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Formulation And Evaluation Of Herbal Hair Dye Containing Nigella *Sativa And Annona Squamosa*

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Abstract

In the present study, we have developed herbal hair dye. Most of the marketed synthetic hair dye formulations contain paraphenylenediamine at 20-25% concentrations. It is known to trigger allergic skin reactions in many people. Hence, there is need of herbal based hair dye which will not contain any harmful chemical constituents and which gives colours to hair. In this study, herbal hair dye containing *Annona squamosa and Nigella sativa* as a chief constituent was used. As *Annona squamosa* is a great natural source of copper and gives hair the dark melanin colour and that it lack. It also helps to deal with premature greying of hair in both men and women. *Nigella sativa* seed has antimicrobial, antioxidant, anti-aging, hair growth promoter, hair dying, sun protection, anti-cancer activity, which make it a novel ingredient for many cosmetic preparations. Other drugs used are Henna, Indigo, Bhringraj, Wallnut hull, Coffee, Amla, Shikakai, Hibiscus etc. for the preparation of herbal hair dye. Totally, 27 formulations were prepared using MINITAB software with factorial design of 5^3. The formulated herbal hair dye was evaluated for organoleptic, physicochemical, phyto-chemical, rheological, patch test, stability test, dye test and colour retention capacity. It was found that herbal preparations is greenish in appearance with characteristic odour and having a fine texture. The pH was 6.7 which is normal to the human scalp and hairs. The rheological parameters were in a acceptable range. All the formulations were found to be a good dying efficacy and colour retention capacity with no irritancy, swelling or redness. The formulation F25 showed highest colour retention capacity in comparison with marketed herbal preparation upto 50 Vs 32 days respectively. The formulated formulation is stable at room and at 40°C temperature.

Keywords: - Herbal hair dye, Dye retention capacity , White hair treatment etc

Introduction

In the present study, we prepared a powdered herbal hair dye that gives colour to hair, with better dyeing effect and greater retention capacity on comparison with marketed herbal hair dye formulation. Most of the marketed synthetic hair dye formulations contain paraphenylenediamine at 20-25% concentrations which is the main ingredient of commercial synthetic dyes. Itis known to trigger allergic skin reactions in many people. Ayurvedic powder hair dye devoid of any synthetic agent was prepared in present work and evaluated for dyeing efficiency. Different combinations of

powdered herbal drug were evaluated as hair dyes. Polyherbal powder hair dye of present investigation is semi- permanent in nature and exhibit better dyeing efficiency than marketed herbal Hair dye. We have developed a herbal hair dye containing *Nigella sativa and Annona squamosa* that will be helpful for treating premature greying of hair, also to treat whitehairs. Medicinal herbs have been used to treat white hairs since ancient times. As *Annona squamosa* is a great natural source of copper and gives hair the dark melanin colour and that it lack. It also helps to deal with premature greying of hair in both men and women. Presence of various natural active ingredients makes *Nigella sativa* seed as great medicinal herb. *Nigella sativa* seed has antimicrobial, antioxidant, anti-aging, hair growth promoter, hair dying, sun protection, anti-cancer activity, which make it a novel ingredient for many cosmetic preparations. We have selected herbal drugsbecause herbal medicines have always been deemed safer than any other medicine due to the absence of side effects comparable to those associated with allopathy. The presence of essential phytochemical components were observed as per earlier study report, during the medicinal herbs screening. Other drugs used are Henna, Indigo, Bhringraj, Wallnuthull, Coffee, Amla, Shikakai, Hibiscus etc. For the preparation of herbal hair dye, the cold pressing method for extraction of *Nigella sativa* and Sieving method for the *Annona squamosa* was used. The properties of herbal hair dye in the field of organoleptic have been further evaluated. It was found that herbal preparationswere free of chemicals, homogeneous mixture and with a smooth texture and the appearance of greenish colour.

Materials and methods: The part of plants of *Nigella sativa* and *Annona squamosa* were freshly collected from local garden from Pandharpur, Maharashtra, India. The plant were identified and authenticated by, Department of Botany, Karmveer Bhaurao Patil, College Pandharpur, India. The Plant materials the cold pressing method for extraction of Nigella sativa and Sieving method for the Annona squamosa was used, then dried .Then powders were passed through sieve no 16, and used for further study.

Preparation of herbs - The Plant materials the cold pressing method for extraction of Nigella sativa and Sieving method for the Annona squamosa was used, then dried .Then powders were passed through sieve no 16, and used for further study. - Herbal drugs namely Nigella sativa, Annona squamosa, Lawsonia inermis, Indigofera tinctoria, Embilica officinalis, Acacia conicinna, Elipta prostrate, Walnut hull, Rosa sinesis, Coffee were screened at a concentration range of 10 gm, 10gm, 2gm, 5gm, 5gm, 4gm, 4gm, 4gm, 2gm,4gm respectively.

Phytochemical Screening-

In order to determine the presence and absence of primary and secondary metabolites, phytochemical screening of plant extracts has been carried out using a standard procedure to verify the presence and purity of herbal medicinal products. Phytochemical tests for Alkaloid, Flavonoid, Carbohydrates, Steroid, Glycoside, Tannins, Vitamins and Anthocyanin were carried out as per specified standards .

Preparation of herbal hair dye: -

The part of plants of *Nigella sativa* and *Annona squamosa* were freshly collected from local garden from Pandharpur, Maharashtra, India. The plant were identified and authenticated by, Department of Botany, Karmveer Bhaurao Patil, College Pandharpur, India. Evaluation of purity and quality of raw materials by morphological, physical and chemical techniques, toxicological studies were performed.The collected herbs were made into powdere, weighed according to the specified

concentrations in Table no.1.A herbal hair dye was prepared by mixing all the herbal powders.For the purpose of a evaluation human white hairs were collected from human voluntaries.The white hair samples were kept in the formulated paste for 30 min, then washed with water and observed for its dyeing effect (colour grade), safety parameters, for all formulations.

Table 1 Formulation chart of Herbal Hair Dye (5^3 factorial design)

Sr No.	Codes	Name of ingredients	Low	Middle	High
1	A	Annona squamosa	8	9	10
2	B	Nigella sativa	9	10	11
3	C	Lawsomnia inermis	1	2	3
4	D	Indigofera tinctoria	4	5	6
5	E	Embilica officinalis	3	4	5

Evaluation

1] Organoleptic Evaluation: -

Organoleptic characteristics for various sensory characters like color, taste, odour etc. was carefully carried out.(Table no.3)

2] Physico-Chemical Evaluation -

The physical and chemical features of the herbal hairdye were evaluated to determine the pH, its moisture content and ash value for the purpose of stability, compatibility and the amount of inorganic matter present in it. Loss on drying was determined by weighing about 2gm of the powder material in previously weighed dried petridish (tarred evaporating dish) and dried in an oven at 105-110℃, take two consecutive weights, which do not differ by more than 5mg. The weight after drying was noted and loss on drying was calculated. The percentage was expressed as %w/w with reference to air dried sample. Total Ash value determined by weighing 2 gm of powder accurately in a previously ignited and tarred silica crucible. The material was then ignited by gradually increasing the heat to 500-600℃ until it appear white indicating absence of carbon. It is then cooled in a dessicator and total ash in mg per gram of air dried material was calculated.(Table No.4)

3] Rheological Evaluation :-

Physical parameters like untapped or bulk density, tapped density, the angle of repose, Hausner's ratio, and carr's index were observed and calculated for the inhouse formulation. Bulk density symbolizes the adjustment of particles or granules collectively in the packed form. The formula for determination of bulk Density (D) is D =M/ V where M is the mass of particles and V the total volume occupied by them. This is determined by taking graduated cylinder. 100 grams of weighed formulation was added to the cylinder with the help of a funnel. The initial volume was noted and the sample was then tapped fully. The bulk density value was obtained from the initial volume and after tapping the volume noticed, from which tapped density was calculated. Hausner's ratio is linked with the interparticle friction and influences the powder flow properties. The Hausner's ratio is calculated as D /D' where D' is the tapped density and D, the bulk density. Carr's index helps to measure powder flow from bulk density. (Table no.5)

4] Patch Test :-

A small amount of the aqueous solution of hair dye behind the ear in an area of 1sq.cm and leaved it to dry. Irritancy, redness, and swelling were checked and noticed for regular intervals up to 24 hours, if any .(Table no.6)

5] Stability Test :-

Stability testing of the prepared formulation was performed by storing it at different temperature conditions for the time period of one month. The formulation were stored at different temperature conditions viz.,room temperature and 40°C and were evaluated for the physical parameters like colour, odour, pH, texture, and smoothness.(Table no.7)

6] Dye test :- Various dye formulations viz F1 to F27 were prepared using different ratios of *Annona squamosa*:*Nigella sativa*:Embilica officinalis:Lowsomnia inermis:Hibiscus rosa sinesis. The herbal powders were weighed accurately, mixed geometrically followed by mixing in a polybag(for 50 g).Each formulated powder (F1 to F27) was applied to the white hair. After 30 minutes the hair were washed and the colour of hair was observed and coded upon standard scale of 1 to 10. (Table no.8)

7] Retention capacity / Colour lasting capacity :-

Retention capacity of herbal hair dye can be defined as ability of hair dye to retain or last particular hair colour on white /blonde human hair for definite period of time.In present investigation, the hair was washed with mild shampoo on alternate days using tap water till 25 shampoo washes (50 days) and retention capacity was determined in terms of number of shampoo washes that a colour can withstand. Simultaneously, we conducted comparative study between formulated hair dye with marketed herbal hair dye and synthetic hair dye.

Result and Discussion: - Evaluation of herbal drugs for purity-

The herbal drugs namely Annona squamosa and Nigella sativa have been used in different proportions to prepare herbal hair dye. As specified in the standards, the results of a phytochemical test for all herbal medicinal drugs show that certain essential phytochemical constituents are present as per specified standards. (Table 2)

Table 2 Phytochemical screening

Sr. No	Test	*Annona squamosa*	*Nigella sativa*
1	Carbohydrate	(+)	(+)
2	Anthocyanin	(+)	(+)
3	Glycoside	(+)	(+)
4	Flavonoid	(+)	(+)
5	Vitamins	(+)	(+)
6	Steroids	(+)	(+)
7	Tannin	(+)	(+)
8	Volatile oil	(-)	(+)
9	Alkaloid	(+)	(+)

(+ presence of chemical constituents; - absence of chemical constituents)

General evaluation of hair dye : -

In order to evaluate herbal hair dye, the evaluation parameters are important tests. It has been established that the pH is satisfactory. The formulation has a pH of 6-7, which corresponds to normal skin pH and is not likely to irritate the skin. It was found that herbal preparations were free of gritiness, homogeneous mixture and with a smooth texture and the appearance of greenish colour. The rheological properties was found to be in the accurate range. The patch test showed the absence of any irritancy, itching and sweeling. The formulation was found a stable at room temperature and also at a 40°C.The results are mentioned in following tables.

Table 3. Organoleptic evaluation of herbal hair dye

Sr. No.	Parameters	Results
1	Colour	Greenish
2	Odour	Characteristic
3	Texture	Fine
4	Appearance	Powder

Table 4. Physico-Chemical evaluation of herbal hair dye

Sr. No.	Parameters	Results
1	pH	6-7
2	L.O.D	1.9%
3	Ash value	0.19

Table 5. Rheological evaluation of herbal hair dye

Sr.No	Parameters	Results
1	Bulk density	0.47
2	Tapped density	0.588
3	Carrs index	20
4	Hausners ratio	1.25

Table 6. Patch test of herbal hair dye

Sr.No	Parameters	Results
1	Swelling	Negative
2	Redness	Negative
3	Irritation	Negative

Table 7. Stability test of herbal hair dye

Sr.No	Parameters	Room temperature	40℃
1	Colour	no change	no change
2	Odour	no change	no change
3	pH	6.7	6.7

4	Texture	fine	fine
5	Smoothness	smooth	smooth

Dye Test :-

The formulated powder (F1-F27) was applied to the white hair. After 30 minutes the hair was washed and the colour of hair was observed of each batch. The presence of a nigeline in a Nigella sative gaves a better dying property. It gave a results ranges from lightest blond to black colour of a hair ,as mentioned in (table 8). Formulation F25 gave following result mentioned in Fig.1.

Figure no.1 Dye test

Sample white hair Hairs after 30 min of application Of formulation no.25

Retention capacity / Colour lasting capacity:

After 25 washes, it was found that , the marketed herbal hair dye has the retention capacity upto the 16th washes i.e. upto 32 days and formulated herbal hair dye showed a better result than marketed herbal formulation, i.e. it can lasts upto 50 days, which shows better dying retention capacity. The marketed synthetic hair dye lasts upto 30 washes i.e. 60 days as mentioned in Fig.2.

Figure no.2 Colour retention test

Marketed synthetic hair dye Marketed herbal hair dye Formulated herbal hair dye

Table 8. Dye test of herbal hair dye

nulation No.	Colour of hair after 30 min	nulation No.	Colour of hair after 30 min	Formulatio No.	Colour of hair after 30 min
F1	1	F10	3	F19	5
F2	1	F11	3	F20	5
F3	1	F12	3	F21	7
F4	1	F13	3	F22	7
F5	2	F14	3	F23	8
F6	2	F15	3	F24	8
F7	2	F16	4	F25	9
F8	2	F17	4	F26	8
F9	2	F18	4	F27	8

(Lightest blonde-1 , Medium blonde-2, Dark blonde-3, Lightest brown-4, Medium light brown-5, Dark brown-7, Darkest brown-8, Black-9)

A one-way ANOVA is used to determine whether or not there is a statistically significant difference between the means of three or more independent groups. One-way ANOVA was performed to compare the effect of Annona squamosa and nigella sativa on a dye test and colour retention capacity. MINITAB software was used to perform the one-way ANOVA. The ANOVA output provides an estimate of how much variation in the dependent variable that can be explained by the independent variable. The first column lists the independent variable along with the model residuals. The Df column displays the degrees of freedom for the independent variable. The Sum Sq column displays the sum of squares between the group means and the overall mean explained by that variable. The Mean Sq column is the mean of the sum of squares, which is calculated by dividing the sum of squares by the degrees of freedom. The F value column is the test statistic from the F test: the mean square of each independent variable divided by the mean square of the residuals. The larger the F value, the more likely it is that the variation associated with the independent variable is real and not due to chance. The Pr(>F) column is the p value of the F statistic. This shows how likely it is that the F value calculated from the test would have occurred if the null hypothesis of no difference among group means were true.
Because the p value of the independent variable, Annona squamosa and Nigella sativa, is statistically significant ($p < 0.01$), it is likely that Annona squamosa and Nigella sativa gives a significant effect on hair dye and colour retention capacity as mentioned in table no.(9,11,13 and 15).

Dye test :
In present study we performed a 3 level study for the better dye efficacy as a 8,9 and 10 for Annona squamosa and 9,10,11 for Nigella sativa. After the first wash the colour of hair was noted down as 1 to 10 based on a commercial hair dye scale. Levels are on the X-axis of the interval plot and numerical (1-10) on Y- axis as a colour of hair(dye test).

Retention capacity :
In present study, to determine the colour retention capacity, we took a 60 days study of hair dye. We took a 30 wash of a hairs on a alternate days i.e. 60 days, and observed results was noted down in terms of days, on which days colour was fade or disappears or changes. The levels of Annona

squamosa and Nigella sativa are presented on the X-axis and colour retention capacity on Y-axis in terms of days .

One-way ANOVA: Dye test versus A.squamosa

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

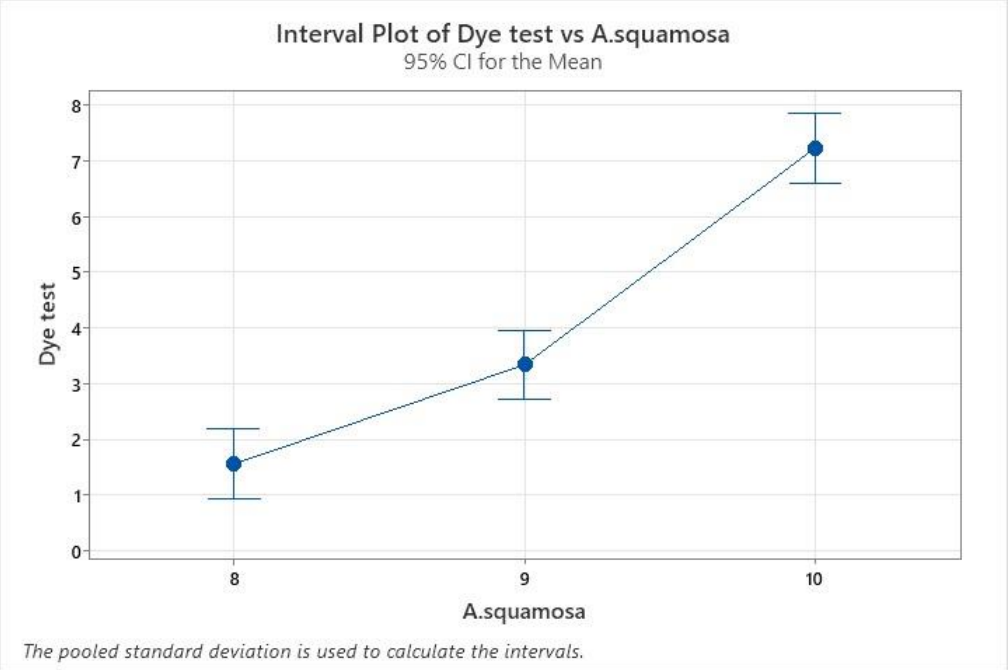
Table 9. Analysis of Variance

A.squamosa	N	Mean	StDev	95% CI
8	9	1.556	0.527	(0.931, 2.180)
9	9	3.333	0.500	(2.709, 3.958)
10	9	7.222	1.394	(6.598, 7.847)

Table 10. Means

A.squamosa	N	Mean	StDev	95% CI
8	9	1.556	0.527	(0.931, 2.180)
9	9	3.333	0.500	(2.709, 3.958)
10	9	7.222	1.394	(6.598, 7.847)

Pooled StDev = 0.907785



A one-way ANOVA was performed to compare the dye test of a Annona squamosa. It revealed that there was a statistically significant difference in a F-value =91.73 and P-value = 0.01. significance level was α = 0.05. The pooled standard deviation was 0.907785. The R.Squared value obtained was 88.43%, R.Squared(adjacent) was 87.47% and R.Squared(prepared) was 85.36% ,As there is no significant change in the values of a R.Squared(adjacent) and R.Squared(prepared), Annona squamosa have a significant effect on hair dye test.

One-way ANOVA: Retention capacity (days) versus A.squamosa
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

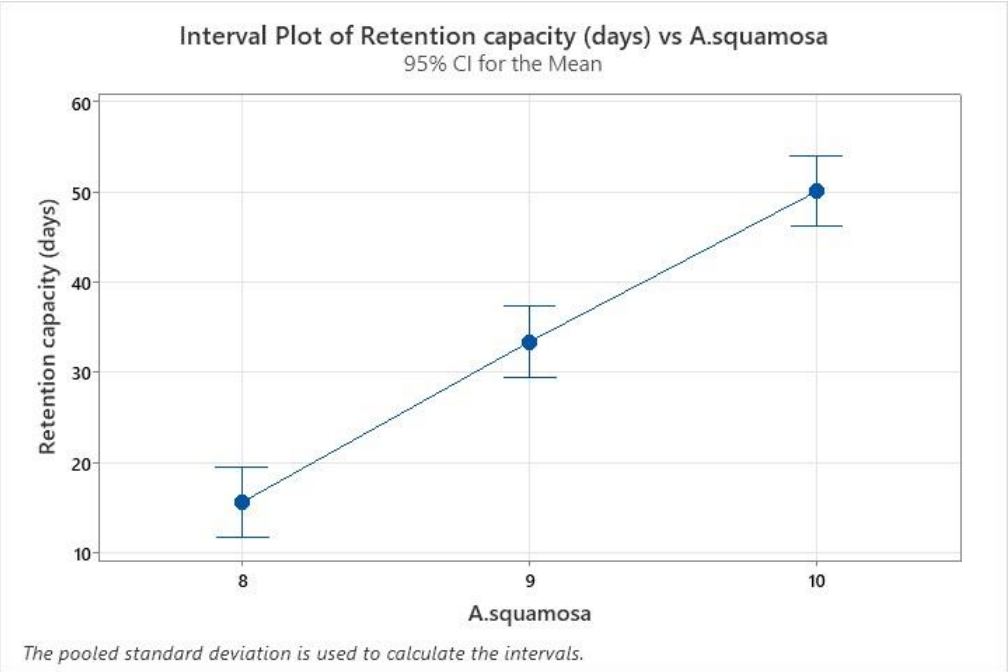
Table 11. Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
A.squamosa	2	5340.7	2670.37	82.99	0.01
Error	24	772.2	32.18		
Total	26	6113.0			

Table 12. Means

A.squamosa	N	Mean	StDev	95% CI
8	9	15.56	5.27	(11.65, 19.46)
9	9	33.33	5.00	(29.43, 37.24)
10	9	50.00	6.61	(46.10, 53.90)

Pooled StDev = 5.67238



A one-way ANOVA was performed to study the retention capacity of a Annona squamosa. It revealed that there was a statistically significant difference in a F-value =82.99 and P-value = 0.01. significance level was α = 0.05.The pooled standard deviation was *5.67238*. The R.Squared value obtained was 87.37%, R.Squared(adjacent) was 86.31% and R.Squared(prepared) was 84.01% .

One-way ANOVA: Dye test versus N.sativa

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

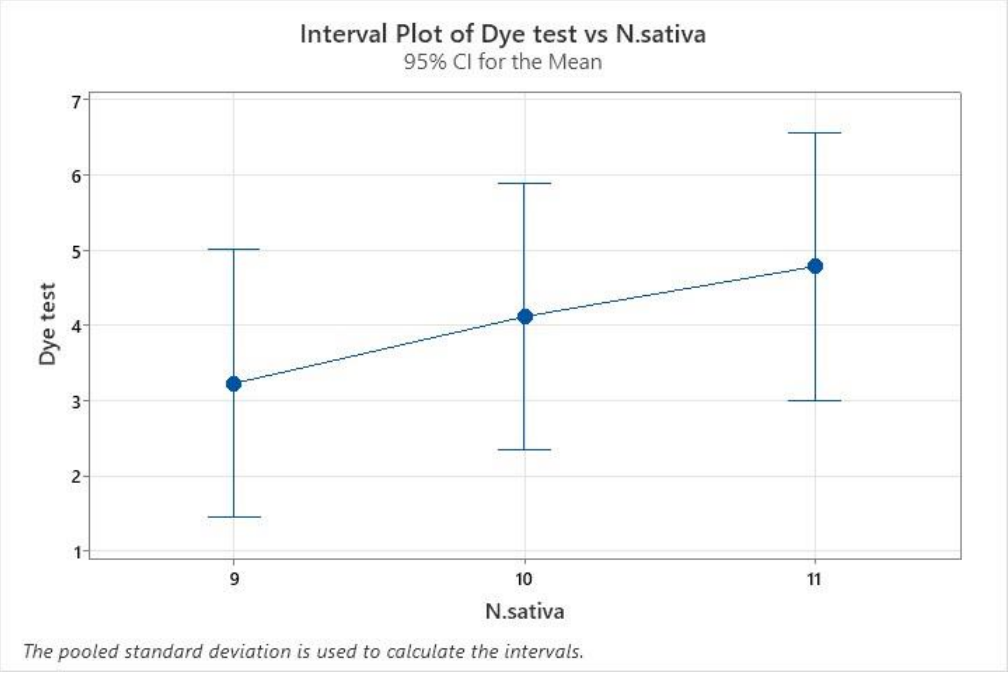
Table 13. Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
N.sativa	2	10.96	5.481	0.82	0.451
Error	24	160.00	6.667		
Total	26	170.96			

Table 14. Means

N.sativa	N	Mean	StDev	95% CI
9	9	3.222	2.108	(1.446, 4.999)
10	9	4.111	2.759	(2.335, 5.887)
11	9	4.778	2.819	(3.001, 6.554)

Pooled StDev = 2.58199



A one-way ANOVA was performed to study the dye test of a Nigella sativa. It revealed that there was a statistically significant difference in a F-value =0.82and P-value = 0.451. significance level was α = 0.05.The pooled standard deviation was *2.58199*.

The R.Squared value obtained was 6.41%, R.Squared(adjacent) was 0.00% and R.Squared(prepared) was 0.00% .

One-way ANOVA: Retention capacity (days) versus N.sativa

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

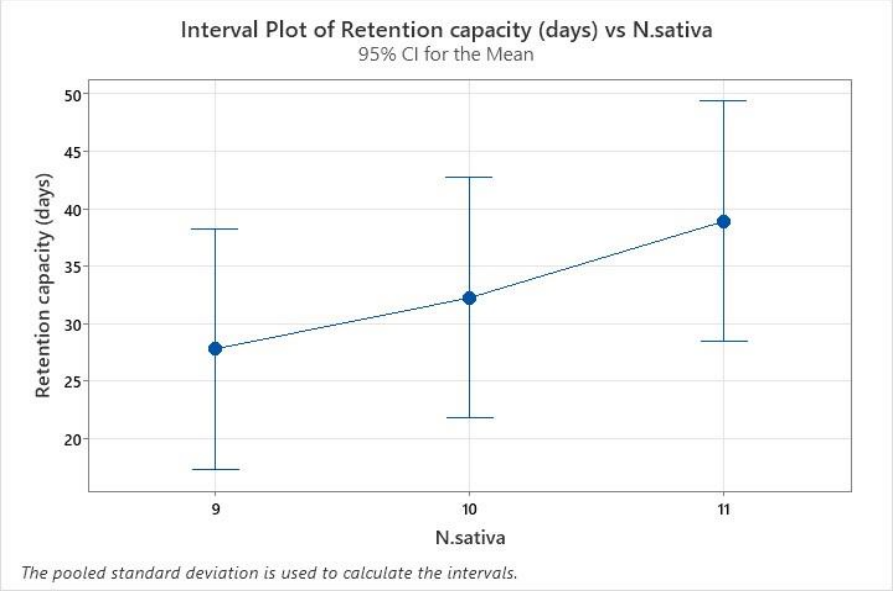
Table 15. Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
N.sativa	2	563.0	281.5	1.22	0.314
Error	24	5550.0	231.2		
Total	26	6113.0			

Table 16. Means

N.sativa	N	Mean	StDev	95% CI
9	9	27.78	14.81	(17.32, 38.24)
10	9	32.22	14.81	(21.76, 42.68)
11	9	38.89	15.96	(28.43, 49.35)

Pooled StDev = 15.2069



A one-way ANOVA was performed to study the colour retention capacity of a prepared hair dye of a Nigella sativa. It revealed that there was a statistically significant difference in a F-value =1.22and P-value = 0.314. significance level was $\alpha = 0.05$. The pooled standard deviation was *15.2069*.
The R.Squared value obtained was 9.21%, R.Squared(adjacent) was 1.64% and R.Squared(prepared) was 0.00% .

Conclusion: -

The prepared herbal hair formulation colours the hair in gentle manner. In this study, we found effectiveness of herbal hair formulation containing *Annona squamosa and Nigella sativa* on blackening of hairs. The herbal hair formulation showed primarily black colour with significant retention capacity. The prepared formulation had shown more effect as compared with marketed herbal hair dye. It has better colour retention capacity, better dyeing effect. The properties of the prepared formulation had been evaluated and were found to be satisfactory. The colour retention time, dyeing effect and effect on skin like irritation and swelling has been evaluated by stability test , dye test, retention capacity test and patch test respectively. The optimized formulation proved good hair dyeing and colour retention capacity as a result of dye test is The formulation F25 had black colour till 25 washes I.e 50 days whereas marketed herbal hair dye shows colour retention only upto 16 washes i.e 32 days and marketed synthetic hair dye shows colour retention till 30 washes i.e 60 days, this reveals that our formulation lasts upto 8 weeks. As per one-way ANOVA ,we found that, the p value of the independent variables i.e. *Annona squamosa* and *Nigella sativa*, is statistically significant ($p < 0.05$), it further confirms that *Annona squamosa* and *Nigella sativa* gives a a significant effect on hair dye and colour retention capacity . The formulation F25 showed higher colour retention capacity in comparison with marketed herbal preparation i.e. upto 50 Vs 32 days. The formulated hair dye has potential to fulfill the desired colour and has optimistic market potential.

Compliance with ethical standards -

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Conflict of interest-The authors declare that they have no conflict of interest.

Informed consent- Not Applicable

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Ethical approval-This article does not contain any studies with human participants or animals performed by any of the authors.

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