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PREPARATION AND EVALUATION OF ANTI-ACNE PHARMACEUTICAL GEL CONTAINING HENNA AND CHAMOMILE EXTRACTS

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ABSTRACT: Acne is a skin-related condition entailing many psychological complications. Although several medications have been developed, natural remedies attracted more attention because of the less side effects and multifunctional. The objective of this study was to prepare and evaluate the anti-acne effects of a pharmaceutical gel containing herbal extracts, including *Lawsonia inermis* and *Matricaria chamomilla*. The total phenolics of extracts were determined with the Folin-Ciocalteu method. Using Hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC) and propylene glycol (PEG), the pharmaceutical gel was formulated and the physical properties of the formulation were specified at 37 ± 2 °C. The release of the active ingredients from the optimum formulation was investigated using Franz cell device. The results showed that the optimum formulation was stable at least for 3 months. The total phenol content of the aqueous extract of Henna leaves, hydroalcoholic extract of chamomile flowers, and the optimum formulation was 57.8 mg/g ex, 181.08 mg/g and 202.75 mg/g, respectively. Nearly 80 % of the phenolic compounds in the optimum formulation were released over 4 h. The phenolic compounds have inhibitory effects on the growth of *S. aureus* and *P. aeruginosa*. It was concluded that the formulation exhibits excellent stability, viscosity, homogeneity, greater extrudability, and enhanced antibacterial activity, which can be employed as a local pharmaceutical gel in acne treatment.

INTRODUCTION: Acne is the most common inflammatory dermatosis that occurs in adolescence and young adults, which involves sebaceous glands [1]. Many factors contribute to the development of acne, including excessive sebum production, hormonal imbalance, inflammation, dead cells [2] and external bacterial infection [3, 4], although other factors such as diet, mental stress, and sunlight can also play major roles [5].

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In principle, acne is characterized by extreme bacterial colonization such as propionibacterium acnes, Staphylococcus epidermidis and *Staphylococcus aurous* besides follicular hyperkeratinization [6, 7].

The ultimate purpose of the acne treatment is to reduce the numbers of inflamed and non-inflamed scars with the least side effects [8]. Besides, prevent the development of scars and undesirable psychological effects from improving the ability of the individual to establish social communication in all stages of the personal life [9]. In this regard, antibiotics play important roles [10]. The common antibiotics widely using for the treatment of acne are erythromycin, clindamycin, and tetracycline, which available as a gel, liquid, lotion, or ointment.

However, the problem caused by the widespread use of these antibiotics is drug resistance [11], which, in addition to the complications of the treatment failure, imposes a financial burden [12]. In this regard, Different strategies have been improvised to reduce the shortcomings of antibiotics and enhance their therapeutic efficiency. For example, a lot of attention has been paid to topical treatment of acne, especially in patients with mild to moderate papulopustular acne [7]comprisesbenzoyl peroxide, topical retinoid; azelaic acid, clindamycin, and erythromycin but their adverse effects like burning, scaling, photosensitivity, and flare-up or even less-therapeutic effect limited their widespread use. Importantly, the long-term use of oral or topical antibiotics could facilitate development of passage antibiotic resistance to other organisms for instance Staphylococcus, Streptococcus, or Chlamydia [13].

Medicinal plants have made significant strides during the past decade through the development of herbal compounds in the pharmaceutical field and natural health care [14, 15], along with extending the potential number of viable solutions to tackle antibiotic resistance [16]. They have a long history of use, and their extracted bioactive compounds have been shown to possess antimicrobial properties, which can use as the best alternative treatments for acne diseases [17, 18]. These plants are a reliable source for the preparation of new pharmaceutical dosage forms [16]. *Matricaria chamomilla* belongs to the family of Compositae, and its flowers have anti-inflammatory and antimicrobial properties [19]. Chamomile extract contains several bioactive compounds such as terpenoids, monoterpenoids, sesquiterpenoids and phenolics [20]. This plant has been used in herbal medicine as an anti-inflammatory and antispasmodic substance. It is also employed in the treatment of antibacterial and anti-fungal diseases [21-23]. Lawsoniainermis (also known as henna), is yet another medicinal plant which belongs to the family of Lythraceae [21]. Henna leaves contain 1.3-22% Lawson or hydroxy naphthoquinone, which is the active ingredient and the color compound [23, 24]. The antimicrobial and fungicidal effects of henna have long been known and reported in Myriad studies [25-27].

The purpose of this study was to investigate the synergism anti-acne effects of hydroalcoholic and aqueous extract of chamomile and henna in a topical pharmaceutical dosage form in order to slow the release of secondary metabolites to the successful treatment of acne.

MATERIALS AND METHODS:

Materials: Mueller Hinton agar, Folin-Ciocalteu reagent, gallic acid, Hydroxypropyl Methyl-cellulose (HPMC), Carboxymethylcellulose (CMC), propylene glycol (PG), and sodium carbonate were purchased from Sigma–Aldrich, USA. Two pathogenic strains of bacteria, namely *Staphylococcus aureus* (ATCC 25923) and P. aeruginosa (ATCC 27853)were supplied from the microbial control laboratory, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Procurement of Plant Material: The samples of *Matricaria chamomillaand* Lawsonia inermis were collected from Kermanshah and Kerman province, Iran, respectively. It was identified by Dr. Mirtajeddini, Bahonar University, Kerman, and a voucher specimen for Matricariachamomilla (No 075 101 003) and Law soniainermis (No 167 003 001) was deposited at the Herbarium of the School of Pharmacy and Pharmaceutical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Preparation of the Plant Extract: Primarily, leaves of henna and aerial parts of Matricaria chamomilla including flowers, leaves, and the end of the stem, were washed to remove dirt via deionized water and then air-dried and ground using a mechanical grinder. The powder was then placed in a dark space at room temperature. Next, 50 g of henna leaves powder was mixed with 500 mL distilled water in an Erlenmeyer flask under stirring at room temperature for 24 h. In the case of chamomile extraction, 50:50 ratios of distilled water and ethanol was added about one-tenth of the weight of the powdered henna plant on the basis of its adhering property for 72 h. The macerated mixture was filtered through Whatman grade No. 42 filter paper to get the appropriate aqueous or hydroalcoholic extract. Finally, the extracts were concentrated, lyophilized, and stored at 4 °C for further studies.

Preparation of Gel Formulation: Different formulations containing henna and chamomile

extract with total concentrations of 5% (w/v) were also prepared. Gels were formulated using hydroxypropyl Methylcellulose (HPMC), carbo-xymethyl cellulose (CMC), and propylene glycol (PG) as excipients. The amount of preservative was considered constant. The gelling agent (HPMC or CMC) was mixed with PG and dissolved in an adequate quantity of phosphate buffer at pH 5.8 (equal to skin's pH) by stirring, after which, the preservative was added to the prepared gel. **Table 1** presents the ingredients used in different formulations. In order to use henna and chamomile extracts in the gel formulation, lyophilized extracts were dissolved in phosphate buffer and added to the gel with a final concentration of 5% w/v in each formulation.

TABLE 1: AMOUNTS (% w/v) OF GEL EXCIPIENTS IN DIFFERENT FORMULATIONS

Role in Formulation	Gelling Agent		Humectant	Preservative	Active Ingredient	
Formulation number	CMC	HPMC	PG	Benzalkonium chloride	Lyophilized henna Ex	Lyophilized Chamomile Ex
F1	2.5	-	10	0.1	5	5
F2	3	-	10	0.1	5	5
F3	3.5	-	10	0.1	5	5
F4	4	-	10	0.1	5	5
F5	4.5	-	10	0.1	5	5
F6	3.5	-	7.5	0.1	5	5
F7	4.5	-	7.5	0.1	5	5
F8	-	3.5	7.5	0.1	5	5
F9	-	4.5	7.5	0.1	5	5

HPMC: Hydroxypropyl methylcellulose, CMC: Carboxymethylcellulose, PG: Propylene glycol

Physical Evaluation of the Gel Formulations: Optimum formulation was chosen according to a comparative evaluation of the physical properties of the prepared gels. These parameters were tested in all formulations:

- pH was measured using a digital pH meter (827PH Lab, Metrohm, Switzerland).
- Color was tested *via* a white surface as the gel background.
- Homogeneity was checked through observing the presence of any particle in the formulations.
- Extrudability was determined by means of Aiyalu *et al.*, method, where 20 g of gel in a collapsible tube was pressed from the crimped end with prevention any rollback. The percentage of the extruded gel was then calculated [28].

  Viscosity was evaluated using the viscometer (DV-111ULTRA, Brookfield, USA).

Stability Studies: The stability studies were carried out in different temperature conditions (25 and 40 °C) for 3 months. All the evaluation parameters, namely pH, viscosity, and appearance, were studied at time intervals of 30th, 60^{th,} and 90th days.

Total Phenolic Assay: The total phenolic compounds (TPC) of henna and chamomile extracts were determined at the concentration of 5% (w/v) using Folin-Ciocalteu method [29]. Briefly, 0.2 mL of henna or chamomile extracts was added to 1 mL of freshly prepared Folin-Ciocalteu reagent, to which 0.8ml of sodium carbonate (7.5 % (w/v) of Na_2CO_3) was added after 10 min. After 2 h incubation at room temperature, the absorbance of the reaction mixture was recorded by UV Spectrophotometer at 760 nm. Gallic acid (GA) was used as standard, and TPC was explained asmg GAE/g extracts equivalents. The calibration equation for GA at different concentrations is as follows (1):

$$Y = 0.0037\ x + .0081 \ldots . 1$$

$$R^2 = 0.9987$$

The Release of Phenolic Compounds: The extract release from optimum formulation was determined using a Franz cell device at 37 °C in the phosphate buffer medium at pH=5.8. The amount of phenolic compounds was determined using the Folin-Ciocalteu method, as already mentioned. The release of the phenolic compound was measured across the dialysis membrane (12 kd) using Franz diffusion cell, with a diffusional area of 2 cm^2 and receptor volume of 50 mL.

The membrane was soaked in the receptor compartment, and 5 g of the gel was placed on the membrane surface in the donor compartment. The receptor compartment of the cell was filled with 50 mL of phosphate buffer pH 5.8 and 37^{o} C.

One mL aliquots we recollected from the receptor side at intervals of 0, 15, 30, 60, 90, 120, 180, and 240 min and replaced by the same volume of fresh buffer in the receptor to maintain the constant volume. The concentration of phenolic compounds in the samples was determined through the use of a UV spectrophotometer and GA calibration curve. All experiments were done in triplicate.

Determination of Antimicrobial Activity of Gel: The antibacterial activity of optimum formulation, 5% (w/v) henna, and chamomile extracts were tested by disc diffusion method against the two pathogenic strains of bacteria *S. aureus and P. aeruginosa*. In this test, clindamycin disc and preservative (0.1%) were used as a positive control. The bacterial suspension (5×10^4 CFU/mL) was swabbed onto sterile Mueller Hinton Agar plates using a sterile cotton swab. Disks soaked in 5% henna or chamomile extract, optimal gel formulation, and 0.1% preservative with clindamycin disc was placed in the environment of Muller-Hinton agar. The plates were incubated at 37 °C for 24 h, after which time, the inhibition zone of bacteria was measured.

The minimal inhibitory concentrations (MICs) of the henna and chamomile extracts were determined using the standard broth dilution method. Live cells of experimented pathogenic bacterial strains at final concentrations of 5×10^4 CFU/mL were inoculated into 48-well plates followed by 100 µL of henna and chamomile extract. After incubation for 24 h at 37 °C, their concentrations were recorded. The pure medium and the medium containing bacteria were respectively used as the negative and positive controls. The MIC was calculated based on the lowest concentration of extract or gel formulations inhibiting the bacterial growth.

RESULTS AND DISCUSSION: Hydrogel masks are 3D networks of polymers with cooling and soothing effects in acne treatment for sensitive skins, which can adsorb water several times the gel weight [30]. For reinforcement of prepared hydrogel, CMC was essential. The extract of henna and chamomile was used as an anti-acne ingredient facial gel. The stability studies of the different parameters of hydrogels are exhibited in **Table 2**.

Among the prepared formulations, formulation number 6 was selected as the optimum formulation for further calculation of drug release due to the better appearance and physical properties. Physical stability parameters of optimum formulation such as appearance, pH N, and viscosity were investigated at 37 ± 2 °C within 3 months. According to the results obtained in this study, after three months, no significant change in pH, viscosity, and appearance was observed in an optimal gel formulation. The pH of hydrogel was measured to be in the range of 5.6-5.9, which was in compliance with human skin pH (4.0 - 6.0).

TABLE 2: PHYSICOCHEMICAL PROPERTIES OF PREPARED GEL FORMULATIONS

	pH	Viscosity (Cp)	Color	Homogeneity	Extrudability
F1	5.8	716	brown	+++	+++
F2	5.8	847	brown	+++	+++
F3	5.9	1650	brown	+++	+++
F4	5.8	2400	brown	++	+
F5	5.8	2740	brown	+	+
F6	5.8	2700	brown	+++	+++
F7	5.6	2823	brown	+	+++
F8	5.8	2940	brown	+++	+
F9	5.7	3261	brown	++	+

+: good, ++: very good, +++: excellent

Determination of Total Phenolic Contents: Total phenolic compounds in plants normally have antioxidant activity. Hence, they are an important factor in health promotion because of their antioxidant and antimicrobial activity. In fact, their beneficial free radical scavenging came from the hydroxyl group of phenolic compounds, which acts as a hydrogen donor [31]. These compounds were studied in the aqueous extract of henna leaves, hydroalcoholic extract of

chamomile flowers, and optimum formulation using Folin-Ciocalteu method. The results are shown in **Table 3**. The total phenolic contents in various compounds such as flavonoids, coumarins, and gallic acid (GA) derivatives, obtained from henna and chamomile extracts is documented by many publications, although just a few studies have investigated the amounts of the phenolic compounds in a mixture of these two plants.

Our observations confirmed the results obtained by Saoudi Mongi *et al*. [32] It could be concluded that the high quantity of phenolic compounds in chamomile flower extract is due to the use of hydro alcoholic solvent as a higher polar solvent than less polar solvents such as alcohol, which is more efficient in extracting the phenolic compounds [33].

TABLE 3: TOTAL PHENOLIC CONTENT OF THE AQUEOUS EXTRACT OF HENNA LEAVES, HYDROALCOHOLIC EXTRACT OF CHAMOMILE FLOWERS AND OPTIMUM FORMULATION (MEAN ±SD)

Sample	Henna Leaves Extract (mg/g)	Chamomile Flowers Extract (mg/g)	Optimum Formulation (mg/g)
Total phenol content	57.8 ± 1.2	181.08 ± 2.57	202.75 ± 3.78

***In-vitro* Phenolic Compounds Release:** Phenolic compounds were considered as the effective ingredient index in the optimum formulation. As shown in **Table 4** and **Fig. 1**, nearly 80% of phenolic compounds in the optimal formulation were released over a period of 4 h due to the slow and regular erosion of the polymeric matrix in the gel and the presence of PG, which can play a penetration enhancer role and facilitate drug release from the 3D matrix of the gel.

In order to investigate the effects of polymer on total phenol content, the base formulation without henna and chamomile extract was checked out similar to its release process.

The results showed that the basic formulation did not interfere with the observed total phenol content.

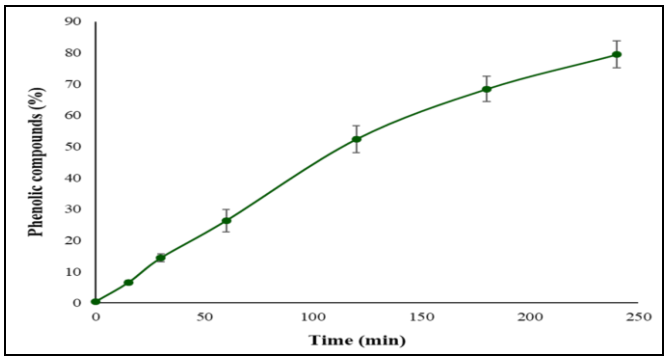

FIG. 1: THE RELEASE PROFILE OF THE PHENOLIC COMPOUNDS IN THE OPTIMUM FORMULATION

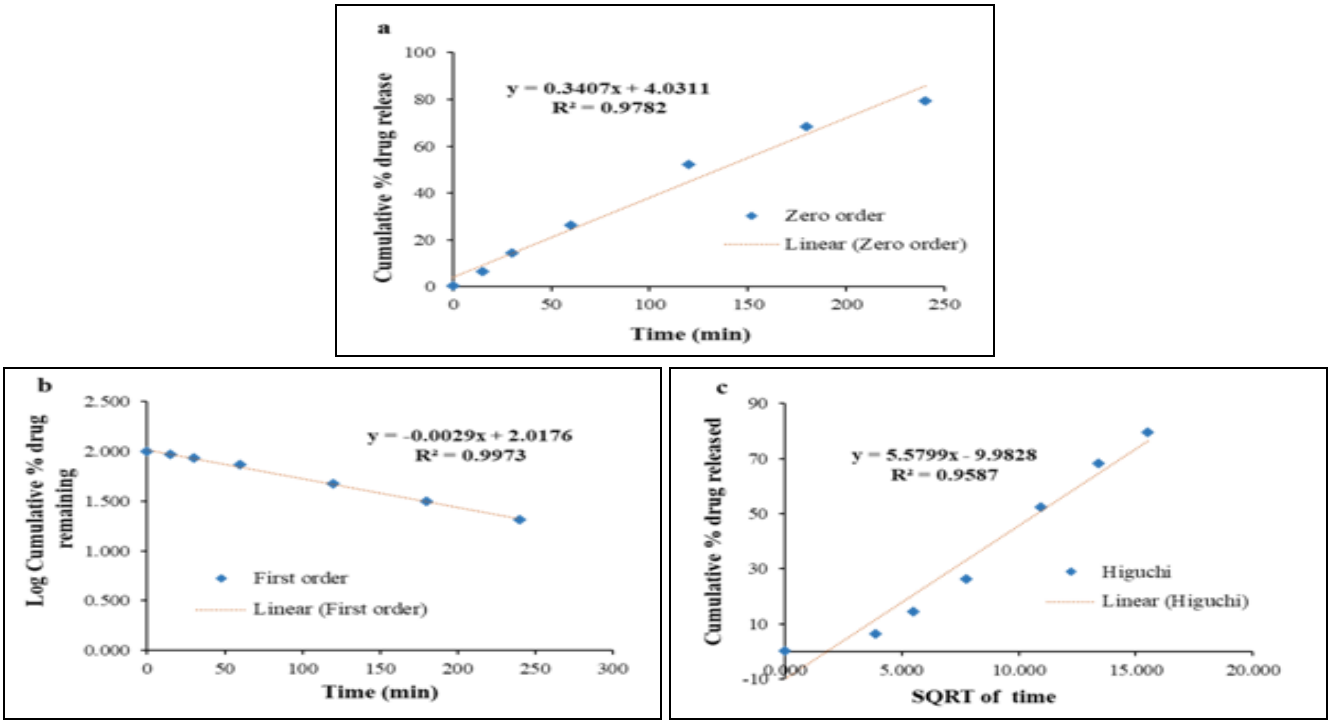

FIG. 2: RELEASE KINETIC MODEL FOR OPTIMIZED FORMULATION; (A) ZERO-ORDER KINETIC, (B) FIRST-ORDER KINETIC AND (C) HIGUCHI KINETIC

TABLE 4: CORRELATION COEFFICIENT OF DIFFERENT KINETIC MODELS FOR PHENOLIC COMPOUNDS RELEASE

Zero order	Higuchi	First-order
$Y = 0.3407x + 4.0311$ $R^2 = 0.9782$	$Y = 5.5799x-9.9828$ $R^2 = 0.9587$	$Y = 0.0029x+2.0176$ $R^2 = 0.9973$

Kinetics and Mechanism of Drug Release: Regarding to the *in-vitro* release profile, data was subjected to different release kinetic models such as zero-order Eq (2), first-order Eq (3) and Higuchi Eq (4) 34, 35: where Q is the phenolic compounds (active ingredients) released at time t, Q_0 is the percent of active ingredients remaining to be released, and k_0, k_1 and k_H are the coefficients of the equations. The experiments to determine the kinetics of release were carried out in triplicate. **Fig. 2** the analyzed release data using mathematical models for zero-order kinetic, first-order kinetic and Higuchi kinetic. The best fit model was determined by highest R_2 value, as shown in **Table 5**. The highest regression value was calculated for first order and zero-order models, indicating the drug release with diffusion-controlled [36].

$$Q=K_0t....2$$

$$Ln\ (100-Q) = Ln\ (Q_0) - k_1t...3$$

$$Q = K_Ht\ 1/2...4$$

Antibacterial Activity: Antibacterial activity of the formulation plays a crucial role in skin acne treatment. Thus, the antibacterial properties of the prepared henna aqueous extract, chamo-milehydroalcoholic extract and optimum formulation against *P. aeruginosa* and *S. aureus* were evaluated in this part. **Table 6** summarizes the results of inhibition zones of phenolic compounds in extracts and optimum formulation against two pathogenic bacteria under disk diffusion. Preservative (0.1%) and clindamycin disk were used as controls. The antibacterial activity of extracts is also presented in **Fig. 3**. The MIC values of *S. aureus* and *P. aeruginosa* were 50 µg/ml (chamomile extract), 5 µg/ml (henna extract), and 1 µg/ml (optimum formulation). According to obtained results, the phenolic compounds had higher effects ongram-negative bacteria *P. aeruginosa* in comparison to gram-positive bacteria *S. aureus*. Interestingly, fewer antibiotics are effective against *P. aeruginosa* [37]. This is may be attributed to the thicker cell wall of gram-positive bacterium, causing more resistance to be attacked by active polyphenol groups than *P. aeruginosa* [38]. The antibacterial activities of the henna and chamomile leaf extracts have been reported against both gram-negative and gram-positive bacteria [26, 27, 39-44]. It is worth mentioning that various experimental conditions comprise extract concentration, type of the solvent, and environmental conditions of plant growth that could affect the antimicrobial activities of extracts [45-47]. The antimicrobial activity of formulation containing chamomile and henna extract is to be caused by synergies and multiple mechanisms. The probable antibacterial mechanism of chamomile is based on the abundant amount of active constituents such as α- bisabolol and chamazulene, which gavedual bactericidal and bacteriostatic antimicrobial action. From the above data, it is assumed that sesquiterpenoid compounds of chamomile with the ability to interrupts cell wall permeability barrier and inhibition of cell membrane enzymes could induce antibacterial effects [48]. Similar to chamomile extract, henna is a rich source of polyphenol compounds such as Lawson, Gallic acid, tannic acid, mucilage, and mannitol [49] which the free hydroxyl group of these compounds with the capability to combine with the bacterial cell wall carbohydrates and proteins and attached to sites of the enzyme rendering them inactive [50-52]. Although, the exact antibacterial mechanism of henna and chamomile bioactive compounds are required tounderst and relied on the promising result, the combination of these extracts shows a synergistic antimicrobial effect.

TABLE 5: ANTIBACTERIAL ACTIVITY OF HENNA, CHAMOMILE EXTRACT AND OPTIMUM FORMULATION (N=3)

Test samples	Inhibition Zone Diameter (mm) , Mean ±SD	
	S. aureus	***P. aeruginosa***
Henna extract (5%)	11.6 ± 0.057	16.3 ± 0.11
Chamomile extracts (5%)	14.3 ± 0.057	22.6 ± 0.057
Optimum formulation	25.1 ± 0.15	36 ± 0.36
Preservative 0.1%	17.3 ± 0.15	19.6 ± 0.057
Clindamycin disk	35.3 ± 0.057	0

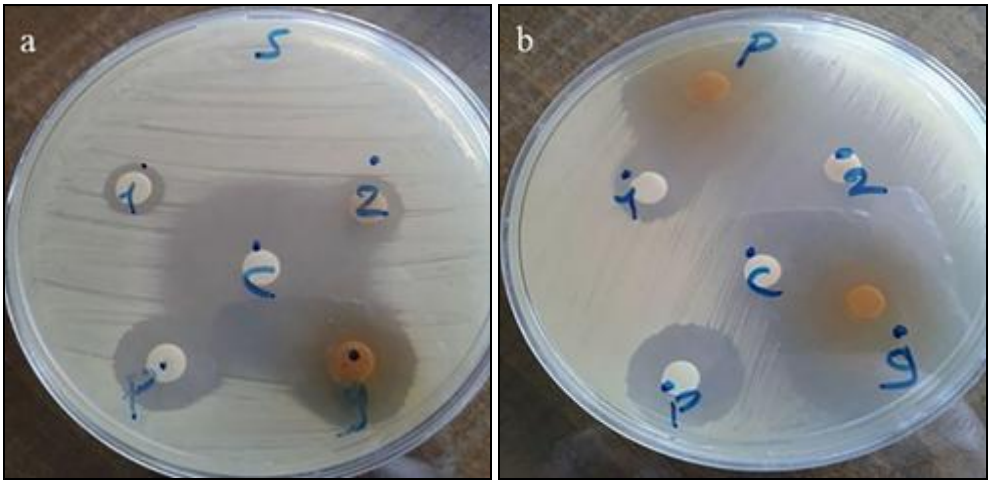

FIG. 3: ANTIBACTERIAL EFFECTS OF PHENOLIC COMPOUNDS AGAINST (A) *S. AUREUS* (B) *P. AERUGINOSA* 1: HENNA EXTRACT DISK, 2: CHAMOMILE EXTRACT DISK, G: OPTIMUM FORMULATION DISK, P: PRESERVATIVE, C: CLINDAMYCIN DISK

CONCLUSION: The chamomile and henna extract were found to have good potency against acne-inducing bacteria. Phenolic compounds were assumed as effective ingredients in both herbal extracts. The gel formulation developed from gelling agent, and these extracts also showed good physical properties and acceptable kinetics in the release of the phenolic compound. It is concluded that the aqueous extract of *Lawsonia inrmis* and the hydroalcoholic extract of *Matricaria chamomilla* act as potential reducing compounds against S. aureus and *P. aeruginosa*, and the formulation exhibits excellent stability, viscosity, homogeneity, greater extrudability, and enhanced antibacterial activity, which can be employed as aqueous-based gel formulation in anti-acne topical dosage forms.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest, financial or otherwise.

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