh buffer solution. The drug permeation kinetics was estimated by using different models such as zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas (Table 2).

Table 2. Permeation kinetic model equations (Iyer *et al*., 2021)

Permeation kinetic model	Equation	Description
Zero-order	$Ct = C_0 + kt$	C_t: Concentration at time t, C_0: Initial concentration, k: Rate constant
First order	$dc/dt = k$	dc/dt: Rate of change of drug, k: Rate constant
Higuchi	$Qt = kt^{1/2}$	Q_t: Amount released at time t, k: Rate constant
Hixson-Crowell	$Q_0^{1/3} - Qt^{1/3} = kt$	Q_t: Amount released at time t, Q_0: Total amount of drug, k: Rate constant
Korsmeyer-Peppas	$Mt/ M\infty^n = kt^n$	Mt/m∞: Fraction of drug released at time t k: Rate constant, n: Release or diffusion exponent

High hydrostatic pressure processing

The gel was treated using high-pressure processing equipment (Hiperbaric, Spain) according to Dang (2021). About 50 g of gel was sealed in the polyethylene bottle and placed in a vessel and distilled water was the pressurizing medium in the chamber. The gel was subjected to a constant pressure of 600 MPa for 2 min of treatment time (excluding pressure rise & decompression intervals). The automated process was conducted at ambient temperature and a thermocouple was used to track the temperature of the pressure medium in the vessel. After treatment, the gels were stored in amber jars at room temperature for 4 weeks.

Microwave pasteurization

Microwave pasteurization was carried out using a microwave oven (Electrolux EMS2540X, Sweden) according to Baroyi (2022). About 50 mL of the gel was put in a 100 mL beaker and microwaved at 700 W until the gel reached 71°C. The time it took for the gel to reach the pasteurization temperature was 80 s. The gel was stored in an amber jar at ambient temperature and analyzed after 4 weeks. After treatments, the physical properties and storage stability of the treated formulated gels were evaluated.

Statistical analysis

The data was presented as the mean ± SD of triplicate results. All the data was statistically evaluated using SAS 9.4 studio software. The significance of the data was determined using the student's t-test and the results were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Qualitative analysis of phytoconstituents by GCMS

The chromatogram of *C. aurantifolia* showed a total of 50 peaks and 78 different compounds were found (Figure 1).

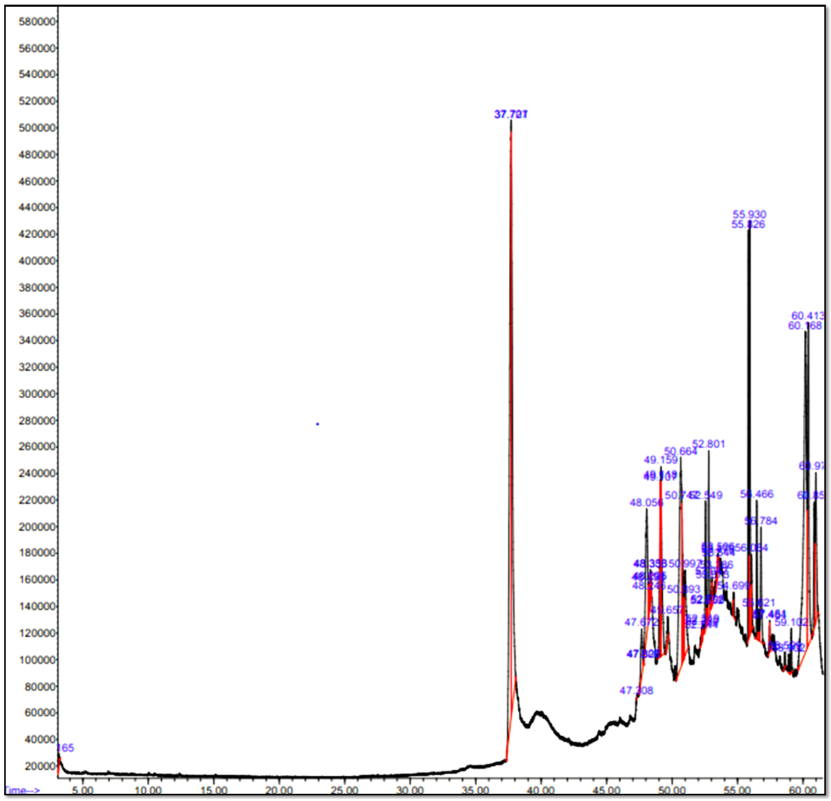

Fig. 1. Chromatogram of *C. aurantifolia* juice extract.

The compounds such as 9-tetradecenal (14.7%), 2-methyl-z,z-3,13-octadecadienol (14.7%), 2-methyl-e,e-3,13-octadecadien-1-ol (14.7%) were recognized as the main compounds followed by 13-hexyloxacyclotridec-10-en-2-one (14.63%), 2-hydroxy-1-(hydroxymethyl) ethyl ester (7.74%), 13-octadecenal (7.74%), oleic acid (4.12%), cis-vaccenic acid (4.12%), cis-13-octadecenoic acid (4.12%), octadecanoic acid (3.92%), 9,17-octadecadienal (3.04%), 2-methyl-z,z-3,13-octadecadienol (2.54%), 1,19-eicosadiene (2.54%), methyl 3-cis,9-cis,12-cisoctadecatrienoate (1.89%), 4-(2-oxo-oxazolidin-4-yl)-piperazin-1-carboxylic acid, ethyl ester (1.72%) and 9,12-octadecadienoic acid (z,z)- (1.14%).

The detected compounds have been reported to possess antibacterial and antifungal activities (Hameed *et al*., 2018). Octadecenoic acid and oleic acid were also present in methanolic extracts of *C. aurantifolia* as reported by Hameed *et al*. (2018). While flavoxate (11.15%), bicyclo[5.3.1]undecane-11-one (11.15%), palmitic anhydride (6.72%), 15-hydroxypentadecanoic acid (6.72%), 2-hydroxy-cyclopentadecanone (5.08%), 2-octylcyclopropaneoctanal (5.08%), bicyclo[10.10]tridec-1-ene (4.03%), methyl 9,12-heptadecadienoate (2.05%), cyclopropane octanal (1.96%) were detected as unknown compounds. The rest of the compounds were found in less than 1% of the extract.

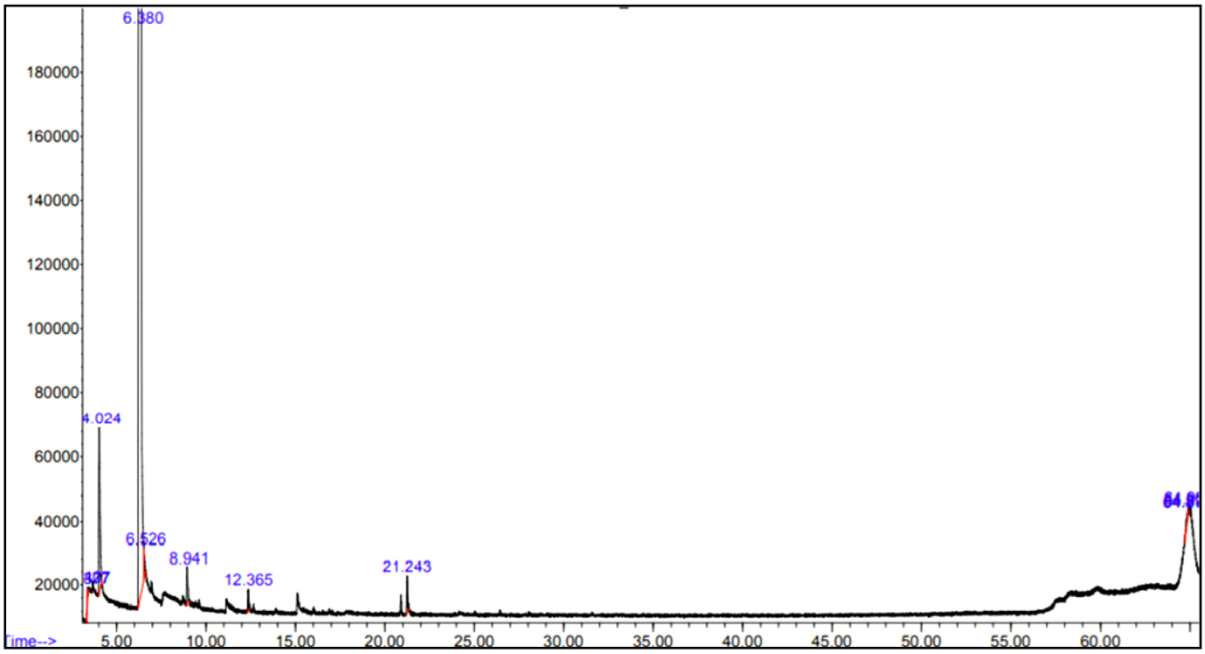

Fig. 2. Chromatogram of *A. barbadensis* leaf gel extract**.**

On the other hand, the chromatograph of *A. barbadensis* extract showed a total of 11 peaks, and 12 different compounds were found (Figure 2). The dihydro-3-methylene-2,5-furandione (96.66%) was recognized as the main compound followed by maleic anhydride (1.92%), methyl cyclobutene (0.39%), 1,2– benzenedicarboxylic acid (0.35%), phthalic anhydride (0.35%), bicyclo [4.2.0] octa-1,3,5-triene-7,8-dione (0.35%), cyclopentene (0.18%), 3,3-dimethyl-2-butanamine (0.12%), N-methyl-1-octadecanamine (0.12%) and hexahydro-1-methy-2H-azepin-2-one (0.01%). These fractions are known compounds and were reported for antibacterial and antifungal activities. The compounds of 1,2–benzenedicarboxylic acid and N-methyl-1-octadecanamine were also present in the previous findings (Bawankar *et al*., 2013; Saljooghianpour & Javaran, 2013). Results showed that different soil types with different nutritional values and chemical compositions have an impact on the chemical composition of plants' bioactive compounds.

Quantitative evaluation of plant extracts

Total phenolic compounds, total tannins, total flavonoids, and ascorbic acid were estimated according to our previous study (Hin *et al*., 2023) and the results are tabulated in Table 3.

Table 3. Secondary metabolites in the *C. aurantifolia* and *A. barbadensis* extracts

Plant extracts	Phenolics (µg GAE/mg extract)	Tannins (µg TAE/mg extract)	Flavonoids (%)	Ascorbic acid (mg/100mL)
C. aurantifolia	58.72 ± 0.021	2.02 ± 0.01	9.83 ± 0.83	48.0 ± 0.10
A. barbadensis	48.27 ± 0.010	2.07 ± 0.01	0.57 ± 0.23	12.8 ± 0.10

*GAE: gallic acid equivalent, TAE: tannic acid equivalent

A higher total phenolic content was observed in *C. aurantifolia* than in *A. barbadensis*. Phenolic compounds contribute to antimicrobial activity due to their inherent ability to denature the cell membrane of acid-resistant bacteria (Halla *et al*., 2018). Similarly, the total tannin content in *C. aurantifolia* was 2.02 µg TAE/mg whereas *A. barbadensis* showed a bit higher value of 2.07 µg TAE/mg. Tannins present antibacterial activity by hindering the development of cell proteins (Gurning *et al*., 2021). Besides, they scavenge free radicals, reduce inflammation, and remove excess oil from pores without causing skin irritation. The mean values of total flavonoid content in both extracts showed a greater difference with respective values of 9.82% and 0.567% for *C. aurantifolia* and *A. barbadensis*, respectively. Flavonoids have been reported for effective antioxidant, anti-allergic, anti-

inflammatory, and antimicrobial activities (Kusuma *et al*., 2018). Ascorbic acid was 4-times greater in *C. aurantifolia* than the *A. barbadensis* which was 12.8 mg/mL. The ascorbic acid holds diversified biological activities including antioxidant, anti-aging, photoprotection, and anti-pigmentation of the skin (Chen *et al*., 2021). The phytoconstituents greatly varied in both extracts which could be attributed to the different plant species, agronomic factors, extraction conditions such as solvent and time and temperature, etc. (Ehiobu *et al*., 2021).

Evaluation of gels

Organoleptic analysis

The formulated gel with 1% of both the plant extracts was evaluated for physicochemical properties and compared with a commercial herbal formulation. The developed formulation was colorless, and odorless, with good phase stability, washability, and good homogeneity (Table 4). Contrary to this, the commercial counterpart was brownish and scented. The brown coloration of the commercial formulation could be attributed to the presence of yellowish Thanakha paste. Besides, the commercial formulation also showed a typical tree-bark aroma. Though color and aroma are the key attributes considered to formulate a commercial cosmeceutical product, synthetic fragrance-free formulation minimize the risk of allergic reactions due to prolonged exposure to the skin. The developed gel and the commercial herbal formulation were found homogenous and lump-free indicating an even dispersion of the plant extracts within the gel body (Rahmawati & Setiawan, 2019). The gel consistency is a crucial parameter in defining the topical application of cosmeceutical products and, hence, a uniform thin layer formation of gel is preferred for good absorption of the bioactive ingredients (Al-Suwayeh *et al*., 2014). The washable nature of the gel is a key concern for halal cosmeceuticals to ensure a complete removal for performing Islamic rituals. Thus, for this purpose, Carbopol-940, an aqua-soluble and washable carbomer, was considered as a gel foundation (Kusuma *et al*., 2018).

Table 4. Comparative evaluation of the formulated and commercial herbal formulation

Parameters	Formulated	Commercial Herbal Formulation
Color	Colourless	Brownish
Odor	No	Aromatic
Homogeneity	Uniform	Uniform
Phase separation	No	No
Consistency	Good	Good
Washability	Good	Good
pH	6.83 ± 0.03	6.01 ± 0.01
Spreadability (mm)	10.76 ± 0.51	14.3 ± 0.23
Viscosity (cP)	7717 ± 90	7240 ± 69
Extrudability (%)	80.88 ± 0.17 (Good)	92.63 ± 0.18 (Excellent)
Drug contents (%)	90.78 ± 3.10	98.58 ± 2.70
Antibacterial activity (inhibition zone, mm)	2.40	1.79

*cP: centipoise

Spreadability

Spreadability is also an interesting parameter to ensure sufficient applicability on the skin. The formulated gels with extracts depicted a spreadability value of 10.38 mm, significantly lower than the commercial counterpart with a value of 15.97 mm (Table 4). This increased spreadability could be correlated to the lower concentration of Carbopol-940 in the commercial herbal formulation as observed in the composition. Nonetheless, the spreadability should be optimized for the desired application and results. Thus, the formulated gel and the commercial formulation are optimized for ample therapeutic efficiency and enhanced bioavailability for topical application (Kola-Mustapha *et al*., 2020).

Viscosity

The viscosity is the direct indicator of the resistance to flow, and it varies with the change in the composition and additives. It was noticed that with increasing the shear (5 to 50 RPM) the viscosity reduced (data not shown) due to the shear thinning behavior of the gels. The formulated gels showed relatively higher viscosity (7717 cP) as compared to the commercial herbal formulation (7240 cP) at 50 RPM, however, it remained statistically similar (Table 4). Nevertheless, the presence of lower carbomer concentration might result in lower viscosity of the latter. The disturbance of the polymeric gel body and chain orientation in the direction of applied shear reduced the viscosity of the formulations (Daneluz *et al*., 2020). The pseudoplastic nature of the gel is preferred which will provide a lower flow resistance upon application to the skin. However, the viscosity of both gels was ample enough and did not depict any runoff during and post-application. Previously, viscosity and spreadability were negatively correlated (R^2= -0.97875) (Okafo *et al*., 2022) and a similar trend was observed in the current study.

Extrudability

Gel extrusion from the tube (packing) is crucial for application compliance and ease of use. The extrudability should not be so low that it may not extrude out from the tube upon compression. Simultaneously, it should not be so high that it automatically flows out with minor compression once stacked post-filling and during storage processes. The formulated gel depicted an 80.1% extrudability value which was lower than the commercial herbal formulation with 92.5% (Table 4). The observed value is in a similar range as reported previously by Borse *et al*. (2020). The presence of a higher concentration of emollient may result in greater extrudability (Varma *et al*., 2014).

pH

The extracts from plant *C. aurantifolia* and *A. barbadensis* showed a pH of 2.74 and 6.07, respectively, and to adjust the pH to 6.84 the formulation triethanolamine was incorporated as a pH adjuster. On the other hand, the commercial herbal formulation depicted a pH of 5.99 (Table 4). Hence, a pH near neutral is preferred for topical applications. The developed gel and commercial herbal formulations are suitable for human skin. The human skin flora is disrupted once the pH is raised towards basic which lowers the production of antimicrobial peptides (Kusuma *et al*., 2018) and supports healthy skin.

Drug contents

The drug content (Aloin) of the formulated gel was 90.78% and of the commercial herbal gel, it was 98.58% (Table 4). The higher drug contents could be due to its lower amount of carbomer compared to the formulation developed using the *C. aurantifolia* and *A. barbadensis* extracts. Results indicated that the drug was distributed evenly throughout the gel body as of the commercial herbal formulation. The polymeric excipients employed in the development of the formulation play a significant role in encapsulating the bioactive agents. However, the lower drug content of formulated gels could be attributed to the volatility and escape of some fractions during preparation and storage which ultimately lead to a relatively lower percentage of the drug. However, to improve drug loading and encapsulation, nano- or micro-carriers are used in formulations that enhance the bioactivity, and antimicrobial effectiveness of gels (Iyer *et al*., 2021). It is reported that aloin has antimicrobial and antioxidant activity which impart functionality to the developed gels based on the *C. aurantifolia* and *A. barbadensis* extracts (Añibarro-Ortega *et al*., 2019).

Antibacterial activity

The agar well diffusion method was adopted for testing the antibacterial potential of the formulated gel and compared with the commercial gel, and the growth inhibition zone of *S. aureus* was measured. The plant extracts showed some antimicrobial activity with bacterial inhibition zones of 1.53 mm and 1.17 mm by *C. aurantifolia* and *A. barbadensis*, respectively. Interestingly, the prepared gels showed better antimicrobial activity (2.40 mm) than the plant extracts and the commercial herbal gel (1.79 mm) (Table 4). The antibacterial activity of formulated gel was greater than individual plant extracts indicating that plant extracts in gel formulation showed a synergistic effect when used in combination. Moreover, polymeric excipients assisted in improving the encapsulation, retention, and bioactivity of the extracts. The antimicrobial activity of the extracts could be due to bioactive fractions such as flavonoids, phenols, and tannins, etc. (Anand *et al*., 2022), which caused the changes in cell shape by damaging bacterial cell wall, thus inhibiting the growth (Adebayo-tayo *et al*., 2016). This indicates the sensitivity of the extracts enhanced with the presence of Carbopol-940 and confirms that the developed new formation is depicting a better anti-acne activity than extracts. However, parabens have been claimed to show antibacterial activity too, especially when their chain length is increased from methyl, ethyl, and propyl, to butyl parabens (Schmitt *et al*., 2022). The antibacterial inhibition zones observed are smaller than in the previous reports which could be due to different concentrations of plant extracts (Adebayo-tayo *et al*., 2016; Kusuma *et al*., 2018).

Particle size analysis

The particle size and polydispersity index (PDI) were estimated with the mean particle size of 2406 nm, which is greater than 600 nm indicating that vesicles prefer to remain on the stratum corneum (Danaei *et al*., 2018). However, this value is widely accepted because the diameter of hydrated Carbopol-940 particles might even exceed 10 µm depending on the stirring conditions (Oelschlaeger *et al*., 2022). The homogenization duration and Carbopol-940 concentration both had an impact on the particle size (Muniraj *et al*., 2020). The result also revealed that the polydispersity index was less than 1, indicating the formulation would have been monodisperse (Bhattacharyya & Reddy, 2019). According to Danaei *et al*. (2018), values of 0.3 and less are regarded as acceptable in drug delivery applications, although a polydispersity index of 0.2 and below is frequently seen to be acceptable in practice. The preparation of a monodisperse population of molecular species is necessary for the greatest bioavailability and the prolonged effectivity of the plant antimicrobial gels.

Gel permeation analysis

The permeation of the bioactive extract (or drug) from the formulated gel was tested in the phosphate buffer media. The formulated gel showed a cumulative percentage of drug release of 14.23% at 30 min (Figure 3a). The release was raised to 17.16% at 60 min, and in 2.5 hr it showed 27.44% which was 10% higher than the release at 1 hr. This slower release of the drug is comparable to the previous findings (Chandira *et al*., 2010). Results showed that skin penetration would be slower and a greater percentage of drug content in the gel formulation would be protected and retained in the gel body. The composition (including the additives employed) has a strong impact on the release properties of the extract besides the extract's chemical nature (Iyer *et al*., 2021).

According to Yen *et al*. (2015), the percentage of drug release is directly proportional to the drug content in the gel formulation. It was also observed that in-vitro drug release from formulation started slowly because polypropylene glycol makes the formulation more viscous and slows the release rate of the extract (Kumar & Eswaraiah, 2020). However, gel formulation containing a permeation enhancer can increase the skin penetration of drugs by promoting transdermal partitioning into the skin layers (Roy *et al*., 2020). In addition, the concentration of the utilized vehicle, Carbopol-940, also has a significant impact on drug permeation. When Carbopol-940 was used in lower concentrations, high permeation efficiencies were obtained for the gel formulation and the drug release was significantly higher (Kumar & Eswaraiah, 2020). The study revealed that Carbopol-940 is an excellent choice for producing drugs in topical formulations and that using appropriate propylene glycol even speeds up the drug release rate (Kusuma *et al*., 2018).

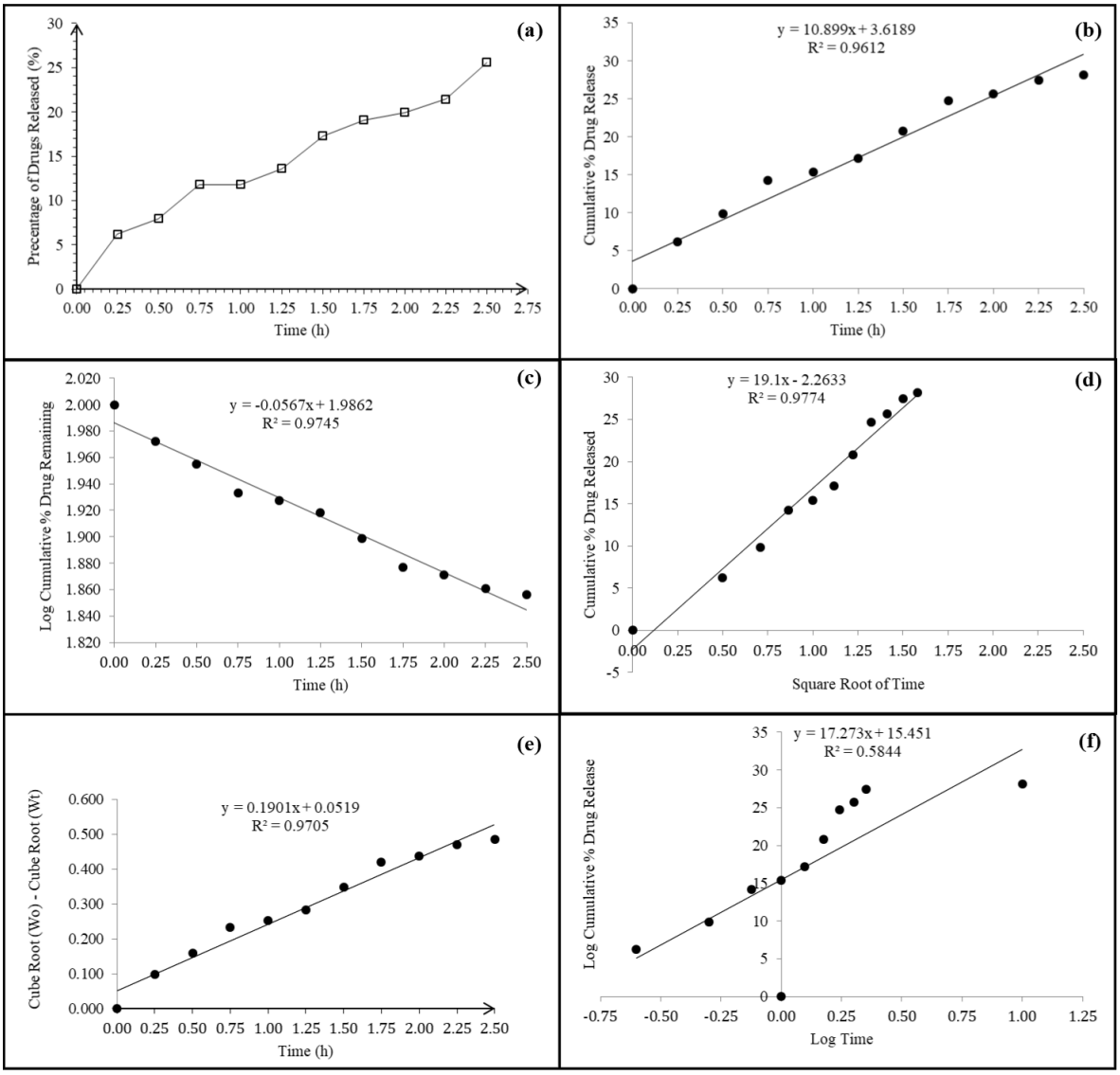

Fig. 3. Permeation of extract from gel to media and kinetic modeling of formulated gel: (a) Cumulative release of the drug, and kinetic models: (b) Zero-order drug release, (c) First order drug release, (d) Higuchi drug release, (e) Hixson-Crowell drug release, (f) Korsmeyer-Peppas drug release.

Permeation kinetic analysis

The rate of permeation of plant extract from the gel to the phosphate buffer was evaluated. The release data was fitted to different models such as zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas (Figure 3). Zero order interprets a constant drug release over time, whereas the first order dictates the drug release depending upon its concentration. On the other hand, the Higuchi model explains that drug release is by diffusion through dispersed vesicles. At the same time, Hixson-Crowell shows drug release by dissolution but not by diffusion, while the Korsmeyer-Peppas model portrays drug release by a combined mechanism of dissolution and diffusion (Iyer *et al*., 2021).

Results showed that the release rate of plant extract (drug) from the formulated gel was best explained by the Higuchi model with a slope of 19.1 and a coefficient of determination (R^2) value of 0.9774. Thus, it was confirmed that the diffusion mechanism drove the drug permeation through the membrane. In transdermal drug delivery systems, Higuchi drug release kinetic is typically observed (Iyer *et al*., 2021). This model is frequently used to explain the release of soluble and slightly soluble drugs from different semi-solid carrier materials which is opposed to first-order concentration gradient-based diffusion (Salamanca *et al*., 2018). However, Sainy *et al*. (2021) reported Korsmeyer-Peppas model explains well the release of desoximetasone from the Aloe vera emulgel prepared by the dispersion technique. Similarly, the aloe vera release in phosphate buffer from the electrospun polyvinyl alcohol matrix followed the Higuchi model (Isfahani *et al*., 2017).

Physical analysis of treated (high-pressure & microwaved) gels

The high-pressure processing and microwave pasteurization treatments of gels were made to test for the possible replacement of paraben (a preservative). The treated gels (with and without paraben), untreated gels, and commercial herbal formulations were evaluated for water activity, pH, viscosity, and spreadability at the start (0 weeks) and after 4 weeks, and results are presented in Table 5.

Water activity

The water activity of treated formulated gels was in the range of 0.982 to 0.988. In contrast to the initial a_w values of the commercial herbal gel, the formulated gel with and without paraben, the treated samples showed a slight decrease in a_w after storage of 4 weeks. However, it was noted that a_w changes post-treatment were insignificant despite the treatment. Hence, it was observed that the treatments and storage time did not impact the stability of gels, and a similar was reported previously (Suksaeree & Chuchote, 2018). All the gels evaluated in this study depicted a_w higher than 0.6 which could be attributed to the presence of water-based carbomers, which may make them susceptible to microbial growth and development (Kusuma *et al*., 2018). However, the presence of antibacterial plant extracts and the treatment might have prevented microbial activity in the gels and thus maintained the gels. Hence, high-pressure processing and microwave pasteurization could be employed to eliminate synthetic additives such as paraben without compromising the stability of gels. According to Dang *et al*. (2021) and Kutlu *et al*. (2022), high-pressure processing and microwave pasteurization are particularly beneficial for food processing in terms of food quality and shelf-life stability.

Table 5. Comparative stability evaluation of the formulated and commercial herbal gels

Parameters	Water activity (a_w)	pH	Spreadability (mm)	Viscosity (cP)	Water activity (a_w)	pH	Spreadability (mm)	Viscosity (cP)
Samples	Week 0				Week 4			
Commercial	0.986	5.99	15.97	7240	0.982	6.02	14.57	7224
Formulation with paraben	0.990	6.82	11.93	7717	0.987	6.84	10.83	7677
Formulation without paraben	0.988	6.83	9.67	7552	0.987	6.82	9.90	7661
HPP	0.988	6.92	11.90	7752	0.982	6.97	11.73	7752
MW	0.988	6.79	10.0	7754	0.987	6.85	11.23	7698

*HPP: high-pressure processing, MW: microwave, cP: centipoise

pH

The pH range for the high-pressure and microwave-treated gels was 6.79 to 6.92. After being stored for 4 weeks at ambient conditions, the pH of the treated gels upshifted (6.85 to 6.97). This rise in the pH could be explained by the dissociation of the weak acids of extract of *C. aurantifolia* which converted to basic fractions and raised the pH. However, the rise in pH to near neutral is desirable as it would be benign and non-reactive to the skin while maintaining the functionality of antimicrobial anti-acne gel. Contrarily, manuka honey and asparagus juice exhibit insignificant pH changes after high hydrostatic processing treatment (Chen *et al*., 2015; Fauzi & Farid, 2015).

Viscosity

The gel formulation without the paraben depicted a drastic change in the viscosity after treatment with high pressure and microwave. In both the samples, an upshift of 200 cP was noticed post-treatment compared to the non-treated counterpart. However, the storage of gels for 4 weeks provided relatively no change in the viscosity for the high-pressure-treated gel while the microwave-treated sample depicted reduced viscosity (7698 cP). On the other hand, commercial herbal formulation showed a minor reduction in the viscosity whereas a strong lowering in the viscosity was noticed for the sample with paraben after storage of 4 weeks. This disparity in the viscosity of gels is interesting and suggests different types of molecular interactions with the presence or absence of paraben which were further ameliorated by high-pressure processing and microwave pasteurization. Parabens are generally employed as preservatives in cosmetics where they may contribute to stabilizing the gel body and texture by interacting with moisture and developing interaction between the polymeric additives such as propylene glycol and carbomers (Schmitt *et al*., 2022). Hence, the processing of gels with the presence and absence of paraben provided different viscosity.

Spreadability

The spreadability is a fundamental characteristic that defines the therapeutic efficacy of gel and also determines consumer compliance (Daryab *et al*., 2022). The spreadability ratio of both the treated gels (with and without paraben) was much higher than the gel at 0 weeks (9.67 mm). However, for freshly high-pressure-treated and microwave-pasteurized gels, a significant increase in spreadability was noticed at 79% and 49%, respectively. However, after storage of 4 weeks, the spreadability of the untreated gels boosted to 9.90 mm from 9.67 mm, while the treated samples depicted variation in the spreadability from 11.23 mm to 11.97 mm which still stood higher than the non-treated gels. This increase in the spreadability could be due to the possible reduction of water-holding capacity by the relaxation of Carbopol-940 molecular chains though there was no apparent syneresis was noticed. Contrary to this, the formulated gel with paraben and the commercial herbal gels showed a reduction in spreadability. Hence, processing has impacted the gel structure and changed the spreadability of the gels after storage of 4 weeks. However, Suksaeree & Chuchote (2018) reported no variation in the spreadability of gels after storage.

CONCLUSION

The formulated polyherbal anti-acne gel was odorless, translucent, washable, homogeneous, and lump-free. It also showed good spreadability, viscosity, extrudability, stability, and a high percentage of drug content. A higher antibacterial activity by the

formulated gel was depicted as compared to commercial herbal formulation against *S. aureus* indicating its suitability to cure mild acne vulgaris. The plant extracts from the gel formulation showed a slower permeability toward the simulated media. Furthermore, high pressure and microwave pasteurization processes improved the viscosity and spreadability of the gel (without paraben) and resulted in similar to the paraben-containing normal gel. Additionally, treated gels showed stability comparable to the commercial herbal formulation and paraben-containing gel, suggesting the suitability of the treatments in developing paraben-free gels. It is concluded that the formulated washable and skin-permeable herbal anti-acne gel containing *C. aurantifolia* and *A. barbadensis* has a high potential as a cosmeceutical. However, in vitro cytotoxicity and antimicrobial evaluation of the developed gel and a comparison with commercial antibiotics should be made to evaluate its safety and effectiveness as a viable cure for acne. Moreover, other than high-pressure processing and microwave pasteurization, the impact of ultrasound and plasma treatment on gel stability should also evaluated in the future.

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ETHICAL STATEMENT
Not applicable

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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