



Total safety management through standardization of formulated ayurvedic Kajal using *Eclipta alba* and *Vernonia cinerea* herbs

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ABSTRACT

Traditionally Kajal is known as Surma or Kohl which is used as an eye liner. Designing Ayurvedic Kajal with medicinal plants as a cosmetic product for beautification was thought of as a novel and an innovative technique. The main advantages of these cosmetic products are more patient compliance, water resistant property, stability and of course economical to formulate. Looking at that the present study was carried out with the aim of formulation of Ayurvedic Kajal with the help of two medicinal plants viz. *Eclipta alba* (EA) and *Vernonia cinerea* (VC) and evaluate their potential for sustained ocular delivery. Standardization of the herbs was performed based on different physiochemical parameters and revealed the values were within the prescribed limits. Ingredients used for this present investigation was standardized using TLC method whereas process standardization was carried out with organoleptic characters and was resulted similar characters in terms of color, odor and consistency. Furthermore product consistency was standardized by penetrometer test, moisture content test, TLC of the different extracts, microbial contamination and finally skin toxicity test. Different extracts like methanolic extract, lamp black extract, methanolic Kajal extract and hexane extracts for both the plants were carried out and revealed good results with all the extracts except

hexane extract. Thereafter all the method revealed significant results without any allergic reaction when tested on New Zealand white strain rabbits. Hence the results concluded that the formulated Kajal with EA and VC is totally safe and can be used for novel cosmetic formulations.

Keywords: *Eclipta alba* (EA); *Vernonia cinerea* (VC); Standardization; Safety; Toxicity study

1. INTRODUCTION

Eyes are the connection between the inner and outer worlds. In Ayurveda, Pitta dosha stands for the element of fire and light that governs our eyes. Hence eyes are most important organ in our body system. Vedic science offers several natural, safe and effective techniques to care for the eyes especially for beautification. With the science of ayurveda, several herbs and floras were used to make Ayurvedic cosmetics that not only beautified the skin but acted as the shield against any kind of external affects for the body. Not only that, plant products are used in cosmetics for useful purposes such as moisturizing, whitening, coloring, sunscreen, antioxidant, immunostimulant, cleansing, preservatives, thickeners, etc. One of that, Kajal is such cosmetic whose role in eye products can't be ignored.

Kajal is the thick, black ointment preparation, used in the Indian tradition for the health of eyes as eye liners. In India, Kajal is usually applied with one fingertip on the inner rim of the lower eyelid and on upper lid as well. Pure homemade Kajal is made from soot of a burning oil lamp and mixed with castor oil, camphor. Kohl is primarily used for keeping the eyes cool and healthy. Furthermore they are improving their appearance. Kajals are also prepared with several synthetic chemicals which cause eye irritation, etching and eye infections. In India, Kajal prices are varied depends on the content, size of stick and the brand for the products. Any branded cosmetic products before comes to market they should pass certain standards given by WHO. Most of the countries testing on animals have banned. Countries in the European Union have decided to stop animal testing by 2013.

The concern in this area is not only the use of animals for laboratory testing but also with the use of materials and ingredients derived from animal sources. The Drugs and Cosmetics Act, 1940, is concerned with the standards and quality of drugs and cosmetics manufactured and sold in India [1]. Hence, there is a prime need for regulation of the "natural" products used in various preparations. Oflate, standardization is the primary concept that can create trust and confidence in the products and increase market relevance. The Practitioner as well as the Consumer now seeks assurance from the manufacturer about quality, safety and efficacy of products. Hence the earlier recommendations related to any cosmetic preparations with herbs for specific condition states may not hold true today unless validated properly.

Thus pharmaceutical research is aimed at meeting the medical needs of the market products for those appropriate usages are not available or at those that are available are unsafe for prophylactic use for various purposes. In this study EA and VC herbs were selected to formulate Kajal by carried out different extracts. EA, commonly known as False Daisy and bhringraj, is a plant belonging to the family Asteraceae. It is widely distributed in tropical and subtropical region of the world [2] and native of Asia especially India, China, Thailand, and Brazil [3].

In ayurvedic medicine, the leaf extract of EA is considered a powerful liver tonic, rejuvenative, and especially good for the hair [4]. A black dye obtained from EA is used for dyeing hair and tattooing.

Medicinally the plant extract is effective against microbes, jaundice, acts as hepatoprotective, antihypertensive, diuretic, antioxidant, etc [5-8]. EA is a major ingredient in the market formulation, Vedic Guard, a polyherbal formulation which is a synergistic combination of 16 medicinal plant extracts [9]. But not much marketed formulations are reported still. Furthermore, VC is commonly called as ash-colored fleabane belonging to the family Asteraceae, found throughout India [10]. The whole VC plant is considered to promote perspiration in febrile condition. The leaves are useful in humid herpes, eczema, ring worm, guinea worms, and elephantiasis [11].

Various pharmacological activities such as analgesic, antipyretic, antioxidant and anti-inflammatory effects are reported [12-14]. Both the herbs were reported to treat conjunctivitis and used as Kohl [15]. In modern ear, eye makeup is an essential item of facial makeup. Eye is a very sensitive organ and hence any cosmeceutical products for use in eye should be made from pure, safe, non toxic and non-irritating materials [16].

Though Kajal is one of the most important in eye makeup, but still the medicinal use of Kajal is bit limited and hence formulating medicated Kajal as a cosmeceutical product to combat eye infections and beautification was thought of an innovative approach. Looking at that the present study was aimed to prepare a contemporary formulation from the preliminary Ayurvedic Kajal called soot/Lamp black, prepared by two herbs namely EA and VC and standardize in terms of identity, physical evaluation and toxicity study for safety usage.

2. MATERIALS AND METHODS

2. 1. Collection of plant materials

The fresh whole plants of EA and VC were collected from the Anamalai hills, Coimbatore district of Tamilnadu, India and were identified and authenticated by Dr. Jawahar, senior taxonomist, Herbarium and Taxonomy division, Foundation for Revitalisation of Local Health Traditions (FRLHT), Bangalore. Voucher specimens kept were designated L/07/08/17 for EA and L/07/08/23 for VC.

2. 2. Proximal analysis

Preliminary macroscopical identification of both the raw plant materials were carried out followed by quantitative standards for the leaves of EA and whole plant of VC, in terms of moisture content, total ash, acid insoluble ash, alcohol and water soluble extractive values for both the samples were performed as per the method described by the World Health Organization (WHO) guidelines [17].

2. 3. Preliminary Ayurvedic formulation of Kajal

The juice of the leaves of EA and VC were prepared hygienically and a cleaned unbleached cloth was soaked in the juice and dried in the dehydrator. The soaking process was repeated until the juice was exhausted. Then the dried cloth piece was used as a wick and was lighted in a mud lamp containing castor oil. The black soot was collected in a clean dry

plate by tapped. The powder then used to apply as surma or mixed with coconut oil to form a paste form i.e. Kajal.

2. 4. Contemporary formulation of Kajal

Modern Kajal was formulated from the preliminary Ayurvedic Kajal with the ingredient given in below Table 1:

Table 1. Modern Kajal formulation.

Phase	Ingredients	% wt
A	Paraffin wax	8.00
	Bees wax	1.60
	Microcrystalline wax	1.60
	Silicone oil	18
	Propyl paraben	5.75
B	Lamp black	15
	Castor oil	44.30
C	Camphor	5.75

All the ingredients of Phase A was weighed in a heated vessel at temperature 85⁰C and stirred until melted and form uniform liquid. Lamp black of Phase B was dispersed first in castor oil until all the powder was well distributed and then added to phase A. Further phase C was added and mixed with constant stirring. Finally the uniform melted ingredients were poured into molds at 75 °C to make the final formulation.

2. 5. Standardization of ingredients, process and products

The quality of the ingredients used in the product was standardized using TLC. Physiochemical characters that includes Penetrometer test (the pressure at which the needle penetrates), Moisture content (using Karl Fischer method), TLC, microbial contamination and toxicity study of the product were performed for the plant extracts, Lamp black and Kajal products of two separate plants.

2. 5. 1. TLC of extracts

Both the EA and VC extracts were to begin with, checked by Thin Layer Chromatography (TLC) on analytical plates over silica gel. TLC was carried out to isolate the principle components that were present in most effective extracts of plant and the final

products. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the suitable solvent system for better resolution [18].

Based on that, 5 g of the product (prepared from EA) was subjected to soxhlet extraction with hexane as solvent to remove the oils in the product and further re-extracted with methanol. Further 5 g of whole plant (VC) refluxed with 100 ml of alcohol for one hour and then evaporated with water bath to reduce the volume. Crude extract then diluted to 100 ml with alcohol.

Finally the methanolic leaf extract of EA, Lamp black, Kajal products and alcoholic plant extract of VC, Lamp black, Kajal products were performed by TLC using two separate mobile phases viz. Toluene: Acetone: Formic acid (11:6:1) and Toluene: Ethyl acetate (95:5) for EA and VC respectively. Both the samples were visualized at 254 nm and 366 nm and identified the bands of similar R_f value.

2. 5. 2. Microbial contamination

The quality of the Kajal products were performed with the microbial test to find out the microbial count, total fungal count and *E. coli* count through plate count method using nutrient agar (for total bacterial count), MacConkey agar (for total *E. coli* count) and Sabouraud dextrose agar (for fungal and yeast count) method by incubated 37° over night, 43° over night and 25 °C for 4 days respectively. Finally the growth were observed and counted by colony counter in per volume plated. Finally results expressed as cfu/g (Colony forming unit per gram of the sample).

2. 6. Evaluation of allergenic studies on Kajal products:

2. 6. 1. Experimental animals

Studies were carried out using New Zealand white strain male rabbits of either sex weighing 25-30 g were obtained from Al-Ameen College of Pharmacy, Bangalore, India after approved by institutional animal ethics committee (Ref No: AACP/IAEC/M-83/2007).

The animals were grouped into 3 and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than three animals per cage and maintained under standard laboratory conditions (temperature 25 ±2°C, 44-56% RH) with dark and light cycle (12:12 hr). These rabbits were fed with standard pellet diet from Hindustan lever limited (Mumbai, India) and water *ad libitum* [19] and the study was carried out based on the laboratory animals guidelines with proper care.

2. 6. 2. Experimental design

The selected rabbits were divided into three groups of three animals each as given below. Group I: Control group (rabbits received 100 mg of standard marketed formulation), Group II: Formulated Kajal with EA and Group III: Formulated Kajal with VC.

To test the materials for the potential ocular irritation, the lower eye lid of the animal was pulled away from the eyeball and the formulations were placed in the conjunctival sac. All the animals were observed for 1 hour then at 24 hours interval for 10 days for sign and symptoms of irritation viz. redness, inflammation, chemosis and the results were recorded which depicted in result section.

3. STATISTICAL ANALYSIS

Results of the proximate were reported as mean \pm SD where n = 3.

4. RESULTS AND DISCUSSION

A diagnostic character in terms of morphology is the preliminary experiment for identification and detection of the pure drug for the quality of the raw herbs. Looking at that morphological identification of both drugs was carried out and the results reported there were no similarities between two drugs. Table 2 has described morphological features of EA and VC.



Eclipta alba (EA)



Vernonia cinerea (VC)

Table 2. Morphological features of two different plants i.e. *Eclipta alba* and *Vernonia cinerea*

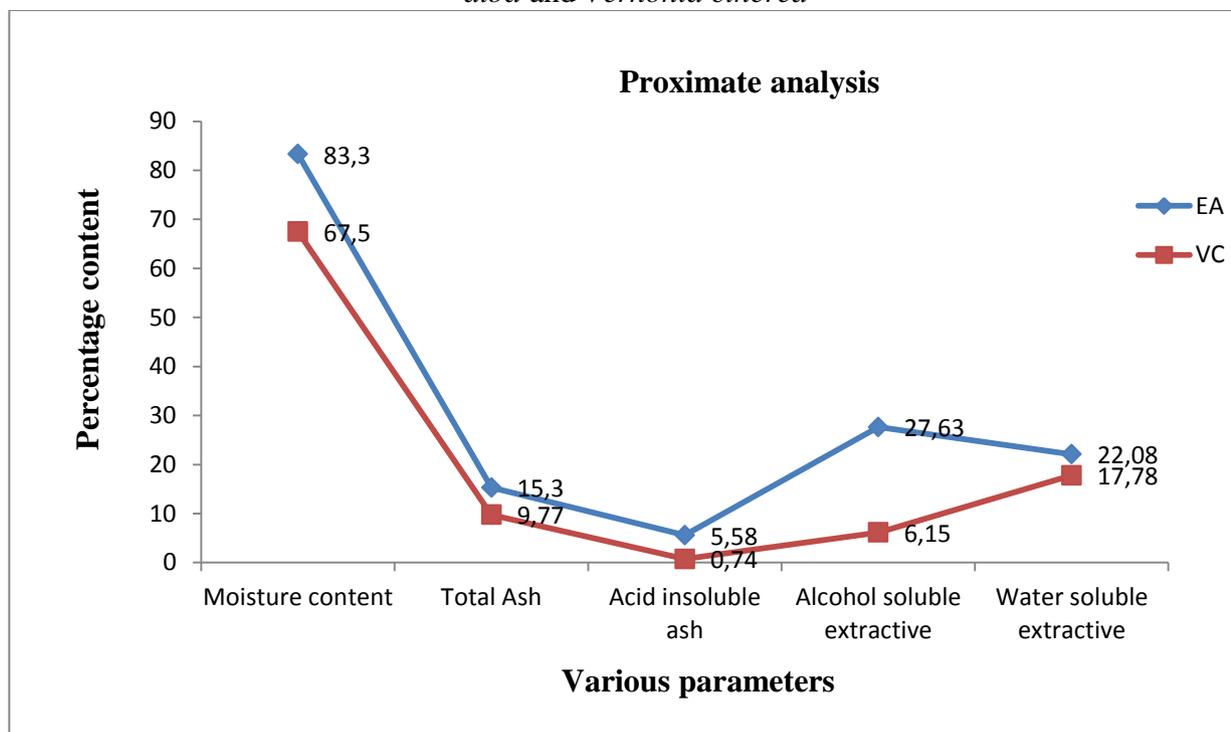
Diagnostic characters	<i>Eclipta alba</i> (Leaves)	<i>Vernonia cinerea</i> (Whole plant)
Color	Dark Green	Erect, slightly branched with pinkish violet flowers
Odor	Slight	Slightly characteristic
Taste	Slightly salty	Characteristic
Form	Oblong	Blunty conical
Size	2-12 cm long	10-20 cm long, varying in thickness

Shape and extra features	Capitulum white 2-6 mm in diameter, achenes elliptical and flattened, 2-3 mm long	Pappus white, the exterior row short, about 0.5 mm long. Achenes 1.2 mm long, slightly narrow at base
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4. 1. Proximate analysis

The proximate values of both the drugs were carried out in terms of moisture content, total ash, acid insoluble ash, alcohol and water soluble extractive and found all the values were with the limit of the specified in the Indian Ayurvedic Pharmacopoeia [20]. Leaves of EA showed more percentage of moisture (83.3 ± 0.02), total ash (15.3 ± 0.01), acid insoluble ash (5.58 ± 0.20), alcohol soluble (27.63 ± 0.11) and water soluble extractive (22.08 ± 0.13) than whole plant of VC. As per “The Ayurvedic Pharmacopoeia of India” the limits of all the parameters such as total ash and acid insoluble ash are not more than 22 and 11 % respectively where as alcohol and water soluble extractive values are not less than 5 and 15% respectively for leaves of EA. The same trend of results also revealed earlier literature reported by Borkataky et al., (2013) [21].

Figure 1. Proximate analysis of two different herbs of Asteraceae family i.e *Eclipta alba* and *Vernonia cinerea*



Furthermore Heritage Amruth, (2006) reported the maximum limits of the various proximate parameters of VC such as total ash and acid insoluble ash are not more than 14 and 2 % respectively where as alcohol and water soluble extractive values are not less than 4 and 15% respectively [22].

The similar study was also reported by Abraham, (2015) where all the proximate parameters are followed the same trend with our present study [23]. The comparative study of both the plants was showed in Figure 1.

4. 2. Standardization of Kajal made from EA and VC

Preliminary formulated Kajal prepared by EA and VC was tested for the organoleptic characters and revealed both the products were black, camphor like odor and solid in form. Formulations were prepared in three different batches separately for both the herbs and showed no variation in its characters. Further Lamp black was prepared with the juices where amount of carbon detected as 7.5 g each from the total leaf sample of EA and VC plant sample (500 g) respectively. The Penetrometer test (in the Penetrometer cup) and moisture content (by Karl Fischer method) of the prepared Kajal by EA and VC were evaluated and resulted same ($2.63 \pm 0.02\%$) moisture content and penetration power (164 mm) for respective herbs which confirms the quality of the Kajal product prepared by EA and VC.

Thereafter microbial contamination of the cosmetic products is essential for the safety uses because various forms and dosage of Pharma cosmetic products are susceptible to contamination by a variety of microorganisms during manufacturing and use [24]. The used contaminated products on body, even where the level of contamination is low may present potential health hazards to human [25] because microorganisms may spoil the products due to loss of its therapeutic properties. In our present study total plate count was performed to detect the microbial load in the Kajal product in terms of total microbial count, total fungal count, *E. coli* and Enterobacteriaceae and resulted absent of all the count and organisms. The result of this research showed that the Kajal tested had satisfactory bacteria levels compared to the British Pharmacopoeia specification of 10^3 CFU/ml (2008) [26]. These results indicated the formulated Kajal is totally safe as use on the eyes.

TLC identification for the detection and separation of the compounds were performed using different solvent system and standardized the best solvent systems for EA and VC. The results from TLC revealed that best resolution for the compounds with similar R_f value (0.67 for methanolic and methanolic lamp black extracts, 0.09 for methanolic Kajal) with combined solvents of Toluene, Acetone and Formic acid (11:6:1), visualized at 254 and 366 nm for EA sample. But no separation observed with hexane extract when observed under both the wavelengths. Thereafter TLC of alcoholic extract of VC resulted same R_f of 0.58 at 254 and 366 nm whereas alcoholic lamp black extracts of VC showed only R_f of 0.6 at 366 nm but no band was detected at 254 nm when standardized mobile phase toluene and ethyl acetate (95:5) was used. The results showed in Table 3 and 4 with Figure 2a, b and 3a, b respectively. TLC profiling of all extracts gave an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gave different R_f values in different solvent system. TLC profile at 366 nm revealed methanolic plant extracts showed many bands. Alcoholic lamp black extract of VC showed 3 bands (Figure 3b). Methanolic extract of Lamp black and alcoholic extract of Lamp black showed few similar bands with respect to the plant extract of EA and VC respectively. TLC fingerprints of methanolic extracts of the two products matched with TLC of the respective lamp black methanol extract.

Furthermore EA and VC extracts were found safe to use in any formulation that revealed by the acute toxicity studies [27-30] and based on that allergenic studies of the Kajal products were carried out on New Zealand white strain rabbits and results revealed total

safety of both the plant extracts and no allergenic symptoms for all the groups which was depicted in Table 5.

Table 3. TLC for ingredients (methanolic extract, Lamp black extract) and product (methanolic and Hexane Kajal extract) of EA at different wavelength.

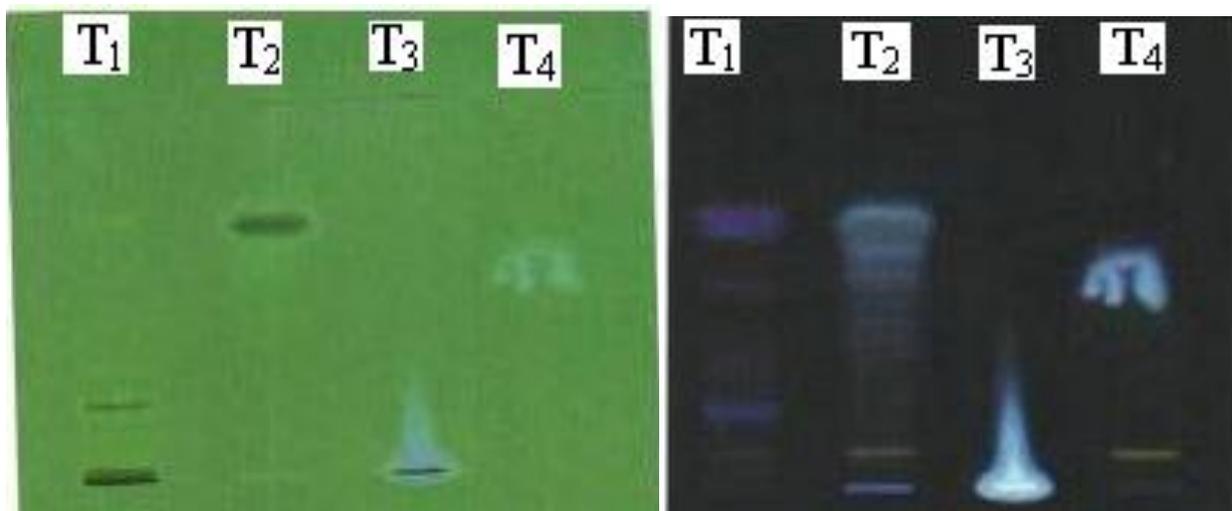
Visualization	Methanolic extract	Methanolic Lamp extract	Methanolic Kajal
254 nm	T ₁ : 0.67 (R _f)	T ₂ : 0.67 (R _f)	T ₄ : 0.09 (R _f)
366 nm	T ₁ : 0.67 (R _f)	T ₂ : 0.67 (R _f)	T ₄ : 0.09 (R _f)

- T₁, T₂, T₃ and T₄ are the tracks of respective extracts

Table 4. TLC for ingredients (alcoholic extract, Lamp black extract) and product (alcoholic and Hexane Kajal extract) of VC at different wavelength.

Visualization	Alcoholic extract	Alcoholic Lamp extract	Methanolic Kajal
254 nm	T ₁ : 0.58 (R _f)	T ₂ : 0.60 (R _f)	T ₄ : 0.60 (R _f)
366 nm	T ₁ : 0.58 (R _f)	T ₂ : 0.60 (R _f)	T ₄ : 0.60 (R _f)

- T₁, T₂, T₃ and T₄ are the tracks of respective extracts



2 a. UV at 254 nm

2 b. UV at 366 nm

Figure 2. TLC plates at different wavelength for EA Kajal extracts

- T₁ = Methanolic plan extract; T₂ = Methanolic Lamp black extract; T₃ = Hexane Kajal extract; T₄ = Methanolic Kajal extract

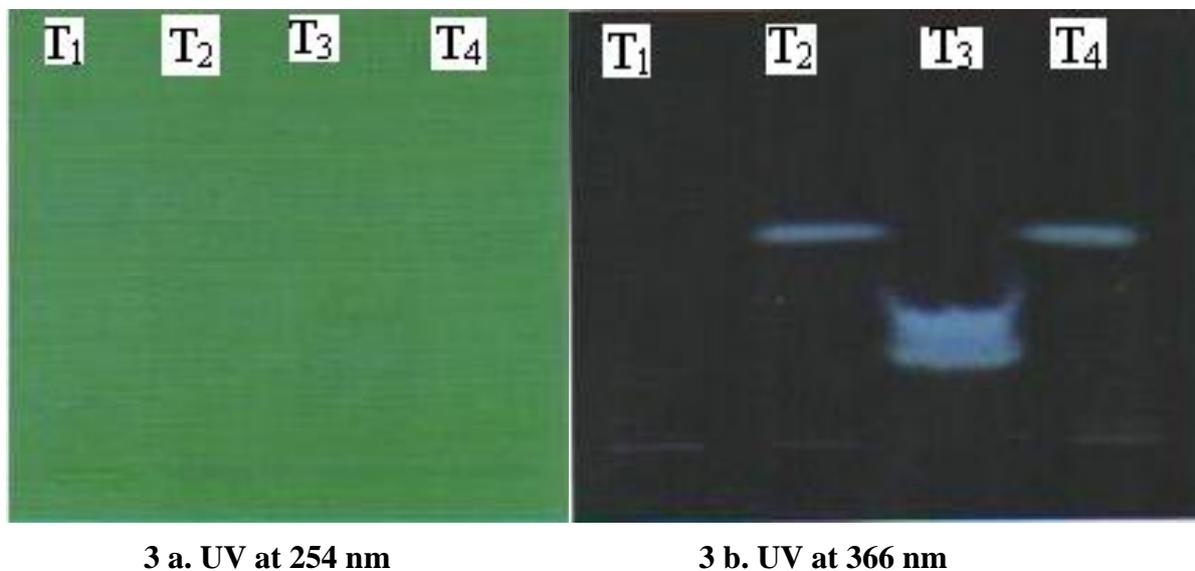


Figure. 3 TLC plates at different wavelength for VC Kajal extracts.

- T₁ = Hexane Kajal extract; T₂ = Alcoholic extract; T₃ = Alcoholic Lamp black extract; T₄ = Methanolic Kajal extract

Table 5. Allergenic tests in New Zealand white rabbits for both the formulated plant products (n = 3).

Groups	Parameters for both the right and left eyes	No. of days									
		1	2	3	4	5	6	7	8	9	10
Group I:	Redness	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Inflammation	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Chemosis	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Group II:	Redness	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Inflammation	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Chemosis	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve

Group III:	Redness	-Ve									
	Inflammation	-Ve									
	Chemosis	-Ve									

(-Ve) = Negative or no reaction

4. CONCLUSION

Standardization of EA and VC plants were carried out with respect to organoleptic characters and founded same characters reported for both the drugs in the official Pharmacopoeia. Various proximate parameters were carried out and revealed similarities in the values for both the herbs.

Chromatographic profile of extracts of the plants showed that there are compounds present had similar R_f values in the duos when they were identified and separated with different mobile phase. Thereafter microbial tests showed absence of microbial load in both the plant products which showed safety of the Kajal products.

Furthermore toxicity and allergenic reactions were also performed in rabbits for both the extracts and compared with that of standard that revealed neither toxicity nor allergenic reactions for all. These studies concluded that the formulated Kajal is safe and can be used as one of the herbal cosmetic products.

Acknowledgements

Authors are thankful to Dr. Padma Venkatasubramanian, Foundation for Revitalisation of Local Health Traditions (FRLHT), Bangalore, India, for provided raw drug materials of *Eclipta alba* (EA) and *Vernonia cinerea* and to the Principal, Al-Ameen College of Pharmacy for provided research facilities to fulfill the project.

Conflict of interest

We declare that we have no conflict of interest.

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(Received 24 May 2015; accepted 06 June 2015)