Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i18.3306

FORMULATION AND CHARACTERIZATION OF *ALKANNA TINCTORIA* LOADED EMULGEL AND ITS IN VITRO AND IN VIVO EVALUATION AS A COSMECEUTICAL PRODUCT

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Abstract

Background: Herbs and plants with enormous levels of phenolic and flavonoid constituents are commonly utilized as antioxidant agents to reduce the impact of UV radiation on the skin and play a significant role in skin treatments.

Aims: *Alkanna tinctoria* extract's potential cosmetic benefits including reduction in melanin, erythema, sebum, anti-aging and sun protection effectswere investigated in this study, which also aimed to create a stable emulgel that contained 4% *Alkanna tinctoria* extract.

Objective: *Alkanna tinctoria* extract was examined and quantified for its phenol and flavonoid content in order to ascertain its cosmetic effects on human skin.

Methods: *Alkanna tinctoria* (AT) loaded o/w emulsion were combined with gel containing carbopol-940 as gelling agent to create the emulgel formulation. Same procedure and formulation

was adopted for control emulgel without extract. For the duration of a 12-week storage period, an in vitro investigation was conducted to assess how color, liquefaction, pH and hardness changed. 13 healthy human volunteers' cheeks were treated with emulgel in order to assess its cosmetic benefits in comparison to control formulations. Utilizing the Mexameter, Elastometer, Sebumeter and Corneometer, and over the duration of the 12-week trial, facial parameters such as skin pigmentation, moisture content, redness, sebum, and skin elasticity were assessed.

Results: *Alkanna tinctoria* extract found to have 48.04 ± 0.0033 mg GAE/g of total phenolics, and flavonoids were 8.64 ± 0.0046 mg QE/g. *Alkanna tinctoria* emulgel significantly efficient at minimizing the sebum, small pores andlarge pores ($p \leq 0.05$) while efficient to maximize skin's elasticity and hydration as compared to control emulgel. Antioxidant activity by DPPH activity was 94.23±0.028.

Conclusion: The flavonoid and phenolic concentration of *Alkanna tinctoria* extract makes it a potent antioxidant that works to protect skin from photodamage and enhance skin conditions.

Keywords: *Alkannatinctoria*, DPPH, Elastometer, Total phenolic contents, total flavonoids contents, skin pores

1. Introduction

Since the dawn of humanity, people have been using drugs, particularly those made from natural ingredients (1).From antiquity, natural remedy made from minerals, animals, and plants used to prevent, treat, and even diagnose a wide range of human disorders and illnesses. Natural materials were used as medicines, according to ancient records from China and India (2). Ayurvedic hymns from manuscripts dating back to 1000 BC in India show that more than a thousand different herbs were used as medicine there (3).We now have access to advanced medications and a variety of therapeutic approaches because of scientific insights and the tireless efforts of scientists. Traditional medicine and therapy, however, are the fundamental causes of this evolution. Future medicine development will include natural items more comprehensively (4).

The beauty and cosmetics sector is seeing a huge shift from inorganic to natural regimens as a result of the lower hazards associated with plant-based products. Skin care items command the top spot with 36% of the worldwide cosmetics market. Natural beauty goods are expected to increase in value to 22 billion USD in 2024, from only 10 billion USD in 2016. This clearly demonstrates increased consumer acceptability for organic skincare products (5). Cosmeceuticals are created to enhance the aesthetic appeal, general health and wellness of the skin and hair. The product comes in both oral and topical forms, including pills, syrups, and powdered supplements as well as creams, emulsions, emulgels and gels (6)

Two immiscible liquids (often water and oil) combine to form an emulsion, which is stabilised by an additional ingredient called the emulsifying agent. One of the immiscible liquids is disseminated uniformly as tiny droplets through continuous phase. In order to create emulsions, different emulsifiers are utilized. Emulsifiers may link to few allergies and irritants. Thus, certain emulsifiers that don't trigger allergies or irritants must be used to make these cosmetic emulsions (7).

Gels are a category of pharmacological preparations created by encapsulating aqueous or hydro-alcoholic liquid in a system of colloidal particles (8). In comparison to the effectiveness of other topical preparations, gels allow for a higher rate of medication disintegration and migration through a carrier. Gels have several benefits, but the main drawback is the delivery of oil base medications(9).To allow therapeutic chemicals of a hydrophobic nature to benefit from the unique qualities of gels, this issue was resolved by creating emulgels. The dosage form created by combining emulsions and gels is known as emulgel (10).

Emulgels are beneficial because they are simple to use, easy to spread, greaseless, moisturizing, having long shelf life, and a decent texture. A gelling agent is added to oil in water emulsion to

create emulgels. Emulgels have therefore recently been employed as delivery systems for the topical administration of medicinal medicines (11).

Emulgels are superior to other topical formulations in a number of ways, including improved stability and loading capacity. The integration of hydrophobic medicines into the oil phase is aided by it, and the o/w emulsion that results from the dispersion of oily globules in the aqueous phase. This emulsion can also be incorporated into a gel base (12). Instead of just mixing medications into the gel foundation, this may demonstrate higher drug stability and release. Drugs with a shorter half-life can have their effects prolonged by emulgels.

Depending on the active chemicals present, cosmetics with medical or drug-like properties may affect the biological functioning of the skin. These cosmetics improving more than solely the appearance of skin and color. Modern medicine's potent tool for disease prevention and treatment is the use of phyto-chemicals produced from plants to deal with various skin conditions. Using cosmetic regularly with antioxidants is an effective way to preserve normal skin (13). The constituents of medicinal herbs include different Poly-phenols and vitamins, such as A, E, and C, and these constituents are related to the antioxidant activity of certain plant parts. Almost every part contain a large number of poly-phenols from different subgroups of plants (14).

Essential oils due to phenolic compounds are more appealing to researchers who are interested in evaluating their antioxidant activities. Numerous plant-derived antioxidants with potent antioxidant capabilities are used in the cosmetics industry (15).

In Western Asia and Europe, *Alkanna tinctoria* (L.) Tausch is widely spread. As a natural remedy for wounds, inflammation, and ulcers, its root has been utilized. Alkannin and its derivatives, among other Naphthoquinone pigments, have been isolated from this plant through previous Phytochemical research. Some of these substances have Cytotoxic, anti-bacterial, anti-leishmanial, and anti-inflammatory effects, among other biological features(16). Shikonins are secondary chemicals of significant commercial value that have a variety of biological properties, including antibacterial, insecticidal, anticancer, and antioxidant properties. Since these substances are typically colorful, they are used in food, textiles, and cosmetics. The Boraginaceae family of plants contains Shikonin and its derivatives, the most significant Naphthoquinone pigments from a commercial perspective (17). Antioxidant activity of Methanolic extract in the literature found to be 93.44% (18).

Figure1. *Alkanna tinctoria*

2. Materials and method

2.1. Plant identification

From the local market, roots of *Alkanna tinctoria* were purchased. An expert from "the department of biological sciences" assigned the identification number 331/ Botany to the plant sample in order to identify it.

2.2. Preparation of *Alkann atinctoria* (AT) Extracts

Following collecting and identification, the chosen roots were washed with tap water, sliced into smaller pieces, and allowed to dry in the shade for four weeks. The dry material was macerated with methanol after being crushed to powder (100 g) (80:20). An eight-layer muslin cloth was employed for rough extraction after the 72-hour maceration period. The filtrate had been subjected to rotary evaporator with reduced pressure until dry mass obtained. It was maintained between 2 and 8 °C while not in use.

2.3. (TPC) Total phenolic counts Estimation

By using the Folin-Ciocalteu Reagent (FCR) method as detailed in the literature, TPC of the crude extract sample was measured (19). A calibration curve was created using various Gallic acid concentrations.

. TPC was quantified in sample extracts as Gallic acid equivalents (mg GAE/g) using the formula below.

TPC = CxV/M

Where V = extract in ml, M = Plants extract in g, and C = mg/ml

2.4. (TFC) Total Flavonoid Count Estimation

With just minor modifications, the described method (20) was used to quantify the total Flavonoids level for the crude extract. 1 % Sodium nitrite solution in Methanol, 10% Aluminum chloride solution and 4% Sodium hydroxide solution in distilled water were made.100 µl of the extract was taken from the stock (1 mg/ml), and they were added to a well of a 96-well Microplate. Next, 25 µl of Sodium nitrite were added, and then there was a 5-minute incubation period. The above combination received 10 µl of Aluminium chloride solution after incubation, and this time it was given another 5 minutes to stand. After that, 30 µl of Methanol and 35 µl of Sodium hydroxide were added to the well containing the sample extract for the same amount of time as the standard curve is being formed. Using an automatic Microplate Absorbance reader, the sample's absorbance at 510 nm was measured in triplicate. By using Catechin linear regression curve, the total Flavonoid level of the corresponding extract samples was represented as µg Catechin equivalents per g (g CE/g) of extract.

2.5. Antioxidant activity by using DPPH

Using the method outlined by Mohsin et al., (21) the effectiveness of the external sample of extract of AT's ability to capture free radicals was determined. In a little amount of methanol, 7.9 mg of precisely measured DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) was dissolved. In a graduated cylinder, Methanol was added to create the final capacity of up to 100 ml. The volume of a graduated cylinder containing 10 mg of ascorbic acid was changed to 10 ml using methanol to create a negative control. The reaction mixture was incubated to 37 degrees Celsius in a dark room. A photometric technique at 517 nm and a Micro-plate reader were used to measure the absorbance drop that occurred a half hour later (synergy-HT BioTech). DPPH scavenging activity was calculated using the formula below.

%age inhibition= $(A_0\text{-}A1)/ (A_0x100)$

Where, A_0 is the absorbance of the control, A1 is the absorbance of the test extract, and A1 is the absorbance of the test extract.

2.6. Control and plant extract (active) emulgels Preparation

Different combinations surfactants and co-surfactants were calculated by using Griffin's HLB method used to create the primary emulsion formulation. And by keeping this final calculation, obtained by Griffin method at center, other different 59 formulation by using Behnken-Box method with increasing and decreasing variable concentration of ingredients were formulated. Then this primary emulsion was incorporated in gel phase, which was then initially screened for sensory attributes and stability characteristics at 50°C. Depending on its physicochemical and organoleptic qualities, a stable preparation was chosen for additional research. After the selection of stable emulgel, emulgel with different concentrations of AT extracts (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10%) were created and tested for antioxidant response. Emulgel having 4% AT extract showed the antioxidant activity same as the higher concentrations (means 5% to 10%) of extracts. So emulgel having 4% AT extract was further investigated for this study to achieve the maximum outcome and minimize the toxicity which may produce in higher doses.
In three processes, the emulgels were made. The first phase involved creating the primary emulsion, the second involved creating the gel, and the third involved dispersing the finished gel with the primary emulsion.

2.6.1 Preparation of primary emulsion

Separate beakers were used to heat the oil phase having AT extract and aqueous phase to 70–80 °C. A homogenizer was used for 20 min. at 2000 rpm to progressively incorporate the oil into the aqua phase. Homogenizer speed was steadily dropped to 1000 rpm for the last 10 minutes to confirm the homogeneity of the mixture. Prior to adding in aqueous phase, methyl paraben, a preservative, was dissolved in a small amount of propylene glycol. Table 1 lists the composition and elements needed to produce an emulsion.

Table 1. Control and AT loaded Emulsion to prepare emulgel

Ingredients	Phase	Control Emulsion	AT loaded Emulsion
Liquid Paraffin		10%	10%
Glyceryl Monostearate		1%	1%
Span-80		0.5%	0.5%
Propyl Paraben		0.06%	0.06%
AT Extract	**Oil Phase**	0%	4%
Tween-80		1.5%	1.5%
Propylene Glycol		10%	10%
Methyl Paraben	**Aqueous**	0.11%	0.11%
Distilled Water	**Phase**	80.83%	76.83%

2.6.2 Preparation of gel phase

In order to prevent lumps from forming, the Carbopol 940 was gradually distributed into water to create the gel phase individually. To guarantee that Carbopol was completely hydrated, the created gel was refrigerated overnight. Triethanolamine was gradually added to the solution to obtain the pH in range of 5.5 to 6.5 (22).

2.6.3 Gel and Emulsion Mixing

The emulsion created in the first step was finally mixed continuously at 1000 rpm to create emulgel. The same procedure was followed in the preparation of the control formulation, but no plant extract was used. The griffin method was used to determine the amount of span 80 and tween 80 surfactant blends used to create emulsion, and the results are shown in Table 2.

Table 2. Formulations of control and AT loaded Emulsions to prepare Stable emulgel

Ingredients	Phase	F1	F2	F3	F4	F5	F6
Liquid Paraffin		10%	10%	10%	10%	10%	10%
Glyceryl Monostearate		1%	1%	1%	1%	1%	1%

Span-80	**Oil Phase**	0.2%	0.3%	0.4%	0.5%	0.6%	0.7%
Propyl Paraben		0.06%	0.06%	0.06%	0.06%	0.06%	0.06%
AT Extract		4%	4%	4%	4%	4%	4%
Tween-80		1.5%	1.5%	1.5%	1.5%	1.5%	1.5%
Propylene Glycol		10%	10%	10%	10%	10%	10%
Methyl Paraben	**Water Phase**	0.11%	0.11%	0.11%	0.11%	0.11%	0.11%
Distilled Water		77.13	77.03	76.93	76.83%	76.73	76.63

2.7 In vitro evaluation of emulgel

Several in vitro studies were carried out in order to get evidence for consistency, stability, efficacy, and safety. The samples of both formulations were stored under stress conditions in a stability chamber for 90 days, and any changes to the organoleptic qualities such color, smell, liquefaction and phase separation were assessed. By putting the gel at different temperatures for 12 weeks, pH, and stability were assessed (7, 22-24). Globule size of the control and AT emulgels was observed.

2.8 In vivo (Non-invasive) evaluation

2.8.1 Study design

13 healthy male volunteers (human) of ages between 20 to 35 were recruited. To ensure that none of the volunteers had any severe skin diseases, a skilled dermatologist checked each volunteer. All research participants underwent patch testing prior to the trial's start to rule out any potential skin irritation or allergic reaction. Volunteers were fully instructed on how to utilize formulations during the trial time.

The formulas' respective application sites were marked by the capital letters "R" and "L" in bold. Volunteers were given instructions to apply the necessary amounts of formulations twice daily for a 12-week period. Throughout the whole trial period, no studied participant received any further therapy. Values of the various test parameters were measured from cheek area. The results were calculated as the average value after each measurement was made in triplicate (n = 3).

2.8.2 Ethical consideration

After authorization by the institutional ethical committee, healthy human volunteers recruited. The study was carried out in accordance with the Helsinki Declaration and with the recommendations for good clinical practices. Everyone who took part in the study was made aware of their ability to discontinue at any moment, with or without cause.

2.8.3 Melanin and Erythema level Estimation

Measurements were performed on study participants using test formulations in order to unbiased assess the therapeutic efficacy of Erythema and melanin levels. Results were recorded after getting readings from the entire cheek area at baseline, the second, fourth, sixth, eighth, tenth, and twelfth weeks after application.

2.8.4 Skin’s elasticity measurements

Elastometer’s probe was placed on the cheeks of the volunteers to evaluate any differences in the skin's elasticity.

2.8.5 Skin’s hydration measurements

Hydration of skin was measured on day one and throughout twelve weeks of the trial. Atopic dermatitis (AD) and other skin dryness issues can be managed by increasing skin moisture content.

2.8.6 Skin’s sebum Measurements

Using the Sebumeter SM815 sebum collection cassette device, the sebum level on the skin was observed. After applying tape to the skin to collect sebum, a photometric evaluation of grease

patches is carried out (25). The amount of skin moisture has no bearing on this method of measuring sebum.

2.8.7 Skin pores Estimation

Each participant in the study had a full-face photograph taken with a Visio face 1000 D at the baseline and at different intervals during 12 weeks of the experiment. The number and size of the skin's fine and microscopic pores were counted and compared in these images by using (CSI) software platform. Selecting a consistent area on the left and right cheeks required the use of CSI software. The same was used to determine the quantity and size of small and large pores.

2.9 Statistical analysis

For the numerical and graphical calculations, Microsoft Excel 2013 was used. Standard deviation was computed for every mean value. The SPSS version 20.00 was used to perform a paired sample t-test. The LSD test and a two-way ANOVA were used to analyze differences in effects over time periods and between people, respectively. 5% (p-value = 0.05) was chosen as the threshold of significance.

3 RESULTS

3.1 *Alkanna Tinctoria* extract's antioxidant activity

The DPPH method was used to assess the antioxidant activity of the *Alkanna tinctoria*(AT) extract, which was found to be 94.23 ± 0.028 % (18).

3.2 (TPC) Total phenolic content measurements

Using the Gallic acid standard curve value and the formula $y = 0.0026x + 0.4327$, $R^2 = 0.994$, the total phenolic content (TPC) of *Alkanna tinctoria* extract was calculated. The TPC value of Methanolic extract from *Alkanna tinctoria* (AT) was 48.03 ± 0.0032 mg GAE/g(18).For different solvents, the measured TPC values were 48.04 mg GAE/g, 30.54 mg GAE/g, 25.54 mg GAE/g and 12.69 mg GAE/g.

3.3 (TFC) Total Flavonoid content measurement

The TFC value of the extracted *Alkanna tinctoria* (AT) was 8.64 ± 0.0046 mg QE/g of AT extract, with the application of the Quercetin standard curve formula ($y = 0.0024x + 0.4434$, $R^2 = 0.9956$). For methanol, ethanol, chloroform, and water, respectively, the TFC values were 8.63 ± 0.0046 mg QE/g, 6.00 ± 0.017 mg QE/g, 5.34 ± 0.031mg QE/g, and 2.54 ± 0.018 mg QE/g. Additionally, **Table 3** displays the obtained TFC values.

Table 3. TPC and TFC values of different extracts with different solvents

Sr. No.	Extract	TPC (mg GAE/g)	TFC (mg QE/g)
1	Water	12.69 ± 0.009	2.54 ± 0.018
2	Chloroform	25.54 ± 0.0027	5.34 ± 0.031
3	Ethanol	30.54 ± 0.0081	6.00 ± 0.017
4	Methanol	48.04 ± 0.0032	8.64 ± 0.0046

3.4 In vitro testing of emulgel

3.4.1 Evaluation of emulgel stability

The created emulgel exhibited no change in color, odor, or heterogeneity under temperature stress conditions. 90 days were spent storing the test product at a variety of temperatures and humidity levels, including 8, 25 and 45°C.To assess stability characteristics, product was kept at 40°C for 5–6 months. When stored at 4°C for a month and after numerous freeze-thaw cycles between 20 and 25°C, there were no indications of instability. Table 4 displays the physical stability of the two emulgels (AT and control) after being stored for 90 days at varied temperatures of 8, 25, 40, and 40°C with 75% RH.

Table 4 Organoleptic Evaluation of AT Emulgel

Organoleptic Parameters	Temperatures	Time (Days)							
		0 h	24 h	7	15	30	45	60	90
Color	**8°C**	R	R	R	R	R	R	R	R
	25°C	R	R	R	R	R	R	R	R
	40°C	R	R	R	R	R	R	R	R
	40°C+75% RH	R	R	R	R	R	R	R	R
Liquefaction	**8°C**	NC	NC	NC	NC	NC	NC	NC	NC
	25°C	NC	NC	NC	NC	NC	NC	NC	NC
	40°C	NC	NC	NC	NC	NC	NC	NC	NC
	40°C+75% RH	NC	NC	NC	NC	NC	NC	NC	NC
Phase Separation	**8°C**	NC	NC	NC	NC	NC	NC	NC	NC
	25°C	NC	NC	NC	NC	NC	NC	NC	NC
	40°C	NC	NC	NC	NC	NC	NC	NC	NC
	40°C+75% RH	NC	NC	NC	NC	NC	NC	NC	NC

Note: NC means= No change, R means= Red

3.4.2 Centrifugation test

The test formulations were all constant over the course of the investigation, and there were no appreciable differences between the test and control formulation.

3.4.3 Determination of pH

The pH of AT emulgel and controls were measured using a pH meter at baseline and various time intervals. Results were averaged after each test was done in triplicate. The pH values were somewhat decreased, however the paired sample t-test produced insignificant findings ($p > 0.05$). Both AT and control, held at varied storage conditions for 90 days, the pH values has been illustrated in Figures 1 and 2 for each. An active test formulation including AT extract had a pH between 5.5 and 6.0 that was acceptable, indicating that it would be a good candidate for cosmetic preparation.

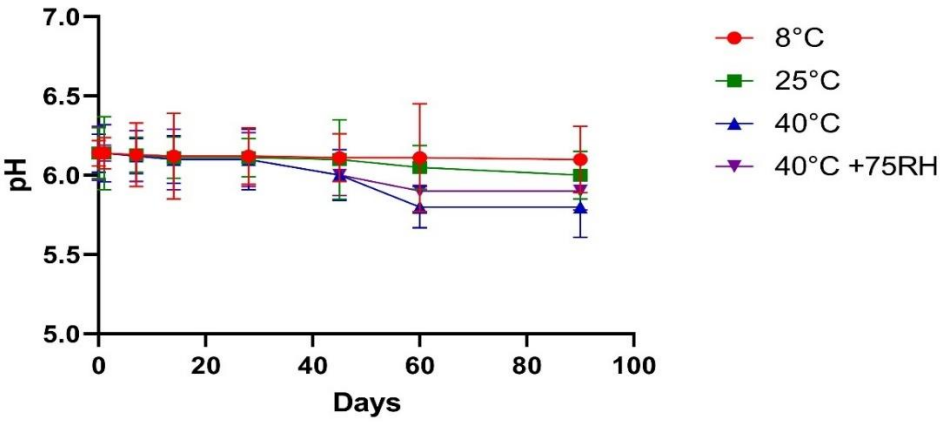

Figure 2. Vicissitude in pH of Control from 0 to 90-days

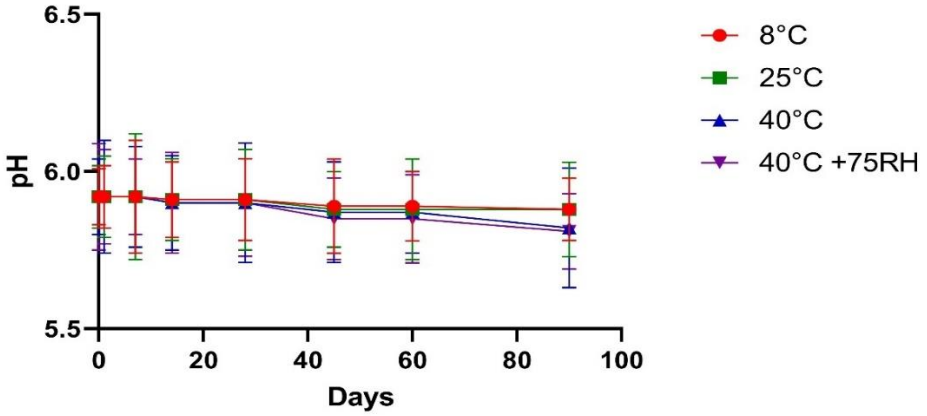

Figure 3. Vicissitude in pH of AT Emulgel from 0-90 days

3.4.4 Globule size determination

The evolution of the emulsion into the gel depends on the microscope measurement of globule size. This test verifies the presence of internal phase in the emulgel that are spherical in shape (26). The mean size of AT fresh emulgel globules was 0.81± 0.112 µm; however, after 12 weeks of investigation, it increased slightly to 0.85± 0.142 µm. Similar to this, globule size of fresh control emulgel was measured, and it was 0.83± 0.121 µm. After a 12-week investigation, it increased up to 0.86± 0.126 µm. In comparison to accelerated temperatures, the rise in AT emulgel globule size was seen at a slower pace at 8, 25°C. The results of this investigation revealed that there was no change in globule shape, but that acceptable, modest growth in globule size occurred within the stability range. Both AT emulgel and control emulgel's altered globule sizes have been demonstrated in (Figures 4 and 5), respectively. AT emulgel's microscopic images of freshly made and after 12-week globules have been shown in (Figure 6 & 7).

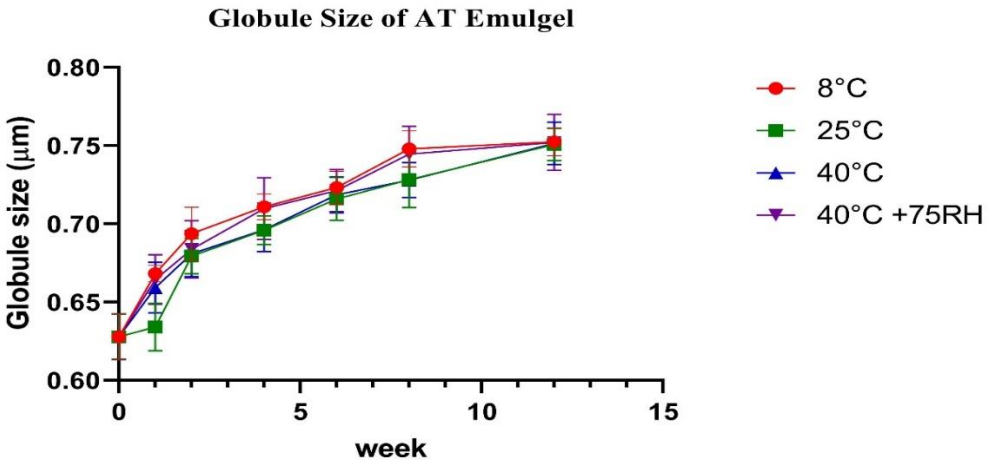

Figure 4. Vicissitude in Globule Size of AT Emulgel

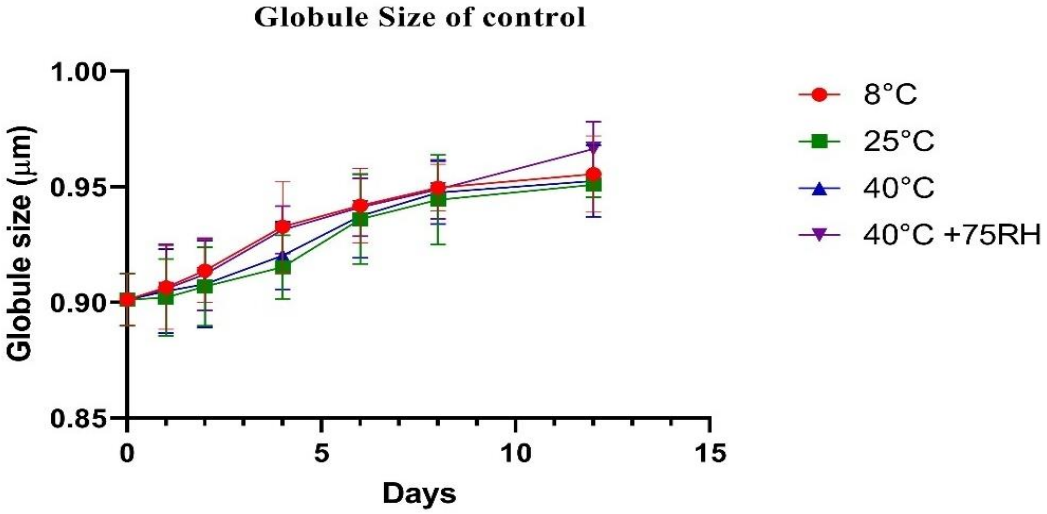

Figure 5. Vicissitude in Globule Size of Control Emulgel

Figure 6. Globule Size of freshly prepared AT Emulgel

Figure 7. Globule Size of AT Emulgel after 90-days

3.5 Melanin and erythema level

Melanin and Erythema levels were evaluated for control and AT emulgel formulations. As indicated in Figures 8 and 9, applying control emulgel (without extract) caused a minor decrease and in

Erythema whereas applying test formulation emulgel with AT extract also caused a decrease in Erythema count. A minor rise in the level of melanin by the control was found by analyzing the two-way ANOVA findings ($p > 0.05$). A formulation including AT extract, however, showed a substantial increase in melanin levels ($p > 0.05$). Its antioxidant potential is what causes the Erythema to reduce.

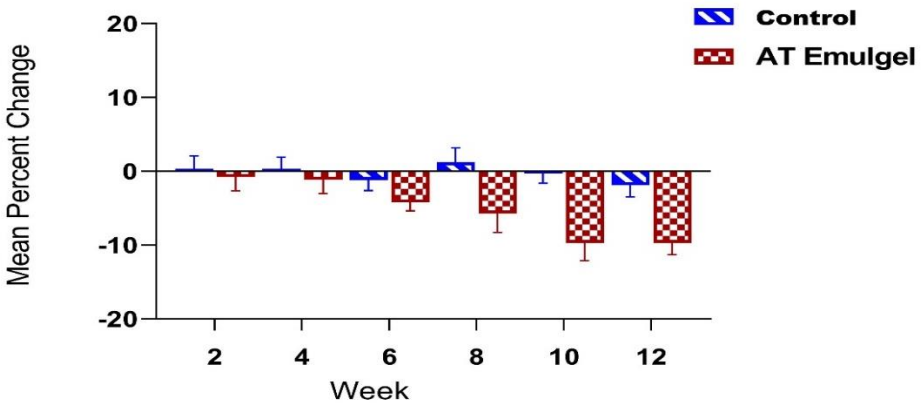

Figure 8. Vicissitude in Erythema counts after treatment with Control and AT Emulgel during course of study

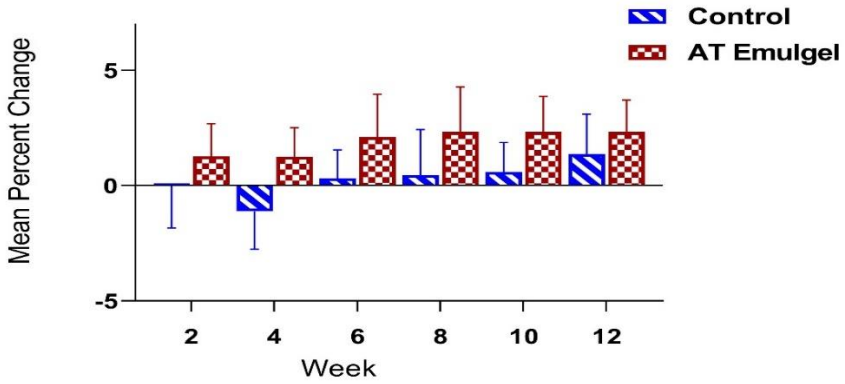

Figure 9. Vicissitude in Melanin counts after treatment with Control and AT Emulgel during course of study

3.6 Moisture level

Application of a formulation emulgel containing AT extract led to a rise in moisture content, while application of a control emulgel (without extract) led to a modest increase in moisture level, as shown in Figure 10. Insignificant rise in moisture level by control was found by analysing two-way ANOVA results ($p > 0.05$). Formulations using AT extract were found to significantly raise moisture level ($p < 0.05$). Due to the AT's protective nature and the layer's development to create a barrier for water loss from the skin layers, the moisture content of the skin has increased.

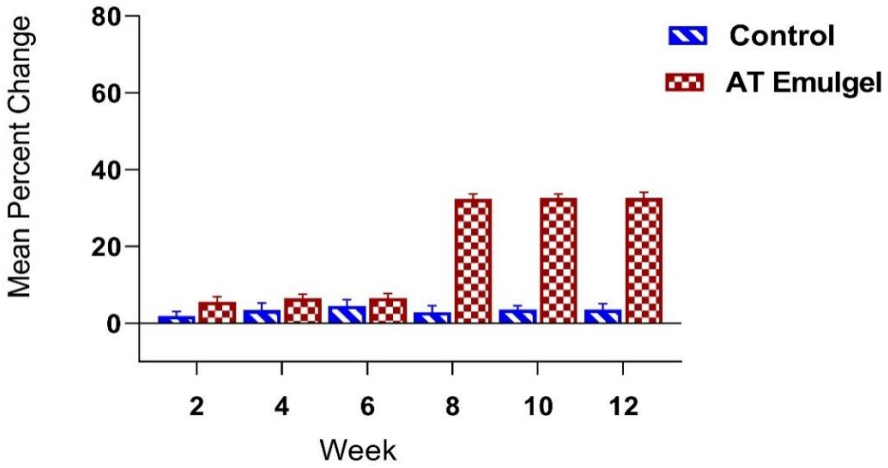

Figure 10. Variation in Moisture counts after treatment with Control and AT Emulgel during course of study

3.7 Sebum level

When control emulgel (without extract) was applied, sebum level increased slightly, while application of formulation emulgel with AT extract caused a modest decrease in sebum content (Figure 11). Sebum level by control showed a negligible increase according to examination of two-way ANOVA findings ($p > 0.05$). In contrast, a test formulation including AT extract showed a substantial reduction in sebum level ($p < 0.05$). Phenolics and Poly-phenols are reason for the decrease in sebum content.

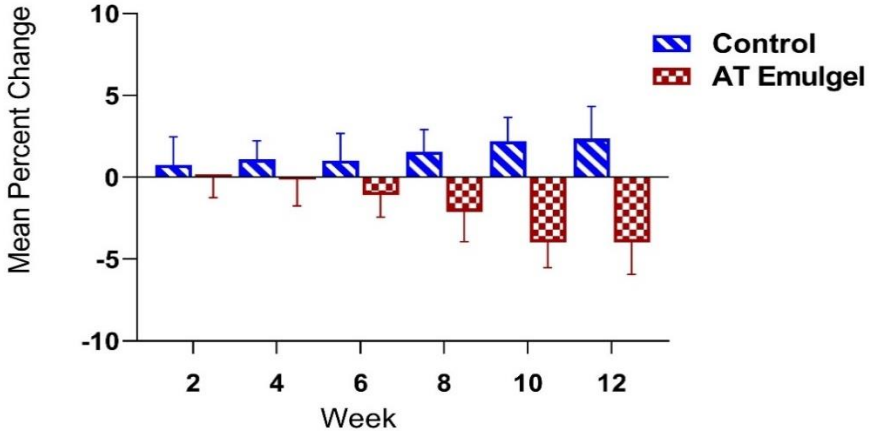

Figure 11. Vicissitude in Skin's Sebum counts after treatment with Control and AT Emulgel during course of study

3.8 Skin elasticity level

According to the findings, applying a formulation emulgel with AT extract increased elasticity but applying a control emulgel (without extract) did not, as shown in Figure 11. Analysis of the two-way ANOVA findings revealed a negligible improvement in control elasticity ($p > 0.05$). While formulations incorporating AT extract showed a substantial increase in elasticity ($p < 0.05$).

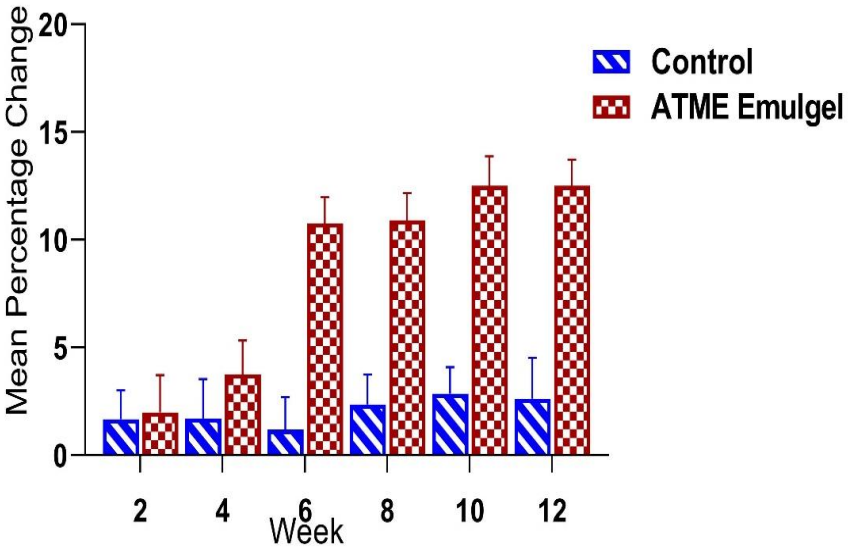

Figure 12. Vicissitude in Skin's Elasticity counts after treatment with Control and AT Emulgel during course of study

3.9 Skin Pores count measurements

Applying a control and emulgel containing AT extract allowed researchers to examine the cheek pores of volunteers using the Visio face® apparatus (courage + khazaka). A three-month timeframe was used to conduct the study (90 days).

3.9.1 Skin Fine and large facial pores

When control emulgel without AT extract is applied, there is an increase in facial pores. On the face, there is a rise in proportion of both small and large pores. Percentage change rise in the number of small facial pores was 11.13% to 15.75% after using control emulgel, it is not statistically significant ($p > 0.05$). And significant fall in both small and large facial pore size has been observed continually. Significant values were obtained ($p < 0.05$) and it was approximately 19.4± 2.3% by applying AT extract loaded emulgel. Results are depicted in figure 13 and 14 respectively. The majority of participants showed11 % increases in the number of pores after using control emulgel, whereas all of the volunteers who used AT-containing emulgel had a fall in the number of facial pores. A volunteers' cheek was depicted in Figures 15 and 16.

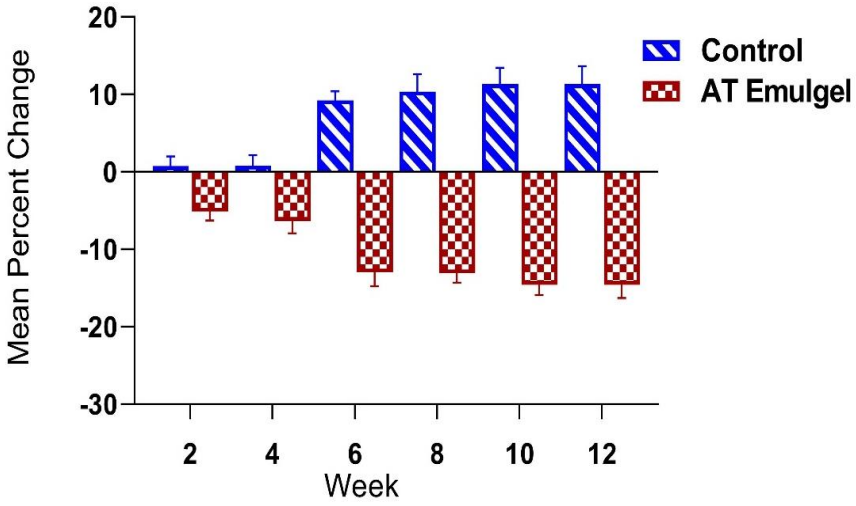

Figure 13. Vicissitude in Skin's Small Pores after treatment with Control and AT Emulgel during course of study

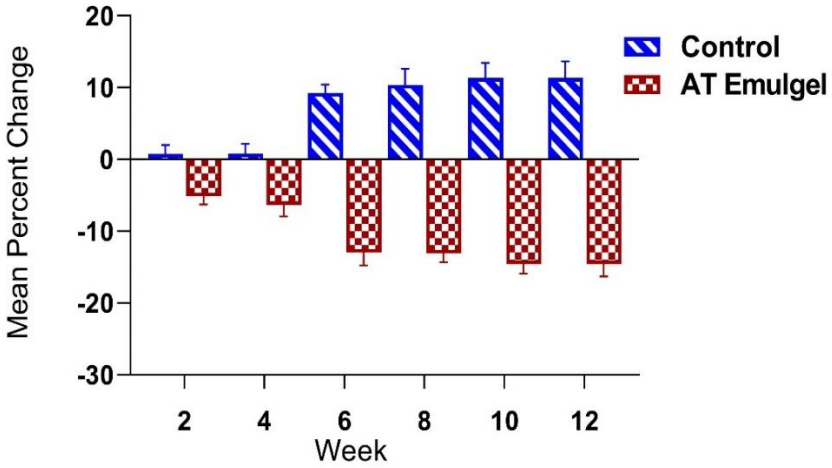

Figure 14. Variation in Skin's Larger Pores after treatment with Control and AT Emulgel during course of study

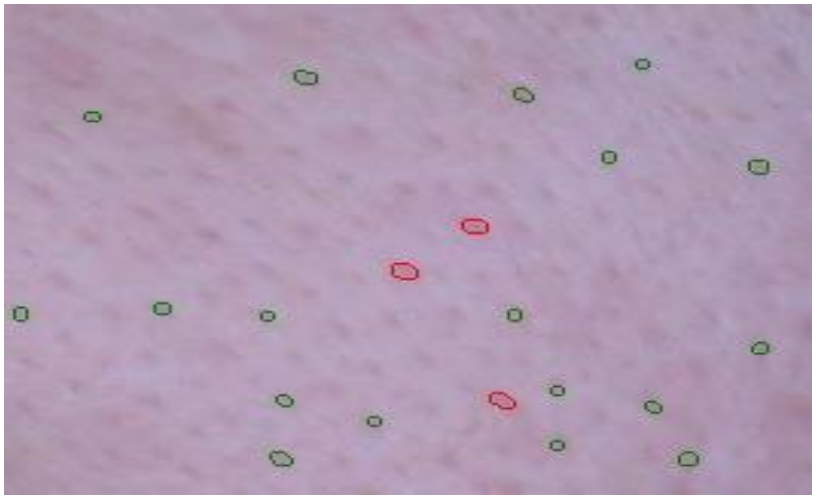

Figure 15. Image of volunteer's Skin Fine and Large Pores before treatment with Control Emulgel during course of study

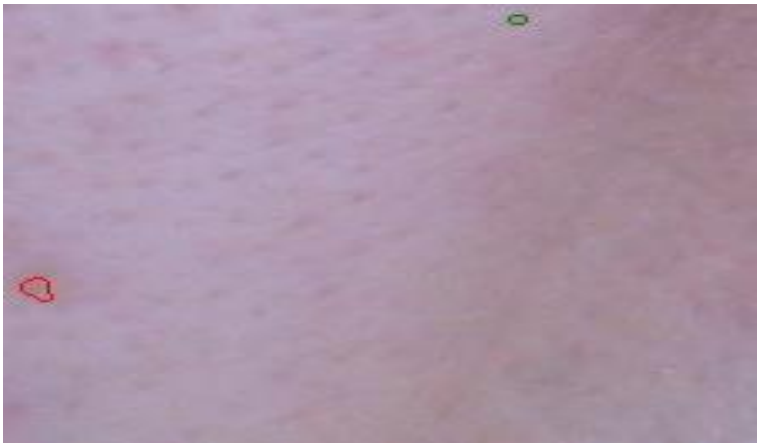

Figure 16. Image of volunteer's Skin Fine and Large Pores after treatment with AT Emulgel during course of study

3.9.2 Effect on fine and large facial pores count

After using control emulgel and emulgel containing AT for a period of 12 weeks, the % improvement in the number of small and large facial pores has been shown in Table 5. After using

control emulgel, the mean percentage vicissitude in small and large pores counts was -11 and 34.2%, respectively.

Table 5 Vicissitude in number of fine and large skin pores

No. of volunteers	Fine pores (AT emulgel)	Fine pores (control emulgel)	Large pores (AT emulgel)	Large pores (control emulgel)
1	-36.5	36.54	13.75	78.3
2	-33.66	39.15	-25.98	39.89
3	-34.14	38.09	19.79	35.19
4	23.3	77.82	-84.57	40.3
5	29.6	34.34	-8.37	86.56
6	-3.45	98	-89.37	55.71
7	-3.13	77.32	-99.39	79.99
8	11.51	80.35	-32.55	49.61
9	-5.21	79.22	-87.45	97.28
10	-6.62	86.44	2.11	99
11	-1.18	64.67	-35.19	73.82
12	-20.19	34.57	2	87.33
13	-3.64	35.34	-4.26	33.41

3.9.3 Fine and large skin pores area determine

The percentage variation in the size of the fine and large face skin pores after applying control emulgel first decreased during the first 14 days, then gradually increased by 6% to 46% over time, however the results were not statistically significant ($p > 0.05$). While the results of using an emulgel containing AT were significant ($p < 0.05$), there has been a gradual decrease in the size of the small and large skin pores (30.11% and 45.23%).

4 DISCUSSIONS

To treat various skin damage disorders brought on by exposure to sunshine, the usage of natural antioxidants has expanded for cosmetic purposes. The skin is damaged by UVA and UVB radiation in particular, which also generate hypersensitivity reactions, hyper pigmentation, and ageing effects. Antioxidants, in particular Flavonoids and phenolic acid, are obtained from various herbal extracts for this purpose. Natural antioxidants like Flavonoids and phenolic acid are very effective at scavenging free radicals. As a result, the quantities of these secondary metabolites in the AT extract from the entire plant were determined. When compared to control emulgel, an extract of *Alkanna tinctoria* was found to be substantially more effective at reducing the effects of skin Photodamage ($p< 0.05$). It has also reported in earlier investigations of AT extract how much of these secondary metabolites are present there.

The skin, the body's outermost defense mechanism, is constantly exposed to pollutants as well as sunlight's harmful UV rays. The skin becomes damaged over time by exposure to these damaging UV rays, which also raises the level of melanin in the epidermis, causing hyper pigmentation and tanning of the skin. Recent research demonstrated that by consistently and considerably reducing the amount of melanin in the skin and improving the condition of the skin due to the presence of Flavonoids, Phenolic content, Shikonin and Alkanin, AT-containing extract emulgel can be applied. According to earlier research, antioxidant-rich botanical extract lowers the amount of melanin in the skin.

The secondary metabolites' anti-oxidant capacity and the Flavonoids and phenolic contents' suppression of Melanogenesis may be the reason for the decrease in melanin content seen following application of the AT extracts emulgel. The Phenolics, Shikonin, Flavonoids, and Alkannin found in AT extract also have anti-inflammatory properties. By reducing the activity of the enzymes Cyclooxygenase (COX) and Lipoxygenase, Flavonoids primarily have the capacity to stop the process of inflammation. Numerous anti-inflammatory medications have been used to reduce inflammatory symptoms, particularly pain, Edoema, redness, elevation of body temperature, and

swelling. Consequently, the capacity for reducing welling and Erythema AT extract has been shown to have a strong ability to calm, soothe, and relax the skin.

The conversion of testosterone is caused by 5-reductase type 1 and androgen receptors. Large androgen receptors located throughout the skin have been demonstrated by a research review into its active state. Previous investigations have demonstrated that lowering the activity of 5-reductase also inhibits the formation of sebum. Quercetin and Kaempferol, two phenolic components found in AT extract, work by suppressing the 5-reductase enzyme's ability to convert testosterone into its active form Dihydro testosterone, hence reducing sebum output. It has been demonstrated in the current study that using AT extract-containing emulgel causes a steady decrease in sebum secretion over the course of the 12-week trial period. Many patients who complained about their oily skin have noticed an increase in the size of their facial pores, both large and small. Because sebaceous glands are present on the skin's surface, sebum production has risen, giving the impression of oily skin. Sebaceous filaments block large skin pores as a result of increased sebum secretion, which also makes skin oily, look shiny, and feel greasy. There is a connection between sebum secretion and pore size, according to earlier studies. This issue is receiving a lot of attention from dermatologists in the fields of cosmetic and dermatitis, who are working to find a solution.

Recent research has shown that the application of control emulgel increases the number of pores on the skin, both small and large, as well as the area of those pores. When AT extract with emulgel was used, there was a noticeable, ongoing decrease in the quantity and size of both small and big pores. After the application of AT-containing emulgel, reduced the size and quantity of both small and larger pores has been seen. Sebum content also improved in a similar manner, rising when control emulgel was applied while falling when AT-containing emulgel was used. This improvement was closely related to pore size, area, and sebum secretion. Therefore, it was discovered that emulgel containing AT was very powerful and promising in reducing sebum output and treating skin conditions linked to face pores.

5 CONCLUSIONS

In conclusion, numerous skin disorders, including elasticity, Erythema level, Sebum content, and pore size, can be improved by long-term use and consistent topical administration of AT extract containing emulgel. Additionally, it is useful in reducing the risks associated with light-induced skin reactions and hypersensitivity. It is a preferred ingredient for dermatological treatments because of its antioxidant properties of Alkannin and Shikonin, which are used to treat a variety of skin issues linked to Photodamage. In the future, this medication may be tested for Dermatitis, Hypersensitivity reactions, Melasma, and anti-acne uses in clinical settings. As a last thought, the created AT emulgel formulation was effectively applied topically and proved to be a good contender as a cosmetic and medicinal agent.

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