



Research Article

EMULSION FORMULATION OF *CAMELLIA SINENSIS* AND *ALLIUM CEPA* FOR *ALOPECIA AREATA*

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ABSTRACT

In this study aim was to prepare characterize the herbal topical emulsion formulation to hair with *Camellia sinensis*, *Allium cepa* extracts and with almond oil. *Allium cepa* extract (Bulb) and *Camellia sinensis* extract (Leaves), Almond oil (*Prunus dulcis*) have the capability to regrowth the hair effected by *Alopecia areata*. Initial the compatibility of herbal ingredients was evaluated and the physical properties of emulsions were studied. Then decided emulsion was prepared as w/o type emulsion. The formulations characterize via several parameters such as; Dye solubility test, piratical size analysis, organoleptic test, centrifugation, viscosity test, PH test, electrical conductivity. The characterization of prepared emulsions shows acceptable properties as per ICH guidelines for three months. The biological activities of prepared emulsion were tested for 10 days on Wistar rats in three groups. Finally, by these findings suggest that the formulated emulsion with extracts of *Allium cepa* and *Camellia sinensis* showed promising results to treat the *Alopecia areata*.

Keywords: *Allium cepa* extract and *Camellia sinensis*: almond oil: preparation of emulsion *Alopecia areata*.

INTRODUCTION

Alopecia areata (AA) is a common form of non-scarring Alopecia involving the scalp and/or body, characterized by hair loss without any clinical inflammatory signs. It is one of the most common form of hair loss seen by dermatologists and accounts for 25 % of all the Alopecia cases¹. Actually, *Alopecia areata* is two types one is *Alopecia totalis* and another one is *Alopecia universalis*. *Alopecia areata* occurs due to genetic tenderness, nutritional deficiency, oxidative stress², chemical exposure, pollution etc. There are so many synthetic drugs are available in markets to treat the *Alopecia areata*. This synthetic drug use satisfactory results but the results are associated with some side effects. Due to side effects of synthetic drugs we looked into medicinal plants to treat *Alopecia areata*. There are so many herbal plants have capacity to cure *Alopecia areata*, in that we selected *Allium cepa*³ and *Camellia sinensis*⁴.

To cure *Alopecia areata* we selected emulsion formulation, due to its pore permeation (rate of contact) and to supply oil soluble and water-soluble nutrients to hair. The *Allium cepa* extract contain water soluble compounds and *Camellia sinensis* compounds extracted with methanol are soluble in oil. So, by using these two medicinal plants compounds and using almond oil gives nutritional supply and medicinal cure to the hair.

MATERIAL AND METHOD

Ethanollic extract of *Allium cepa* (Authenticated by Dr. P. Satyanarayana Raju Department of Botany, Acharya Nagarjuna University): Dichloride methane extract of *Camellia sinensis* (Green Tea) were active ingredients, Almond oil as oil phase, distil water as aqueous phase, gum acacia as emulsifying agent, methyl paraben used as preservatives.

Method of Preparation

Water phase

Different concentration of *Allium cepa* extract was soluble in water to prepare three formulations. Quantities are mention in formulation Table 1.

Oil phase

Different concentration dichloride methane extract of *Camellia sinensis* were added or soluble in oil phase. Quantities are mention in formulation Table 1.

Mixing

Mortar and pestle were taken for mixing the two phases. The gum acacia was taken into mortar and triturate with almond oil for few minutes after that add water phase drop wise to the oil phase. The water extract was added drop by drop and the mixer was being mixed continuously. This was done until the click sound. The final volume was making up to 120 ml, by using homogenization.

Table 1: Compositions of emulsion formulations of *Camellia sinensis* extraction and *Allium cepa* extraction

S. No.	Ingredients	F1	F2	F3
1	Almond oil	57.14 ml	57.14 ml	57.14 ml
2	Water	28.57 ml	28.57 ml	28.57 ml
3	Acacia	14.28 g	14.28 g	14.28 g
4	Methyl paraben	0.1 g	0.1 g	0.1 g
5	<i>Allium cepa</i> extraction	3 g	1 g	2 g
6	<i>Camellia sinensis</i> extraction	1 g	3 g	2 g
7	Total makeup volume	120 ml	120 ml	120 ml

Evaluation parameters of prepared emulsion

The characterization of the emulsion is done in four steps those were compatibility studies and microscopic observation, short term stability studies.

Compatibility studies by FT-IR

FTIR studies were performed to detect possible molecular interaction between extracted compounds and utilized excipient

Microscopic observation

The microscopic observation is done to identify the type of emulsion by dye solubility test. The dye solubility test was done by using amaranth red dye.

Organoleptic tests

The emulsion was divided into two samples, which were kept at 8°C in refrigerator, 40°C. They were organoleptically evaluated to note alteration in odor, color, and phase separation for a period of 3 months at definite time intervals.

Centrifugation

Normally, flocculation and creaming are slow processes. However, these processes can be hastened by inducing stress conditions using centrifuge.

Particle size analysis for 100 particles

For the particle size analysis and particle size distribution, the stage microscope is used. In the stage microscope the 45th division

of the eyepiece micrometer coincides with 9th division of the stage micrometer (point “x”). Another point of coincidence is the 73rd division of the eyepiece micrometer and 10th division of stage micrometer and 10th division of stage micrometer (point “y”).

Viscosity test

The emulsion viscosity was measured using viscometer (Brookfield Rotational Digital Rheometer Model LV/DV-II) with spindle 42 at 1 rpm.

pH of the emulsion

The pH of the emulsion is determined by using pH meter

Ex-vivo hair growth activity assessment

The study was performed using male and female a white Wistar rats. We are taken permission to test the sample has been approved by the Institutional Animal Ethics Committee (IAEC); the project proposal no. HCOP/IAEC/PR-5/2018. The tested wistar rats are divided into three groups, each group has three rats these rats are placed in a cage. These animals are housed in cages with husk bed and nutritional supply up to 12 days. Hair at the back of rats was shafted at the area of 2.5 cm × 2.5 cm. The testing samples were applied onto the shafted areas to 3 groups with 3 rats. The hair growth in the tested areas was observed for 12 days.

RESULTS AND DISCUSSION

Compatibility studies by FT-IR

FTIR data was showed in following depicted Table 2.

Table 2: IR spectra data of extracts and mixtures

Functional groups	Standard ranges (cm ⁻¹)	Observed frequency for ethanol extraction of <i>Allium cepa</i>	Observed frequency for dichloromethane extraction of <i>Camellia sinensis</i>	Observed frequency for emulsion
O-H stretching	3590-3050	3289	3390	3394
Ar-H	3000-2800	2926	3009	2920
C-O-	1050-1350	1256	1166	1235
C=O	1550-1850	1639		1746
S=O	1050-1030	1022		1021
C-S	650-100	630		

Microscopic observation

Dye Solubility Test

The test emulsion shows that the droplets are in red colored and continues phase is colorless. So it means that emulsion is w/o type.

Stability studies

Organoleptic tests

No differences were observed in the organoleptic properties among emulsion for the three months of the experiments under different conditions.

Centrifugation

Centrifugation tests were performed by taking 10 ml for 10 minutes to check phase separation for 3 months at different time intervals. There was no phase separation found after centrifugation in any of the sample of emulsion. A good emulsion does not exhibit detectable separation of oil phase, until certain time period.

Particle size analysis for 100 particles

In this study we examined particle size distribution for 3 months. For particle size distribution we don't maintain any temperature conditions, but we take sample from actual stored emulsion container.

The calculations in calibration are given in the below.

1. point "X" on eyepiece micrometer $X = 45$ (a)
2. point "Y" on eyepiece micrometer $Y = 73$ (b)
3. no of eyepiece micrometer division between X & Y = $a-b = c = 73-45=28$
4. no of stage micrometer divisions between X and Y = 10 (d)
5. Distance between X and Y on stage micrometer = $10 \mu\text{m}$. (d) = $e = 10$. $10 = 100 \mu\text{m}$
6. "c" eyepiece division is equal to $c = e = 100 \mu\text{m}$
7. One eyepiece division is equals to $= e/\text{no of eyepiece micrometer divisions} = 100/28 = 3.57 \mu$

The bellow histogram represents the particle size distribution that formulated emulsion was shows smaller size (range between 1-5) above 80 compared to particle size range 5-10 and 11-15. Due to the smaller size particle size distribution the formulated emulsion shows more stability up to 3 months.

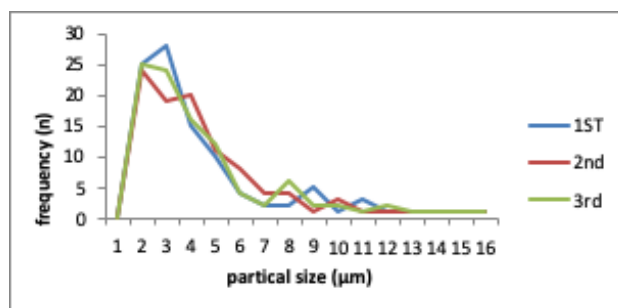


Figure 1: Histogram of partial size analysis

Viscosity test

The emulsion used in this study was shows no much variation in viscosity, so it indicates that emulsion shows stability up to 3 months.

Table 3: Viscosity parameters for 3 months at 8°C, 40°C temperature

S. No.	Months	Viscosity in Cp at 1 rpm		Viscosity in Cp at 5 rpm	
		8°C	40°C	8°C	40°C
1.	1 st month	105	101	102	98
2.	2 nd month	105	102	102	98
3.	3 rd month	106	102	101	98

pH of the emulsion

There were no significant changes in pH values of emulsion at 8°C and 40°C storage temperatures as a function of time. Minor changes of pH values as a function of time and storage temperatures indicated that the examined emulsion was stable.

Ex-vivo hair growth activity assessment

The hair growth tested samples images and results are showed in Table 4, Table 5.

Table 4: Hair growth images

Days	Blank	Marketed product	Test (herbal emulsion)
1 st day			
2 nd day			

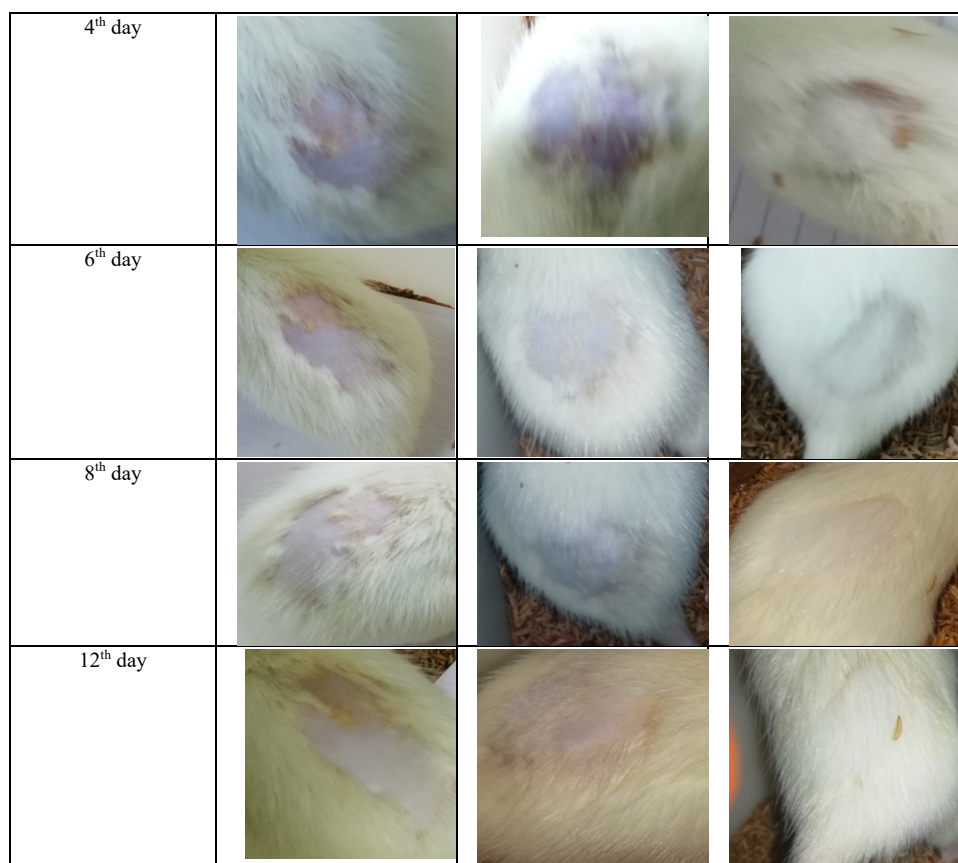


Table 5: Observed hair growths by graph paper in mm

Days observed	Blank growth in mm	Marketed product in mm	Test in mm
1 st day	0	0	0
2 nd day	0	0	0
4 th day	0	0	0.5
6 th day	0	0.1	0.89
8 th day	0	0.12	1.3
12 th day	0	0.2	1.9

CONCLUSION

From the present study, it can be concluded that the used extracts of *Camellia sinensis* and *Allium cepa* emulsion preparation with almond oil shows good stability up to 3 months as per ICH guidelines. For F3 formulation we performed animal studies for its biological activities. The emulsion is applied on to the hair shafted area of the wistar rats for 12 days shows faster growth compared with standard and blank.

Finally, by these findings the formulated emulsion is with extracts of *Allium cepa* and *Camellia sinensis*, showed best results to treat the alopecia areata.

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