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Effect of traditional herbal products of Kajal on isolated bacteria from eyelids of eye cosmetics users

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Abstract

Traditionally, kajal is used as eyeliner and is referred to as kohl or surma. A new, creative method would be to create Ayurvedic kajal using medicinal plants as a cosmetics product. These cosmetic product's key benefits include being more patient-friendly, water resistant, stable, and cost-effective to create. Taking this into consideration, the current study was conducted to formulate Ayurvedic kajal using two medicinal plants, *Eclipta alba* (EA) and *Rosa indica* (RI), and to assess their ability for prolonged ocular administration. A qualitative phytochemical study was carried out to identify employing a herbal plant extract. Alkaloids, Phenols, Triterpenoids, Steroids, Flavonoids, Saponins, and Tannins are screened for in aqueous extracts of both herbal extracts. Based on various physiochemical criteria, standardisation of the herbs was carried out, and the results showed that the values were within the permitted ranges. TLC technique methodology and results were utilised to standardise the ingredients employed in this inquiry. Penetrometer testing, moisture content testing, TLC testing of the various extracts, microbiological contamination testing, and finally total bacterial count testing were used to standardise product consistency. Different extracts, including methanolic, lamp black, kajal, and hexana extracts for the plant's leaves and petals, were tested, and the findings were promising. The disc diffusion method was used, with the parameters chosen and the antibacterial activity taken into consideration. *Echerichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* are all inhibited by herbal cosmetics.

Keywords: *Eclipta alba, Rosa indica,* TLC, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* Antibacterial activity, Phytochemical screening.

Introduction

The organs in humans that give us sight are called eyes. The eyes are not only the windows to the soul but also a potent tool for communication the structure known as the eyelids. A structure with an almond shape an essential link between the external and internal worlds is provided by the eyes our health is governed by the pitta dosha. **(Cicekli U 2003).** Wearing kajal is traditional for several reasons including aesthetics defence against the evil eye and other factors is medically beneficial for the eyes and it is encouraged by the sunna which contains the traditional Islamic social mores consumers. However are frequently confused by the range of goods available consumers are still unaware of the precise raw material concentration or the toxicity levels of the additives many consumers are carelessly using and buying these goods **(Al-Ashban RM *et al.*, 2004).**

The word "kohl" is primarily of Arabic origin, really called it "kahal." In the Eastern (Unani/Ayurvedic) and Greco-Arabic systems of medicine, kohl is a term used to describe an ultra-fine powder that contains one or more ingredients (such as galena, herbs, pearls, gemstones, etc.) **(Snodgrass *et al.*, 1973).** Eye cosmetics kajal and kohl are often made using heavy metal salts like stibnite and galena a lead sulphide kohl surma and kajal have been utilised traditionally in many parts of the world for a number of reasons for example to enhance the look, beauty of the eyesand is applied to the eyes for the prevention and treatment of various eye diseases**(Balsam MS *etal.*, 1972).** *Bacillus species, Staphylococcus species, Pseudomonas species, P. vulgaris species*, and *Serratia marcescens* species have been isolated from samples of Al-Kohl that have been utilised as well as samples that have not. The conjunctivae of the young toddlers absorbed germs after having kohl applied to them. *E. coli* and gram-positive (*S. aureus, S. epidermidis*, and *S. capitis*) bacteria have been discovered on the eyelids of people who use natural eyeliner and eye cosmetics **(Akrayi HFS 2012).**

To preserve the health of the eyes, Hindu culture indicates that kajal, a thick, black ointment, be applied as eyeliner. In India, the inner rims of the upper and lower eyelids are frequently covered with kajal with one fingertip. The soot from an oil lamp is burned along with castor oil, camphor, and pure home made kajal. Kohl is mostly used to keep the eyes cool and healthy. They are also improving how they seem. Given this, the purpose of the current study was to create Ayurvedic kajal using the herb *Eclipta alba* (EA) and *Rosa indica* (RI) see whether continuous ocular administration was possible.

Materials and Methods

Collection of eye sample

Totally 10 eye infected samples (swab) were collected from eye makeup women's. The eye samples were inoculated into nutrient broth and incubate. After incubation samples were analysis by Gram staining, Biochemical test, carbohydrate utilization test and specific selective media.

Collection of plant material and screening

The fresh whole plants of *Eclipta alba* leaves and petals of *Rosa indica* was collected from Tiruvannamalai district of Tamilnadu.In order to identify different classes of active chemical elements in the collected plant extracts, a qualitative phytochemical analysis was performed by aqueous extraction.

Methods for *Eclipta alba* and *Rosa indica*

S.No	Test	Positive interpretation
1.	Alkaloids (Wagner's test)	Reddish brown ppt
2.	Phenols (Ferric chloride test)	Deep blue color
3.	Triterpenoids (Liebermann-Burchard's test)	Deep red color (lower layer) for EA
		Yellow colour (lower layer) for RI
4.	Sterols (Liebermann-Burchard's test)	Green colour (upper layer) for EA
		Red colour (lower layer) for RI
5.	Flavonoids (Alkaline test)	Yellow to colorless
6.	Saponins (Foam test)	Foam formation
7.	Tannins (Braymer's test)	Deep blue / dark green precipitate

Proximal and preliminary Ayurvedic formulatation of kajal

Preliminary macroscopical identification of both the raw plant materials were carried out followed by quantitative standards for the leaves of *Eclipta alba* and petals of *Rosa indica* (RI), 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 1998).** The sample material dried powder for preparing the extract was acquired.The extract was hygienically prepared and an unbleached cloth was soaked in the leaves of *Eclipta alba* and petals of *Rosa indica* extract separately and dried in a hot air oven. The dried cloth piece was used as a wick and was lighted in a mud lamp containing castor oil both samples.The black soot was collected in a clean, dry sterile box. The power was taken then mixed with cow ghee to form a paste (i.e., Kajal) stored in plastic or glass air tight container.

Contemporary formulation of kajal

All the ingredients of *Eclipta alba* and *Rosa indica* was weighted and formulated in same manner as preliminary ayurvedic kajal and the ingredients are given in table.

Quantitative composition of the formulation of herbal kajal

S.No	Ingredients	Quantity taken
1.	Leaves extract of *Eclipta alba*	50 mL
	Petals extract of *Rosa indica*	
2.	Castor oil	6g
3.	Cow ghee	7g
4.	Camphor	1.2g
5.	Black soot	10g
6.	Bees wax	4g

Finally the uniform melted ingredients were poured into molds at 75º C to make the final formulation of kajal.

Standardization of ingredients, processes and products

The quality of the ingredients used in the product was standardized using Thin layer chromatography (TLC). The physiochemical characters that includes penetrometer test (the pressure at which the needle penetrates), moisture content (using karl fisher method), TLC, microbial contamination and toxicity study of the product were performed for the plant extracts, lamp black and kajal products of plant.

Thin layer chromatography

Both the EA and RI extracts were to begin with, checked by TLC on analytical plates over silica gel. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the suitable solvent system for better resolution. Based on that, 5 g of the EA product was subjected to soxhlet extraction with hexane as solvent to remove the oils in the product and further re-extracted with methanol. Further 6 g of the RI product 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 RI, 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 RI respectively. Both the samples were visualized at 254 nm and 366 nm and identified the bands of similar R_f value.

Microbial analysis of kajal

Microbial tests were performed to check the quality of the kajal product to determine the total bacterial count (nutrient agar), total coliform count (MacConkey agar) and total fungal count (Sabouraud dextrose agar) by standard plate count method.

Evaluation of allergenic studies on kajal products

- **Physical Evaluation:** The formulated ayurvedic product was of a shiny black color, with a characteristic odor and smooth in texture with a semisolid consistency.
- **P^H Determination :** To determine the 10 g of kajal sample was dissolved in 75 ml of distilled water. The solution was reading and recorded with a P^H meter read as 7.1.
- **Evaluvation of Base:** For acid, saponification, and ester values, vegetable ghee was evaluated as per Indian Pharmacopoeia (I.P) 1996.
- **Acid Value:** Acid value = 5.61 × (N / W), where N is normality of the potassium hydroxide or sodium hydroxide solution of 0.1 ml and W is the weight of the sample taken (g); N = 0.6 and W = 2.5. Thus, the acid value was 5.61 × 0.6/ 2.5 = 1.3464.
- **Saponification Value:** Saponification Value: Saponification value = 28.05 (b − a)/w, where w is the weight in grams of the substance, b is the blank solution reading, and a is the sample solution reading; b = 22, a = 4.1, and w = 2.5 Therefore, the saponification value was 28.05 (22 − 4.1)/2.5 = 200.838.
- **Ester Value:** Ester value = Saponification value – Acid value. Thus, ester value was 200.838 − 1.3463 = 199.4916

Antibacterial potential of Kajal sample (Disc diffusion method)

The antibacterial activity of kajal samples was assessed by disc diffusion methods. Muller hinton agar (MHA) was prepared and sterilized the media was poured into sterile petriplate and get solidified. Sterilized swab are used to inoculated the culture on surface of the agar plates. The suspensions of kajal samples were prepared by adding one gram of kajal in 1 ml of sterile distilled water and vortexted well and dried in hot air oven for 10 minutes. The disc were placed on the agar surface of the place with help of sterile forceps. The plates were incubated at 37º C for 24 – 48 hours. After the zone of inhibition was measured with transforant ruler, the results were tabulated.

Results

The present study reveals that a total of 10 different eye swab samples were collected from eye makeup women's. The sample inoculated in to nutrient broth (NB) and incubated. After incubation observe the microbial growth. Further analysis by staining technique, biochemical test and carbohydrate utilization test. The result of the Gram stain were bacterial growth of 7 Gram negative rod and 3 Gram positive cocci observed.

Biochemical, Carbohydrate utilization test and selective media given table below:

Gram staining and Biochemical tests				
S.no	**Name of the test**	***E. coli***	***P. aeruginosa***	***S. aureus***
1.	Gram staining	Gram (-) ve rod	Gram (-) ve rod	Gram (+) ve cocci
2.	Indole	+	-	-
3.	Methyl red test	+	-	+
4.	Voges - proskauer test	-	-	+
5.	Citrate utilization test	-	+	+
6.	TSI	Yellow slant and butt, gas production and no H2S production	Red slant and butt, no gas and H2S production	Red slant with yellow butt with bubble and black precipitate
7.	Urease test	-	-	+
8.	Catalase test	+	+	+
9.	Oxidase test	-	+	-
10.	Coagulase test	-	-	+
Carbohydrate fermentation test				
S.no	**Types of sugar**	***E. coli***	***P. aeruginosa***	***S. aureus***
1.	Glucose	+	+	-
2.	Sucrose	-	-	+
3.	Fructose	+	+	-
4.	Lactose	+	-	-
5.	Mannitol	+	+	+
6.	Galactose	-	-	+
Selective media				
S.no	**Selective media**	**Colony Morphology**		
1.	Eosin methylene blue (EMB)	Green metallic sheen colonies		
2.	Cetrimide agar	Pyocyanin pigmented colonies		
3.	Mannitol salt agar (MSA)	Golden yellow color colonies		

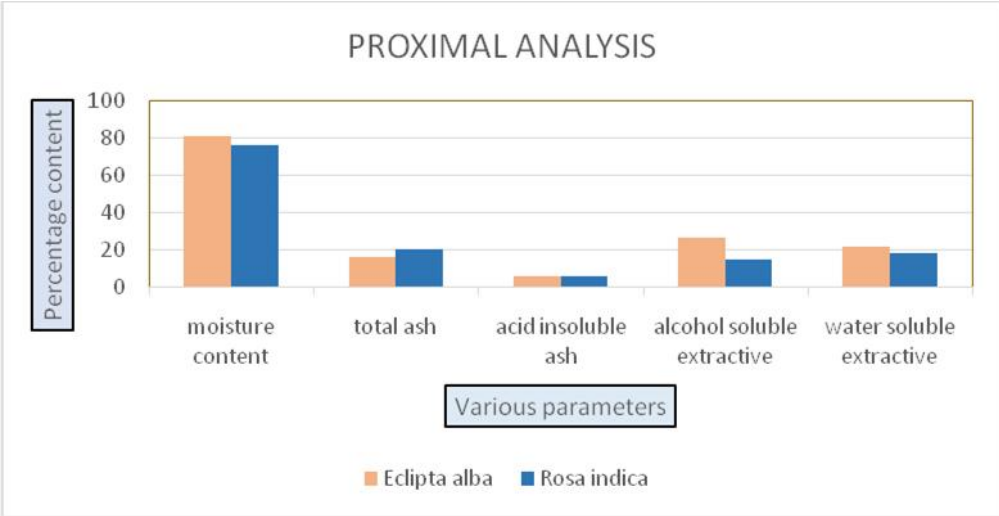
A organoleptic characters in terms of morphology is the preliminary experiment for identification and detection for the quality of the raw herbs.

S.no	**Characters**	***Eclipta alba***	***Rosa indica***
1.	Color	Dark green	Dark pink
2.	Taste	Slightly salty	Slightly sweet
3.	Odor	Slight	Dark pink
4.	Size	2 – 12 cm long	1.5-4 cm wide and 2-4cm high

The current study reveals that phytochemical screening of the leaves of *Eclipta alba* contains alkaloids, flavonoids, saponins, tannins, triterpenoids, steroid and phenol test and petals of *Rosa indica* contains alkaloids, flavonoids, saponins, triterpenoids, steroid and phenol test in aqueous solvent extracts.

S.no	Phytochemical compound	*Eclipta alba*	*Rosa indica*
1.	Alkaloid test	+	+
2.	Phenol test	+	+
3.	Triterpenoids	+	+
4.	Steroids	+	+
5.	Flavonoids	+	+
6.	Saponins	+	+
7.	Tannins	+	-

The proximate values of both the herbs were carried out in terms of moisture content, total ash, acid insoluble ash, alcohol and water extractive and found leaves of *Eclipta alba* showed more percentage of moisture (80.5 ± 0.02), total ash (16.1 ± 0.02), acid insoluble ash (5.9 ± 0.15), alcohol soluble (26.53 ± 0.13) and water soluble extractive (22.1 ± 0.14) than petals of *Rosa indica* showed percentage of moisture (76.1 ± 0.02), total ash (20.4 ± 0.02), acid insoluble ash (6.0 ± 0.15), alcohol soluble (15.3 ± 0.13) and water soluble extractive (18.6 ± 0.14). The instructed guidance are followed to make kajal used the herbal traditional ingredients.



Thin layer chromatography identification for the detection and separation of the compounds were performed using different solvent system and standardized the best solvent systems for *Eclipta alba* and *Rosa indica.*

Thin layer chromatography (TLC) for *Ecliptaabla*at different wave length.			
Visualization	**Methanolic extract**	**Methanolic lamp extract**	**Methanolic kajal**
254nm	T_1: 0.66 (R_f)	T_2: 0.66 (R_f)	T_4: 0.10 (R_f)
366nm	T_1: 0.66 (R_f)	T_2: 0.66 (R_f)	T_4: 0.10 (R_f)
Thin layer chromatography (TLC) for *Rosa indica* at different wave length			
Visualization	**Alcoholic extract**	**Lamp extract**	**Kajal extract**
254nm	T_1: 0.67 (R_f)	T_2: 0.67 (R_f)	T_4: 0.09 (R_f)
366nm	T_1: 0.67 (R_f)	T_2: 0.67 (R_f)	T_4: 0.09 (R_f)

The results from TLC revealed that best resolution for the compounds with similar R_f value (0.66 for methanolic and methanolic lamp black extracts, 0.10 for methanolic Kajal) with combined solvents of Toluene, Acetone and Formic acid (11:6:1) and toluene and ethyl acetate (95:5) was visualized at 254 and 366 nm for *Eclipta alba* sample. Methanolic extract of *Eclipta alba* resulted same R_f of 0.66 at 254 and 366 nm whereas methanolic, lamp black extracts of *Eclipta alba* showed only R_f of 0.10 at 366 nm and 254 nm But no separation observed with hexane extract when observed under both the wavelengths. Thereafter TLC of alcoholic extract of *Rosa indica* resulted same R_f of 0.67 at 254 and 366 nm whereas water, lamp black extracts of *Rosa indica* showed only R_f of 0.09 at 366 nm and 254 nm whereas kajal extract. 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. Water, lamp black extract of *Rosa indica* showed 3 bands (Figure 3b). Methanolic extract of Lamp black and Water extract of Lamp black showed few similar bands with respect to the plant extract of *Eclipta alba* and *Rosa indica* respectively. TLC fingerprints of methanolic extracts of the two products matched with TLC of the respective lamp black methanol extract.

The quality of the kajal products were performed with the microbial test to find out the total bacterial count (nutrient agar), coliform count (MacConkey agar) and total fungal count (SDA). Finally the microbial growth was not observed. Evaluvation of allergenic studies on kajal products analysis by physical evaluvation, P^H determination, evaluation of base, acid value, saponification and ester values. The study revealed that herbal kajal were tested against some clinically important pathogens (*E. coli, P. aeruginosa, S. aureus*). The highest activity was reported against *P. aeruginosa* and *S. aureus* and less activity for *E. coli.*

S.no	Organism	Zones of inhibition (mm)	
		Eclipta alba	***Rosa indica***
1.	*Escherichia coli*	12 mm	9 mm
2.	*Pseudomonas aeruginosa*	20 mm	18 mm
3.	*Staphylococcus aureus*	25 mm	23 mm

Discussion

In the present study antibacterial activity and phytochemical properties of *Eclipta alba* and *Rosa indica* investigated. Plant materials were dried and extracted using aqueous extract valuable compounds. The phytochemical screening of *Eclipta alba* and *Rosa indica* showed the presence of different groups of secondary metabolites like alkaloids, phenol, triterpenoids, steroids, flavonoids, saponins, tannins. When the leaves of *Eclipta alba* and petals of *Rosa indica* was extract in aqueous extract **(Lunavath V *e tal.*, 2013).** The whole plant part of EA is reported to have shown the presence of bioactive compound viz., alkaloids, phenol, triterpenoids, steroids, flavonoids, saponins, while the aqueous extract resulted in the identification of six bioactive compounds in both extract. Tannins is present only EA and not present in RI.

The proximate values of both the herbs were carried out in terms of moisture content, total ash, acid insoluble ash, alcohol and water extractive and found all the values were with the limit of the specified **(Indian ayurvedic pharmacopoeia 1999).** The current study was conducted to assess the containing zinc, sulfur, lead, cadmium and to find out the effects of kajal on systemic circulation after absorption through eye and ensure subsequent effect on vital organs. So, herbal kajal products which are safer and even good for eyes (**Draize *et al.*, 1952).**

The isolation of the principle components that effective extract of the plants and the final products were carried out using TLC. Based on that 7.5g of *Eclipta alba* and *6g Rosa indica* of the product was subjected to Soxhlet extraction with water as a solvent. Finally the leaf extract of *Eclipta alba* and *Rosa indica*, lamp black and kajal product separately for both samples were subjected to TLC using two separate mobile phases, viz., toluene: acetone: formic acid (11:6:1) and toluene: ethyl acetate (95:5), visualized at 254 and 366nm for both extracts. TLC profiling of all extracts gave an impressive result that directing towards the presence of number of phytochemicals **(Sajithaputhalath *et al.*, 2015).** Various phytochemicals gave different R_f values in different solvent system. Visualization at 254nm and 366nm for *Eclipta alba* sample no separation observed with hexane extract observed both wave length. Extract and lamp extract R_f values are 0.67 and methanolic kajal has 0.09 in both wave length. Visualization of *Rosa indica* has 0.67 value for both wavelength in water and lamp extract and 0.09 in kajal extract for both wavelength **(Sandeep waghulde *et al.*, 2018).**

The microbiological examination concluded that home-made kajal samples have the required total bacterial count, coliform count, fungal count as per specification and they so not promote any microbial contamination **(Indian standard 2002).** Furthermore *Eclipta alba* and *Rosa indica* extracts were found safe to use in any formulation that revealed by the acute toxicity studies **(A.M.Rahman *et al.*, 2012)** and based on that allergenic studies of the kajal products were carried out and results revealed total safety of both extracts and no allergenic symptoms. Amongst 75 % of herbal kajal revealed good antibacterial activity for eye infecting organism. Finally, concluded that the use of herbal kajal prevent the eye infection, which are safe and keep eyes healthy as well as beautiful **(Pandey D. Agarwal *et al.*, 2019).**

Conclusion

The present study to isolate three different bacteria from eye makeup women's clinical samples. The microorganism identified by using Gram stain and biochemical tests etc. We are selected two herbal plants that are *Eclipta alba* and *Rosa indica* analysis by phytochemical screening have medicinal activity as well as exhibiting physiological activity. The phytochemical screening shows the presence of medicinally active compounds in leaves of *Eclipta alba* and petals of *Rosa indica*.

The two selected herbal plants for preparing homemade kajal. The standardization of *Eclipta alba* and *Rosa indica* plants were carried out with respect to organoleptic characters reported for both the herbals in the official pharmacopeia. Various proximate parameters were carried out and revealed similarities in the values for both the herbs. Thin layer chromatographic (TLC) 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.

Furthermore toxicity and allergenic reactions and antibacterial activity were also performed 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.

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