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Effect of Excipients on the Phenolic Content in Pumpkin Leaf Extracts after Their Introduction into Semisolid Pharmaceutical Forms and Evaluation of In Vitro Stability

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ABSTRACT

The present study aimed to formulate semi-solid pharmaceutical forms from pumpkin leaves (Cucurbita pepo Casper). Total phenolic content was measured after extracting the leaves from Ghouta, Syria. Plant extracts were prepared from Cucurbita pepo Casper leaves using four solvents: ethanol (70%), ethanol, aqueous, and methanol extract. The phenolic content of each extract was quantified. The most effective extracts were incorporated into semi-solid pharmaceutical forms. The influence of excipients on phenolic content was evaluated by measuring phenolic levels in the semisolid formulations and analyzing changes in viscosity and consistency on the shelf. The aqueous extract of Cucurbita pepo 'Casper' leaves exhibited the highest phenolic content, with a concentration of 2.21 ± 11.77 mg/g dry powder. This extract was selected for formulation into three distinct pharmaceutical bases. Among the formulations, the water-in-oil (w/o) cream demonstrated the highest phenolic content and superior stability compared to the oil-in-water (o/w) cream. Stability tests conducted over a three-month period confirmed that the w/o cream maintained optimal stability. Thus, the w/o cream was determined to be the most effective formulation.

Keywords: Cucurbita pepo Casper leaves, aqueous extract, phenolic content, Gallic acid.

1- INTRODUCTION

In recent years, medicinal plants have gained prominence due to their biologically active compounds, which offer various benefits for skincare and overall health. Plant extracts are abundant in vitamins, antioxidants, essential oils, proteins, and other bioactive compounds, providing a range of biological effects including antioxidant, anti-inflammatory, antiseptic, and antimicrobial properties [1-2].

Phenolic compounds are of particular interest due to their ability to neutralize free radicals [3] and their potential in protecting skin from ultraviolet radiation [4]. The composition of phenolic compounds is significantly influenced by the extraction method and solvent used [5].

Cucurbita pepo 'Casper' was selected in this study due to its high carotenoid content, antioxidant capacity, and substantial levels of carbohydrates and minerals, which are beneficial for skin health [6]. Additionally, the plant contains polysaccharides, para-aminobenzoic acid derivatives, sterols, proteins, and peptides, contributing to its medicinal properties, including anti-tumor, immune-enhancing, antibacterial, cholesterol-lowering, anti-parasitic, and anti-inflammatory effects [7-10].

The primary objective of this research was to extract and quantify the phenolic content of Cucurbita pepo

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'Casper' leaves and to incorporate these extracts into semisolid pharmaceutical formulations. The study also aimed to evaluate the impact of excipients on the stability and efficacy of phenolic compounds within these formulations.

Study focus: Cucurbita pepo 'Casper' leaves.

2- MATERIALS AND METHODS:

2-1- Devices and Tools Used: Rising coolant extraction device (Büchi, Flawil, Switzerland), Soxhlet extractor device (Sigma-Aldrich, St. Louis, Missouri, USA), Frosted volumetric flasks 1000 ml, 500 ml, 50 ml (Pyrex, Corning, New York, USA), Sensitive electronic scale AX200 (Shimadzu, Kyoto, Japan), Spectrophotometer device, Hitachi-U-1800(Hitachi, Tokyo, Japan), Mill. Thermometer (IKA, Staufen, Germany), Magra 50 ml and 100 ml (Wilmad-LabGlass, Buena, New Jersey, USA), Frozen micropipettes with different capacities (Eppendorf, Hamburg, Germany), Test tubes - micropipette tips (Gilson, Middleton, Wisconsin, USA), Spectro device T80+ (PG Instruments, Leicester, United Kingdom), Rotary evaporator (IKA, Staufen, Germany).

2-2- Materials:

Cucurbita pepo Casper leaves (Purity: Not applicable (natural material), Farm-grown, Western Ghouta, Damascus, Syria), Distilled water (100% pure distilled water, Generic Laboratory Supply, Syria), Absolute ethyl alcohol (99.5%, Eurolab, London, United Kingdom), Methanol (99.8%, Sigma-Aldrich, St. Louis, Missouri, USA), Anhydrous sodium carbonate (99-100%, PanReac AppliChem, Barcelona, Spain), Standard Gallic acid (98%, Titan Biotech, Rajasthan, India), Folin-Ciocalteu reagent, (Reagent grade, Fluka, Buchs, Switzerland).

2-3- Methods:

2-3-1. Preparation of Plant Samples: Cucurbita pepo* 'Casper' leaves were collected, dried, and ground into a fine powder. Then it was stored in tightly sealed containers to prevent moisture absorption.

2-3-2. Extraction Methods: Four types of extracts were prepared

- Methanolic Extract: 30 g of dried plant powder was extracted with 250 ml of methanol for 2 hours using a Soxhlet extractor. The extract was then concentrated and dried using a rotary evaporator [11].
- Aqueous Extract: 30 g of plant powder was extracted with 200 ml of distilled water, heated under reflux for 1.5 hours, and then evaporated to dryness.[12]
- Ethanolic Extract: 30 g of plant powder was extracted with 300 ml of ethanol for 2 hours. The extract was concentrated and dried using a rotary evaporator.[11]
- Ethanolic 70% Extract: 30 g of plant powder was extracted with 300 ml of a 70/30 mixture of ethanol and distilled water for 2 hours. The resulting extract was dried using a rotary evaporator.[11]

2-3-3 Determination of Total Phenols (T.P.)

The total phenolic content was determined using the Folin-Ciocalteu method. In this process, phenolic compounds reduce the tungsten-molybdate-phosphoric acid complex in an alkaline medium, resulting in the formation of a blue-colored solution. The absorbance of this solution was measured at a wavelength of 760 nm, as a result of a series of reactions where one or two electrons are transferred from the phenolic compounds, leading to the formation of blue-colored complexes.[13]

Preparation of the Standard Series: Gallic Acid Solution: Dissolved 0.5 grams of gallic acid in 10 ml of ethanol, then diluted with distilled water to a final volume of 100 ml in a volumetric flask. The solution can be used daily but is best stored in a closed container in the refrigerator for up to two weeks to prevent degradation.

Preparation of the Calibration Curve: A standard series was prepared by adding 0, 1, 2, 3, 4, 5, and 10 ml of the gallic acid solution to 100 ml volumetric flasks, and then diluting each to the mark with distilled water.

This results in phenol concentrations of 0, 50, 100, 150, 250, and 500 mg/L of gallic acid.

Twenty microliters (20 µL) of each standard solution

were pipetted into separate cuvettes. To each cuvette, 1.58 mL of distilled water and 100 µL of Folin–Ciocalteu reagent were added, and the mixture was thoroughly mixed. The samples were incubated for 8 minutes and 30 seconds, after which 300 µL of 20% sodium carbonate solution was added. The mixtures were stirred again and left to stand for 2 hours at 20 °C. Absorbance was then measured at 760 nm using a spectrophotometer, with distilled water serving as the blank. [14]

Preparation of Sodium Carbonate Solution: To prepare a 20% sodium carbonate solution, 200 grams of anhydrous sodium carbonate was dissolved in 800 ml of distilled water. The solution was boiled, cooled, and left to stand for 24 hours before being filtered and diluted to a final volume of 1 liter.[13]

The analysis was conducted in triplicate, and the average values were calculated along with the standard deviation.

2-3-4. Preparation of Pharmaceutical Forms: Three semisolid formulations were prepared: water-in-oil (w/o) cream, oil-in-water (o/w) cream, and hydrophilic cream. Each formulation was analyzed for phenolic content, viscosity, consistency, and stability.

Table (1): Pharmaceutical Formulations (content and ingredients table to follow).

the number	Materials	Formula1	Formula2	Formula 3
1	stearic acid	-	3%	10.5%
2	Tri ethanolamine	-	-	2.5%
3	PolyethyleneGlycol200	40%	-	-
4	Polyethylene glycol 4000	25%	-	-
5	beeswax	-	5%	
6	OgreCytosteryl	-	22%	7%
7	Paraffin oil	-	5%	15%
8	Glycerin	-	10%	15%
9	The spans80%	-	5%	-
10	Preservative	0,1%	0,1%	0,1%
11	Almond oil	-	15%	15%
12	Aqueous extract	35%	35%	35%

Preparation Methods:

A. Hydrophilic Cream (Formula 1):

Polyethylene glycol 4000 was melted in a water bath. Polyethylene glycol 200, the aqueous extract, and the preservative were then added and mixed continuously until the desired cream consistency was achieved.

B. Water-in-Oil (w/o) Cream (Formula 2):

Stearic acid was melted, followed by the addition of beeswax and cetostearyl alcohol. Paraffin oil, almond oil, and sulfur 80 were incorporated at 70°C. Concurrently, the aqueous phase was prepared by dissolving the preservative and glycerin in the aqueous extract. This aqueous phase was then added to the oil phase with continuous stirring in a water bath until the mixture cooled (room temperature).

C. Oil-in-Water (o/w) Cream (Formula 3):

Stearic acid was heated in a water bath until fully melted, after which triethanolamine was added. The remaining ingredients (beeswax, cetostearyl alcohol, paraffin oil, almond oil) were heated to 70°C. This mixture was then combined with the aqueous extract, which had

been mixed with glycerin and heated to 75°C. Stirring continued gradually until the mixture cooled.

2-3-5- Determination of Phenolic Content in Prepared Pharmaceutical Forms:

The phenolic content of each formulation was determined by weighing 1 g of each cream, dissolving it in 100 ml of phosphate buffer (pH 5.4), and stirring the mixture for one hour using a magnetic stirrer. The mixture was then centrifuged at 4500 rpm for 10 minutes, and the supernatant was filtered through a 0.45-micron filter. Optical absorption was measured at 760 nm, and the phenolic content was quantified using a standard curve for the extract. The analysis was performed in triplicate, and the average value along with the standard deviation was calculated.

2-3-6- Statistical Studies:

Statistical analysis was performed on the experimental data using GraphPad Prism software (GraphPad Prism, San Diego, USA). The analysis involved conducting a one-way ANOVA test to assess whether there were significant differences among the groups being compared. After the one-way ANOVA test, Tukey's post-hoc test was applied to determine which specific groups differed from each other. A p-value of less than 0.05 was considered to indicate a statistically significant difference between the groups.

3- RESULTS

3-1- Phenolic Titration Results in Plant Extracts:

Table 2 shows the gallic acid content in various extracts, with mean values and standard deviations, The letters indicate which values are statistically different from each other

Table 2 presents the results of phenolic titration for the four extracts of Cucurbita pepo 'Casper' leaves.

extract type	Ethanol extract	Ethanolic extract70%	Methanolic extract	Aqueous extract
Gallic acid content (sd)	11.10 ± 4.67^{b}	11.57 ± 2.06^{a}	11.07 ± 3.34^{b}	11.77 ± 8.43^{a}

Notes: Values are expressed as milligrams of gallic acid per gram of dry powder ± standard deviation (± sd).

Similar letters indicate that the statistical differences are not significant The different letters indicate that the statistical differences are significant, and the P value was relied upon to indicate the statistically significant difference.< 0.05

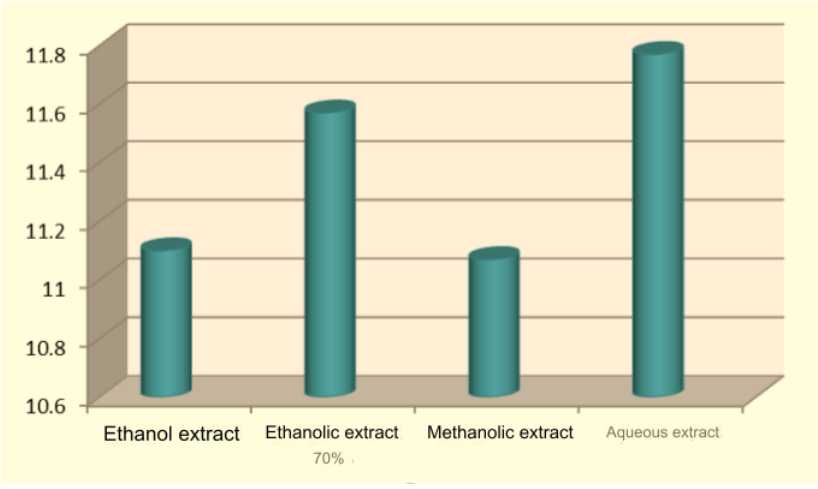

Figure (1) flowchart Phenols in extracts in four casper leaves

Table (3): Average readings of the standard series for Gallic acid

the concentration mg/L	300	250	200	150	100	50	0
Average absorption	0.86	0.72	0.59	0.49	0.31	0.16	0

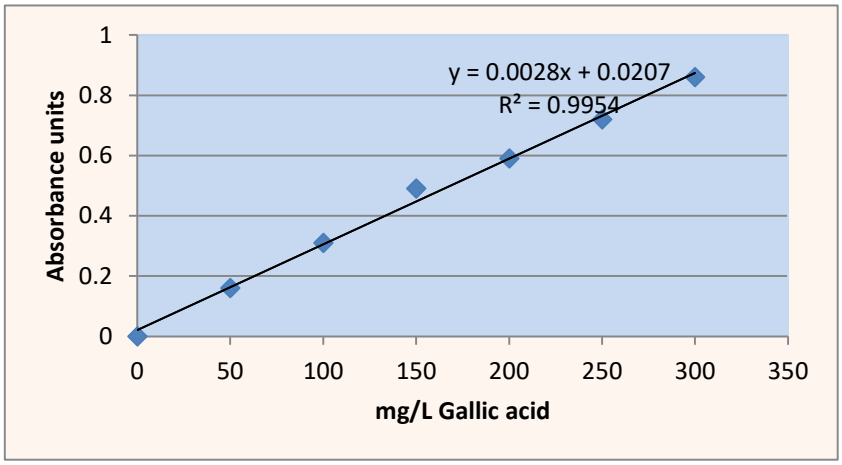

Figure 2: Graphical Curve for Gallic Acid Titration

The graphical curve illustrates the relationship between Gallic acid concentration and its absorbance. The results indicate that the aqueous extract of Casper leaves exhibited the highest gram equivalents of Gallic acid (mg/L) compared to the other extracts.

3-2- Results of the Effect of Excipients on the Phenol Titration in Prepared Pharmaceutical Forms: Table 4 presents the assay results for the phenolic content in the three prepared pharmaceutical forms.

Table 4: Gram Equivalent of Gallic Acid in the three Pharmaceutical Forms

Prepared pharmaceutical form	**Gram equivalent of gallic acid%**
Formula 1	**$\pm$3.64^{b}76**
Formula 2	**$\pm$2.42^{a}92**
Formula 3	**$\pm$1.64^{a}86**

Similar letters indicate that the statistical differences are not significant The different letters indicate that the statistical differences are significant, and the P value was relied upon to indicate the statistically significant difference.< 0.05

3-3- Test Results on the Prepared Pharmaceutical Forms:

3-3-1-Viscosity Examination of the Prepared Pharmaceutical Forms:

This test was performed to assess the ease of application of the prepared pharmaceutical forms at a temperature of 24°C using a viscometer. The results are summarized in Table 5.

Table 5: Viscosity of Prepared Pharmaceutical Forms

Viscosity±SD (Santi Boaz)	The type of pharmaceutical form prepared
2863±23.62 [a]	Formula 1
3266± 17.96 [b]	Formula2
3244±17.962[b]	Formula3

Similar letters indicate that the statistical differences are not significant As for the different letters, they indicate that the statistical differences are significant, and we relied on considering the P value to indicate the statistically significant difference.< 0.05.

Viscosity Results: The viscosity of the prepared pharmaceutical formulations was deemed appropriate for their ease of application on the skin. Formula 1 exhibited the lowest viscosity, while Formula 2 (w/o cream) displayed the highest viscosity.

3-3-2- pH Measurement Results:

Table 6 provides the pH values of the prepared pharmaceutical formulations.

Table 6: pH Values of Prepared Pharmaceutical Forms

The value of splendor pH	Type of pharmaceutical form prepared
5.08 ± 0.22 [b]	Formula 1
5.7±1.02[b]	Formula2
7.4±2.12[a]	Formula3

Similar letters indicate that the statistical differences are not significant The different letters indicate that the statistical differences are significant, and we relied on considering the P value < 0.05 to indicate a statistically significant difference.

pH Results: The pH values of the prepared pharmaceutical formulations were within acceptable ranges, closely matching the skin's natural pH (5.0-5.5), with the exception of the third formulation.

3-3-3- Shelf Stability Examination Results:

The three cream formulations were assessed for stability over one, two, and three months at 25°C. Throughout the testing period, the formulations demonstrated consistent homogeneity, hardness, consistency, and pH stability, indicating that all formulations maintained their stability.

4- DISCUSSION:

The study established that Cucurbita pepo 'Casper' leaves exhibit the highest phenolic content in the aqueous extract, followed in descending order by the 70% ethanolic extract, the ethanolic extract, and the methanolic extract. The observed differences in extraction efficiency are likely due to the interaction between the chemical properties of phenolic compounds and the extraction solvents. The solubility of phenolic compounds is influenced by the polarity of the solvent and the degree of ionization [15]. The aqueous extract exhibited the highest phenolic content, significantly surpassing that of 70% ethanol, likely due to its ability to extract a broader range of active compounds as a result of its polarity.

The aqueous extract was formulated into three distinct pharmaceutical bases, each incorporating different excipients. Among these, the water-in-oil (w/o) cream formulation preserved the highest phenolic content, followed by the oil-in-water (o/w) cream. The hydrophilic cream exhibited the lowest phenolic content. This underscores the crucial role of excipients in maintaining phenolic concentrations within pharmaceutical formulations.

In the formulation containing polyethylene glycol 200 and 4000 (Formula 1), a moderate level of phenolic content was observed. The reduction in phenolic concentration in this formulation may be attributed to the chemical reactivity of polyethylene glycol [16], which can enhance oxidative processes due to the presence of peroxides and secondary oxidation products. This oxidative activity may compromise the efficacy of the phenolic compounds and influence the formulation's physical properties, as polyethylene glycol increases mixture fluidity, potentially leading to phenolic degradation.

The w/o cream (Formula 2) (cream w/o) emerged as the most effective formulation, demonstrating superior phenolic content retention. The protective effect of the oil phase minimized phenol oxidation, and comprehensive stability, viscosity, and pH assessments confirmed its suitability for dermal applications. The excipients used in this formulation were compatible with phenolic compounds, facilitating optimal preservation.

In contrast, the o/w cream (Formula 3), while similar to the w/o cream but with the aqueous phase as the external phase, showed some degree of phenol oxidation due to environmental exposure, though it remained more effective than the hydrophilic cream.

These findings highlight the importance of selecting suitable excipients to optimize phenolic content and formulation stability. The w/o cream formulation was identified as the most effective in preserving phenolic compounds, ensuring the desired therapeutic properties such as anti-inflammatory, anti-allergic, antioxidant, tissue-strengthening, and disinfectant effects [17].

5- CONCLUSION:

Among the four extracts of *Cucurbita pepo* 'Casper' leaves, the aqueous extract exhibited the highest phenolic content, followed by 70% ethanolic, ethanolic, and methanolic extracts. The w/o cream formulation was the most effective in preserving phenolic content, showing suitable viscosity, pH, and stability. The o/w cream ranked second, whereas the hydrophilic cream, although functional, showed reduced phenolic content due to excipient incompatibility.

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تأثير المواد المضافة على محتوى الفينول في مستخلصات أوراق القرع بعد إدخالها في الأشكال الصيدلانية شبه الصلبة وتقييم ثباتها المخبري

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ملخص

هدفت هذه الدراسة إلى تحضير أشكال صيدلانية شبه صلبة من أوراق قرع الكوسا (Cucurbita pepo Casper). تم قياس المحتوى الفينولي الكلي بعد استخلاص الأوراق من غوطة دمشق/سوريا. أُعدت المستخلصات النباتية باستخدام أربعة مذيبات: الإيثانول (70٪)، والإيثانول النقي، والمائي، والميثانولي. ثم قُدر المحتوى الفينولي لكل مستخلص. أُدخلت المستخلصات الأكثر فعالية في الأشكال الصيدلانية شبه الصلبة. تم تقييم تأثير المواد المضافة على المحتوى الفينولي من خلال قياس مستويات الفينول في المستحضرات شبه الصلبة وتحليل التغيرات في اللزوجة والتماسك على مر الزمن. أظهر المستخلص المائي لأوراق القرع أعلى محتوى فينولي (2.21 ± 11.77 ملغم/غم مسحوق جاف)، واختير لتحضير ثلاثة قواعد صيدلانية مختلفة. من بين المستحضرات، أظهر كريم زيت/ماء (w/o) أعلى محتوى فينولي وثباتاً متفوقاً مقارنةً بكريم ماء/زيت (o/w). أكدت اختبارات الثبات لمدة ثلاثة أشهر أن كريم w/o حافظ على ثبات مثالي، مما يجعله الصيغة الأكثر فعالية.

الكلمات الدالة: أوراق قرع الكوسا، مستخلص مائي، محتوى فينولي، حمض الغاليك.

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