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FULL PAPER

Formulation and characterization of polyherbal anti-acne gel containing *Citrus aurantifolia* and *Aloe barbadensis* extracts for the management of acne vulgaris

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Abstract

The antibiotic used to treat acne may develop antibiotic resistance and side effects. Meanwhile, treating acne-causing bacteria by applying *Citrus aurantifolia* juice to the skin immediately is ineffective. *Citrus aurantifolia* juice was employed in an anti-acne gel formulation to treat acne that was caused by *Cutibacterium acnes* and *Staphylococcus epidermidis* has no synergistic therapeutic effect shown. Additionally, polyherbal formulations are currently in high demand in the global market. The herbal industry's global market value has risen dramatically from US$60 billion in 2000 to US$105 billion in 2017. The worldwide market will increase at a rate of 7.6% each year until it reaches $5 trillion in 2050. This study aims to formulate a polyherbal anti-acne gel containing *C. aurantifolia* and *Aloe barbadensis*, and to characterize the formulated gel for its physicochemical properties. The phytoconstituents found in plant extracts were also analysed. The antibacterial activity of plant extracts against *Staphylococcus aureus* was also investigated using an agar well diffusion method. After plant extracts were discovered, gels with varying concentrations of plant extracts, and excipients were prepared along with compatibility studies. Parameters like colour, odour, homogeneity, phase separation, consistency, washability, pH, viscosity, spreadability and antibacterial activity were evaluated. The results showed that the levels of phenol, tannins, and flavonoids in plant extracts varied, the antibacterial activity of plant extracts was studied with a clear inhibition zone demonstrated, and plant extracts were compatible with all excipients. Gel formulations containing *C. aurantifolia*, *A. barbadensis*, the excipients Carbopol-940, methylparaben, propyl paraben, propylene glycol-400, triethanolamine and water have been developed. The formulated gels were clear, odourless, uniform, consistent, without lumps, and washable. It was found that pH was closer to the skin, good viscosity and spreadability. The formulated gel, F4 has greater antibacterial activity than plant extracts and other formulations with a larger diameter inhibition zone. It is concluded that the polyherbal anti-acne gel containing *C. auranfolia* and *A. barbadensis* can be formulated which has a high potential for halal cosmetics product development. However, more research should be done before it can be commercialised by comparing it to commercially available marketed formulations to treat acne vulgaris.

1. Introduction

Acne vulgaris is another name for common acne. Acne vulgaris is a skin disorder that occurs when dead skin cells, oil, and bacteria such as *C. acnes*, *S. epidemidis* and *S. aureus* clog hair follicles (Borse *et al*., 2020). This results in blackheads, whiteheads, nodules, papules, and pustules on the skin (Sharma and Dev, 2018). Hormonal changes produced by the adrenal glands of both males and females throughout puberty are one of the key causes. Acne vulgaris was ranked eighth among the top ten most common diseases worldwide in 2010 (Hay *et al*., 2014). It affects teenagers the most, causing them to lose confidence due to their looks and interfering with their everyday activities. They are

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looking for a way to get rid of the acne. This increasing frequency of taking various antibiotics as treatment can lead to the development of antibiotic resistance and a variety of adverse effects, including erythema, photosensitivity, allergic dermatitis, excessive skin irritation, urinary problem, joint and muscle pain, headache, depression (Hou *et al*., 2019).

According to previous findings, *C. aurantifolia* juice was found to be an effective antibacterial in the treatment of two acne-causing bacteria, *C. acnes* and *S. epidermidis* (Aini *et al*., 2018). However, it is not practicable to apply *C. aurantifolia* juice to the skin immediately. To overcome this problem, *C. aurantifolia* juice can be made into a gel using Carbopol 940 ($(C_3H_4O_2)n$), a synthetic polymer made up of cross-linked carbomers that form a microgel structure. Gels are a semisolid preparation that may be applied to the skin more easily than lotions and creams. Creams and lotions are quickly washed away from the skin due to stickiness, resulting in low drug bioavailability (Naga Sravan Kumar Varma *et al*., 2014). The use of a gel can lengthen the time that medications stay on the skin and speed up the release of good active substances (Dantas *et al*., 2016). It had also been established previously that an anti-acne gel containing only *C. aurantifolia* juice was formulated to treat acne caused by *C. acnes* and *S. epidermidis* (Kusuma *et al*., 2018). Single plants may not be able to provide the desired therapeutic effects, due to the inadequacy of their active bioactive compounds (Kola-Mustapha *et al*., 2020). As a result, it is important to research to develop a gel-based formulation that uses poly medicinal plants, *C. aurantifolia* and *A. barbadensis* to treat acne vulgaris since it will have a better therapeutic effect and less toxicity. Bioactive compounds such as phenol, flavonoids and tannins found in *C. aurantifolia* juice (Adebayo-tayo *et al*., 2016). The aloe vera gel also contains secondary metabolites such as phenol, flavonoids, and tannins (Manna, and Rudra, 2020). Its secondary metabolites have properties of anti-inflammatory, antimicrobial, antioxidant, immune boosting, anticancer, anti-diabetic, anti-ageing, and sunburn relief (Nejatzadeh-Barandozi, 2013). This work aimed to formulate a polyherbal anti-acne gel containing *C. aurantifolia* and *A. barbadensis* and characterize the prepared polyherbal anti-acne gel for its physicochemical properties.

2. Materials and methods

2.1 Materials

Citrus aurantifolia fruits and *A. barbadensis* leaves were harvested from a home garden. *C. aurantifolia* fruits with a diameter of 4.33 cm and a weight of 47.25±0.005 g, while *A. barbadensis* leaves with a length of 53 cm and a weight of 166.68±0.77 g was used. *S. aureus* (ATCC 43300) was used as the test microorganism in this study and was provided by the Microbial Culture Collection Unit (UNiCC) of UPM's Institute of Bioscience. Other chemicals were purchased from an online platform (Shopee), with the brand name Hairduta.

2.2 Preparation of lime juice

Prior to preparation, *C. aurantifolia* fruits were washed with distilled water. Then, the fruits were cut in half. Fruit chunks were placed in a juicer and squeezed (Adebayo-tayo *et al*., 2016). The juice was filtered through a filter funnel using filter paper before being pasteurized for 30 mins at 71°C (Kusuma *et al*., 2018). Pasteurized juice was covered with aluminium foil and kept refrigerated at 4°C for further analysis (Aini *et al*., 2018).

2.3 Extraction of leaves of Aloe barbadensis

Fresh leaves were cleaned with distilled water (Mate *et al*., 2021). The resin was drained for 10 mins. The green skin of the leaves was removed with a peeler. The gel was scooped out with a spoon. The gel was blended thoroughly in a blender (Doshi *et al*., 2017). The gel was filtered through a filter funnel using filter paper. It was then put into a sterilized, clean glass jar and kept chilled at 4°C (Manna and Rudra, 2020).

2.4 Quantitative analysis on phytoconstituents

2.4.1 Determination of total phenol content

Total phenol content was determined by heating 5 g of the plant extract with 50 mL of diethyl ether ($(C_2H_5)_2O$) for 15 mins (Bharathiraja and Chandran, 2020). 10 mL of the boiled extract and 10 mL of distilled water were pipetted into a 50 mL conical flask (Bharathiraja and Chandran, 2020). Approximately 2 mL of ammonium hydroxide (NH_4OH) solution and 5 mL of strong amyl alcohol ($C_5H_{11}OH$) were also added to the mixture. The extract was made up to 50 mL by adding distilled water and left to react for 30 mins to allow for colour development. A spectrophotometer was used to measure the absorbance of the sample in triplicate at 725 nm (Onyema *et al*., 2016). The total amount of phenol in the extract was determined using a gallic acid solution calibration curve and represented as gallic acid equivalent in μg/mg (Mahmood *et al*., 2011).

2.4.2 Determination of total tannins content

Total tannin content was determined by pouring 0.5 g of the plant extract into a 50 mL beaker followed by 50 mL distilled water, and the mixture was stirred for 1 hr. This was filtered and made up to 50 mL in a 50 mL

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volumetric flask by adding distilled water. Then, 5 mL filtrate was pipetted into a test tube containing 2 mL of 0.1 M iron (III) chloride ($FeCl_3$) in 0.1 M hydrochloric acid (HCl) and 0.008 M potassium ferrocyanide ($C_6FeK_4N_6$) (Olagoke and Oyewale, 2019). Within 10 mins, a spectrophotometer was used to measure the absorbance of sample in triplicate at 725 nm (Singh *et al*., 2017). The total amount of tannins in the extract was determined using a tannic acid solution calibration curve and represented as tannic acid equivalent in µg/mg (Oikeh *et al*., 2020).

2.4.3 Determination of total flavonoid content

Total flavonoid content was examined with 10 g of the plant extract with 100 mL of 80% aqueous methanol (CH_3OH) at room temperature (Gupta *et al*., 2013). After that the entire solution was filtered through filter paper, the filtrate was transferred to a water bath, and the solution evaporated to dryness (Adeyemi *et al*., 2014). The sample was then weighed until it reached a constant weight, and the percentage of flavonoids was computed (Onyema *et al*., 2016).

$$\text{Percentage of flavonoids} = \frac{[(\text{weight of crucible + residue}) - \text{weight of crucible}]}{\text{weight of plant extract}} \times 100\% \quad (1)$$

2.4.4 Preparation of Staphylococcus aureus culture

Antibacterial activity was evaluated against *S. aureus* as a model bacterium since it causes acne lesions (Borse *et al*., 2020) and was obtained from the Microbial Culture Collection Unit (UNiCC) of UPM's Institute of Bioscience. The loop was sterilized over the flame. The loop was cooled by stabbing it into a clean part of agar. A single colony was picked up and scratched on the surface of the agar in a petri dish using the quadrant technique. A double layer of parafilm was used to seal the plate. The plate was incubated upside down for a day (Kusuma *et al.*, 2018).

2.4.5 Preparation of inoculum

The 24-hour fresh culture of bacteria colonies were picked with a cotton swab (Chen *et al*., 2016). The colonies were suspended in sterile water and blended with a vortex mixer to achieve equal turbidity (Mate *et al*., 2021). The inoculum suspension had a density of 0.5 McFarland standard (Chen *et al*., 2016).

2.4.6 Determination of zone of inhibition

A zone of inhibition test was carried out by inoculating 20 mL liquid Mueller Hinton Agar (MHA) medium with 1×10^8 CFU/mL of *S. aureus* and transferred into the sterile petri dish with an internal diameter of 8.5 cm (Sharma and Dev, 2018). The medium was allowed to harden (Borse *et al*., 2020). The wells were then drilled with a sterile cork borer with a 6 mm diameter (Prabu *et al*., 2017). The well was filled with 20 µL of each diluted plant extract (100 mg/mL) (Mate *et al*., 2021). The petri dishes were incubated for 24 hrs at 37°C (Kusuma *et al*., 2018). For antibacterial activity, the diameter of inhibitory zones was measured in millimetres using a vernier calliper (Bhaskar *et al*., 2009).

2.4.7 Preparation of gels containing plant extracts

The gels were made using various amounts of plant extracts. A total of four formulation batches were prepared according to the composition of formulations as shown in Table 1. Carbopol 940 ($(C_3H_4O_2)_n$) was dissolved in distilled water in a separate beaker with continuous stirring to avoid air entrapment (Chandrasekar and Kumar, 2020). Paraben was dissolved in distilled water in another beaker using a water bath and then cooled (Kola-Mustapha *et al*., 2020). The plant extract was added to the aforementioned mixture (Borse *et al*., 2020). To this solution, the Carbopol mixture was added and thoroughly mixed. After that, propylene glycol ($C_3H_8O_2$) and triethanolamine ($C_6H_{15}NO_3$) were added and the pH was adjusted to 6.8-7 with continuous stirring (Bhaskar *et al*., 2009). The final volume was made by adding distilled water (Al-Suwayeh *et al*., 2014). To eliminate bubbles, the prepared gels were kept at room temperature for a day (Kumar and Eswaraiah, 2020).

Table 1. Formulations of anti-acne gel.

Samples	F1	F2	F3	F4
C. aurantifolia juice	1%	-	1%	1%
A. barbadensis gel	-	1%	1%	1%
Carbopol-940	1%	1%	1%	1%
Methylparaben	0.1%	0.1%	0.1%	0.1%
Propylparaben	-	-	-	0.1%
Propylene glycol- 400	2%	2%	2%	2%
Triethanolamine	q.s	q.s	q.s	q.s
Water	q.s	q.s	q.s	q.s

q.s = Quantum satis.

2.4.8 Drug-excipients compatibility studies

Drug-excipient compatibility studies for the plant extracts and formulated gel were carried out using a differential scanning calorimeter (DSC) (Mettler Toledo, USA) and analysed using Star-e software (Rahman *et al*., 2014). 20 mg sample was placed in a closed aluminium pan and heated from 25°C to 300°C at 10°C/min under nitrogen gas (Al-Suwayeh *et al*., 2014). The same methodology was used to examine all the samples.

2.4.9 Physicochemical evaluation

The formulated gels were evaluated for various parameters as follows: -

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2.4.9.1 Colour

The colour of the prepared gels was examined by the naked eye against a white and black background (Sucharita *et al.*, 2020).

2.4.9.2 Odour

By dissolving the prepared gels in water, the odour of the gels was tested by smelling (Keshri, 2020).

2.4.9.3 Homogeneity

By applying the prepared gels to a transparent glass plate, the presence or absence of particles that have not been mixed homogeneously was examined using visual inspection (Kusuma *et al*., 2018).

2.4.9.4 Phase separation

By applying the prepared gels to a transparent glass plate, the presence or absence of aggregates was examined using visual inspection (Kumar and Eswaraiah, 2020).

2.4.9.5 Consistency

By applying the prepared gels to a transparent glass plate, the presence or absence of coarse particles was examined using visual inspection (Chen *et al*., 2016).

2.4.9.6 Washability

Formulated gels were applied to the skin and then ease and extent under running tap water was subsequently examined using visual inspection (Keshri, 2020). Washability is part of halal product development criteria as this is an important aspect of halal cosmetics, whereby washability is essential for wudu' (ritual purification).

2.4.9.7 pH determination

The pH meter had previously been calibrated with a standard buffer solution (pH 6.86) (Chen *et al*., 2016). Within 24 hrs of production, the pH of plant extracts and formulated gels was measured with a calibrated digital pH meter at a constant temperature. The pH of each plant extract and formulated gels was measured three times and mean values were computed (Kumar and Eswaraiah, 2020).

2.4.9.8 Viscosity measurements

The formulated gels' viscosity was determined by using Brookfield Programmable DV-II + Viscometer with spindle C S93 spinning at 50 rpm at 25°C (Prabu *et al*., 2017). The measurement of viscosity was repeated three times and mean values were computed (Kola-Mustapha *et al*., 2020).

2.4.9.9 Spreadability

The spreadability of formulated gels was determined by measuring the spreading diameter of 0.5 g of gel across two 125 g glass plates measuring 2.54 cm × 7.62 cm each (Kola-Mustapha *et al*., 2020). The gel was placed on a circle of 2 cm diameter marked on the first glass plate and then the second glass plate was placed over the gel (Maqsood *et al*., 2015). For 5 mins, a 500 g weight was applied to the upper second glass plate (Dantas *et al*., 2016). The weight was removed and the diameter of the circle after gel spreading was measured by using a vernier calliper from the three sides of the circle. The mean value of the diameters measured was computed. This procedure was repeated three times for all the formulated gels (Estanqueiro *et al*., 2015). The percentage of spreading diameter was calculated by the following equation, percentage of spreading diameter = d cm ÷ 2 cm × 100%, where d is the increased diameter (Al-Suwayeh *et al*., 2014).

2.4.9.10 Antibacterial activity studies

Exactly 20 mL liquid Mueller Hinton Agar (MHA) medium was inoculated with 1×10^8 CFU/mL of *S. aureus* and transferred into the sterile petri dish with an internal diameter of 8.5 cm (Sharma and Dev, 2018). The medium was allowed to harden (Borse *et al*., 2020). The wells were then drilled with a sterile cork borer with a 6 mm diameter (Prabu *et al*., 2017). The well was filled with 20 µL of each diluted formulated gel (250 mg/mL) (Mate *et al*., 2021). The Petri dishes were incubated for a day at 37°C (Kusuma *et al*., 2018). For antibacterial activity, the diameter of inhibitory zones was measured in millimetres using a vernier calliper (Bhaskar *et al*., 2009).

2.4.10 Statistical analysis

The data was presented as the Mean±SD (Maqsood *et al*., 2015). All the data were statistically evaluated using SAS Studio software. The significance of the data was determined using the student's t-test (Al Namani *et al*., 2018). The results were considered significant when $P < 0.05$ (Ali, 2018). The total amount of phenol and tannins in plant extracts, as well as correlation coefficients, were determined using linear regression analysis (Loizzo *et al*., 2012).

3. Results

3.1 Quantitative analysis on phytoconstituents

The total contents of phenol, tannins, and flavonoids were evaluated in plant extracts. Table 2 shows the mean values of total phenol, tannins, and flavonoid content in plant extracts. The total phenol content of plant extracts

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was evaluated using a standard curve of the gallic acid solution, y = 0.02249x + 0.21347 with a coefficient, R^2 = 0.9696, where y is absorbance and x is the amount of gallic acid concentration in µg (Al Namani *et al*., 2018). The total phenol content in *C. aurantifolia* was 58.729±0.021 µg of GAE/mg of extract, whereas *A. barbadensis* had 48.276±0.010 µg of GAE/mg of extract. The total tannins content of plant extracts was evaluated using a standard curve of the tannic acid solution, y = 1.53056x + 0.26452 with a coefficient, R^2 = 0.9906, where y is absorbance and x is the amount of tannic acid concentration in µg (Singh *et al*., 2017). The total tannins content in *C. aurantifolia* was 2.015±0.005 µg of TAE/ mg of extract, whereas *A. barbadensis* had 2.073±0.005 µg of TAE/mg of extract. The percentage of flavonoids in *C. aurantifolia* is 9.825±0.826%, whereas *A. barbadensis* is 0.567±0.231%.

3.2 Determination of zone of inhibition

Table 2. Quantitative analysis on phytoconstituents.

Samples	Total phenol content (µg of GAE/mg of extract)	Total tannins content (µg of TAE/mg of extract)	Flavonoid (%)
C. aurantifolia	58.729±0.021	2.015±0.005	9.825±0.826
A. barbadensis	48.276±0.010	2.073±0.005	0.567±0.231

Table 3 shows the antibacterial activity of plant extracts and formulated gels represented by the inhibition zones. The plant extracts of *C. aurantifolia* and *A. barbadensis* displayed an antibacterial effect against *S. aureus* with inhibition zones of diameter 7.53±0.06 mm and 7.17±0.21 mm respectively. Meanwhile, the inhibition zone of diameter on the formulated gels F1, F2, F3 and F4 were 8.30±0.10 mm, 7.50±0.36 mm, 7.80±0.17 mm, and 8.40±0.10 mm respectively.

Table 3. Antimicrobial activity of plant extracts and formulated gels.

Samples	Inhibition zone (mm)
C. aurantifolia	7.53±0.06
A. barbadensis	7.17±0.21
Formulated gel, F1	8.30±0.10
Formulated gel, F2	7.50±0.36
Formulated gel, F3	7.80±0.17
Formulated gel, F4	8.40±0.10

3.3 Drug-excipients compatibility studies

Plant extracts and formulated gel were subjected to differential scanning calorimeter analysis. The plant extracts *C. aurantifolia* juice and *A. barbadensis* gel, as well as the formulated gel, displayed similar exothermic peaks at identical temperatures as Table 4. In differential scanning calorimeter analysis, a sharp exothermic peak was produced by *C. aurantifolia* at 111.19°C *A. barbadensis* at 113.21°C, formulated gels, F4 at 111.29° C.

Table 4. Temperature of exothermic peaks.

Samples	Exothermic peaks (°C)
C. aurantifolia	111.19
A. barbadensis	113.21
Formulated gel, F4	111.29

3.4 Physicochemical evaluation

Table 5 shows the physicochemical evaluation of plant extracts and gel formulations. Physical parameters such as colour, odour, homogeneity, phase separation, consistency, washability, pH, spreadability and viscosity were evaluated. The formulated gels were found colourless with transparent, odourless, homogenous, lump-free, good consistency, and washable. All formulated gels had a pH of 6.82 to 6.85 and a spreading diameter of 9.12 to 10.38 mm. It was also observed that the viscosity of all formulated gels remained within the range of 7376.00 to 7690.67 cps when the gels were rotated at 50 rpm.

4. Discussion

4.1 Quantitative analysis on phytoconstituents

According to the literature review, the secondary metabolites found in *C. aurantifolia* and *A. barbadensis* are phenol, tannins, and flavonoids (Manna and Rudra, 2020). In the current study, those bioactive compounds were identified in varying amounts in *C. aurantifolia* and *A. barbadensis*.

The total phenol content in *C. aurantifolia* was similar to the previously reported value of 56 µg of GAE/mg of dry extract (Adebayo-tayo *et al*., 2016). While total phenol content in *A. barbadensis* is higher

Table 5. Evaluation parameters of plant extracts and gels.

Samples*	pH	Spreadability (mm/s)	Viscosity (cps)
C. aurantifolia juice	2.74±0.02		
A. barbadensis gel	6.07±0.05		
F1	6.85±0.01	9.69±0.33	7512.00±112.85
F2	6.84±0.01	9.12±0.64	7690.67±300.22
F3	6.82±0.02	9.82±0.54	7461.33±157.24
F4	6.84±0.01	10.38±0.57	7376.00±146.24

*F1-F4 were reported colourless, no odour, uniform homogeneity, no phase transition, good consistency, and good washability.

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than the previously reported values of 30.53±0.30 and 14.29±0.44 mg GAE/g when *A. barbadensis* power was extracted by methanol and ethanol solvents respectively (Bista *et al*., 2020). It could be due to the different forms of *A. barbadensis* that were used (Bista *et al*., 2020). In comparison to *A. barbadensis* extract, *C. aurantifolia* extract has the most phenol. Phenol is also known as microbicide since it may kill a variety of microorganisms that cause infection and disease (Adebayo-tayo *et al*., 2016). It comes in a wide range of structures, ranging from a simple aromatic ring to a highly complex polymeric structure, and is frequently found in glycosidic forms (Manach *et al*., 2005).

The total tannins content in *C. aurantifolia* is lower than the previously reported value of 525 mg GAE/100 g of dry extract (Adebayo-tayo *et al*., 2016). While total tannins content in *A. barbadensis* is higher than the previously reported values of 1.13±0.19 and 0.844±0.004 mg GAE/g when *A. barbadensis* power was extracted by methanol and ethanol solvents respectively (Bista *et al*., 2020). The difference is most likely due to the different forms of the plant used, as well as the chemicals used to construct the standard curve (Bista *et al*., 2020). *A. barbadensis* extract contains a bit higher amount of tannins compared to *C. aurantifolia* extract. Tannins have been shown to have antibacterial properties (Ali, 2018). Tannins have the ability to disrupt bacterial cell protein production (Bista *et al*., 2020).

Flavonoids are effective antimicrobials against a wide range of microorganisms. This is due to flavonoids being capable of forming complexes with extracellular and soluble proteins, as well as bacterial cell walls (Adebayo-tayo *et al*., 2016). The results revealed that the percentage of flavonoids in *C. aurantifolia*, is higher than in *A. barbadensis*. The mean values of total flavonoid content in plant extracts were computed using a formula and presented in percentages which could not be compared to previous findings expressed in different equivalence, mg QE/g (Bista *et al*., 2020) and mg GAE/g (Adebayo-tayo *et al*., 2016).

4.2 Preparation of gels containing plant extracts

Citrus aurantifolia juice and *A. barbadensis* gel were formulated in the form of a gel with Carbopol-940 ($(C_3H_4O_2)n$). The use of Carbopol can lengthen the time that medications stay on the skin and speed up the release of good active substances. In the preparation of formulated gels, excipient such as paraben was used as preservatives. This is due to the use of water-based media that is vulnerable to microbial growth (Kusuma *et al*., 2018). The Expert Panel for Cosmetic Ingredient Safety found that parabens are safe for use in cosmetics when the total parabens in each formulation do not exceed 0.8%. Meanwhile, the use of propylene glycol ($C_3H_8O_2$) is used as a humectant. It works well as a permeation enhancer (Naga Sravan Kumar Varma *et al*., 2014). The pH of the formulation was adjusted to 6.8-7 with the addition of triethanolamine ($C_6H_{15}NO_3$) (Chandrasekar and Kumar, 2020).

4.3 Determination of zone of inhibition

According to the findings, it was particularly intriguing, given that *S. aureus*, the acne-causing bacteria, was sensitive to plant extracts and all formulated gels. This could be due to the antibacterial activity of plant extracts and formulated gels as reported in previous studies by Cherian *et al*. (2020), Ali (2018), Adebayo-tayo *et al*. (2016), which caused changes in cell shape in the form of damaged and hollow bacterial cell walls, inhibiting the growth of dangerous bacteria (Adebayo-tayo *et al*., 2016).

In comparison to the plant extracts *C. aurantifolia* and *A. barbadensis*, as well as the formulated gels F1, F2, and F3, the formulated gel, F4 exhibits stronger antibacterial potential against *S. aureus*, with an inhibition zone of diameter 8.40±0.10 mm. The antibacterial activity of all formulated gels was higher than individual plant extracts, indicating that plant extracts in gel formulation were more efficient as anti-acne against *S. aureus*. The formulated gel, F4 exhibited a synergistic effect with the use of combined plant extracts and paraben to treat mild acne vulgaris (Mate *et al*., 2021).

However, the values of these inhibition zones were tiny as compared to those previously reported values 24±1 mm (Shakya *et al*., 2019), 10.46±0.01 mm, 18.21±0.05 mm, 20.26±0.01 mm and 24.76±0.01 mm (Kusuma *et al*., 2018) and 20 mm (Adebayo-tayo *et al*., 2016), due to varied parameters employed, such as volume of liquid Mueller Hinton Agar medium and formulated gels, as well as the size of cork borer (Borse *et al*., 2020).

As a result, all plant extracts and formulated gels were found to have antibacterial potential, making them useful in the treatment of bacterial infections. F4 was shown to be the most efficient against *S. aureus*, with the largest inhibition zone.

4.4 Drug-excipients compatibility studies

The compatibility studies provide the framework for combining plant extracts with excipients in the preparation of gels. In differential scanning calorimeter analysis, the position of the peak produced in plant extracts alone and a mixture of plant extracts with excipients showed no significant differences. This

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demonstrates that plant extracts and excipients have no interaction (Kumar and Eswaraiah, 2020). The excipients in the gel formulation were compatible with the plant extracts.

4.5 Physicochemical evaluation

Based on the evaluation, the prepared gels were found colourless with transparent, odourless, homogeneous, lump-free, free of fibre and particles and washable. When compared to *C. aurantifolia* and *A. barbadensis*, which had a pH of 2.74 and 6.07, respectively, all developed formulations had a pH of 6.82 to 6.85, which is within the typical pH range for skin (Kusuma *et al*., 2018). The spreadability values were more than 9 mm/s indicating the formulations are better bioavailability (Chen *et al*., 2016). Whereas the viscosity of gels was high enough which was more than 7000 cps to prevent it from flowing off the skin during or after application (Chen *et al*., 2016). The same amount of Carbopol was employed as the foundation for all formulations causing the spreadability and viscosity measurements to stay within the range (Kola-Mustapha *et al*., 2020).

4. Conclusion

The main goal of this study was to formulate a polyherbal anti-acne gel containing *C. aurantifolia* and *A. barbadensis* and characterize the formulated polyherbal anti-acne gel for its physicochemical properties. The findings revealed that F4 was the best-formulated polyherbal anti-acne gel, outperforming the formulated gels, F1, F2 and F3. The polyherbal formulated anti-acne gel, F4 contains a combination of both plant extracts of *C. aurantifolia* and *A. barbadensis* as well as the excipients Carbopol-940, methylparaben, propylparaben, propylene glycol-400, triethanolamine and water. The plant extracts were compatible with the excipients in the gel formulation. The best-formulated polyherbal anti-acne gel, F4 was odourless, homogeneous, lump-free, free of fibre and particles and washable. It was found that pH was closer to the skin, with good spreadability and viscosity. It also showed greater antibacterial activity against *S. aureus* to cure mild acne vulgaris. However, more investigation is required to strengthen this study and demonstrate the effectiveness of polyherbal anti-acne gel before it can be marketed to end users. This investigation should compare the formulation of polyherbal anti-acne gel with the commercially available marketed formulation in the management of acne vulgaris.

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