

Carbon based kajal formulations: antimicrobial activity and feasibility as a semisolid base for ophthalmics

Abstract

Study aimed to prepare and evaluate carbon based kajal formulation and carry out its comparative evaluation with the marketed formulations of kajal.

Initially, carbon soot was deposited on Aloe vera mucilage spreaded on a copper plate, in specifically designed apparatus. Subsequently, sun dried plate was allowed to expose to the flame of cow ghee for 1 hr. Soot which was deposited on the copper plate was scrapped and Characterization of Soot by SEM, TEM, FTIR, XRD and EDX was carried out and kajal was prepared by adding few drops of cow ghee to it. The kajal was compared with the five marketed kajal formulations available in India, on the basis of physical characteristics their antibacterial potential was also assessed against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*.

All the formulations exhibited almost similar physical characteristics. The prepared kajal showed pH (7.06±0.13). The antimicrobial activity of the marketed formulations was comparatively less than that of freshly prepared exhibiting zone of inhibition 13.05±0.11, 20.08± 0.13 and 22.06±0.18 mm against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *E. coli* respectively. EDX spectra, revealed that the cow ghee soot have the weight percentage of carbon and copper as 79.20 and 17.10 % respectively and Confirmed the absence of Lead (Pb). The eye irritation test (OECD 405) also showed no any irritation, redness, watering of the eye or swelling was not observed for prepared formulation in test animal. Prepared Kajal can be safely used for beatification and also in the treatment of eye diseases especially in pediatrics

Key words: *Kajal, antimicrobial activity; Pseudomonas aeruginosa; draize eye test; ophthalmic base.*

1. INTRODUCTION

Personal care products and facial cosmetics are commonly used by millions of consumers on a daily basis. These products are directly applied to human skin and mainly produce local exposure to certain ingredients [1]. According to the Federal Food and Drug Cosmetic Act criteria (2015), cosmetics mean the articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering the appearance and articles intended for use as a component of any such articles; except that such term shall not include soap [2]. To look stunning and beautiful, as a natural aspiration, increased awareness of looking good has also brought in more sensitivity to problems of the skin especially the face.

Eyes are known as 'The Window of our Soul' [3]. Eye cosmetics have been used since prehistoric times to emphasize and highlight the eyes to enhance perceived attractiveness and beauty [4]. Eye makeup has been used since long time to improve personal appearance, to get better self-worth or attract the interest of others. Amongst the commonest causes leading to eyelid dermatitis, cosmetics play a major role. Cosmetics used for eyes include eyeliners, mascara, eye shadow, eyelash, curlers, makeup removers etc. could all responsible to eyelid Blepharitis [5]. Ancient civilizations particularly Hindu and Muslim religious countries namely Rome, India, Egypt, China, Japan, etc used to use numerous type of eye formulations, not only for beautifying purposes, but also for prevention and treatment of eye diseases. From the 1000s of years, the human being decorated their eyes by using colorful substances in order to improve their facial look. Eye paints were used universally throughout North Africa, Middle East, and South Asia. Reports state, black paints gives relief from the obtrusive sun and reflection from the sand before invention of sunglasses and Kajal was out of them!

Kajal (Kohl) has been defined as ultra fine powder comprising one or more substances like herbs, gemstones, pearls etc. particularly used for the eye, as deciphered in Unani, Ayurveda, Greko-Arabica systems of medicine [6]. In the science of Ayurveda, several herbs and floras have been used to make Ayurvedic cosmetics that not only beautify the skin, but also act as the shield against harmful external factors. Amongst those, Kajal is such a cosmetic, whose role in eye products has been delineated till date. Traditionally, in Middle East, kohl was also applied to

the umbilical stump of newborn babies as an astringent and antiseptic, and to the eyes of infants and small children to improve eye health. Also most women used to apply kohl/ kajal once a week, or on special social occasions, apart from during Ramadan, when kohl/kajal/ surma and all hennas were place aside. Kohl was worn for a multiplicity of reasons mainly traditional, beautification, to prevent form the “evil eye,” the extensive belief that kohl is medicinally valuable for the eyes and even applying kohl is encouraged within the sunna, the traditional behavioral principle of the Islamic religion. Means, its usage is hundred years-old traditions with religious, esthetic, and therapeutic significance

In India, numerous procedures have been followed for the formulation of Kajal. Methods vary on the basis of metal plates used (copper, silver, bronze, gold, etc.) For deposition of soot generated by burning different oils like almond, peanut oil, sesame oil, palm oil etc. Gupta et al., (2016) [7] have reported the novel techniques to prepare Kajal using almond oil soot and plant extracts viz: *Mentha piperita* Linn, *Ocimum sanctum* Linn, and *Vitex negundo* Linn., However, no traditional resources and scientific literature has documented making of kajal using cow ghee. More importantly, cow ghee has been recommended for its medicinal importance (antioxidant, antiatherogenic, anticarcinogenic, antidiabetic and antiadipogenic), attributed to presence of conjugated linoleic acids. Thus, reviewing multiferrous medicinal importance of cow ghee, it has triggered the necessity of its formulation in kajal. Further, it may reduce the side effects of the kajal mainly, lead intoxication after its application [8]. Thus, work has been planned to formulate and evaluate cow ghee based kajal preparation using copper plate and *aloe vera*, and standardize the prepared formulation using some marketed formulations available in India. Especially, antimicrobial potential of the prepared kajal formulation and marketed formulations will be assessed. Because, kajal is used for the prevention/treatment of eye ailments namely conjunctivitis, chalazion, blepharitis, trachoma, cataract, ectropion, pterygium, etc. Sweha (1982) [9]. Predominantly, bacterial conjunctivitis is the second most common cause [10-12] responsible for the majority (50%-75%) of eye affected cases in children [13].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Dimethyl sulfoxides (DMSO), ethyl alcohol, nutrient agar were procured from Loba chem. Ltd, Mumbai, India. The solid media and broth were purchased from Hi-Media Pvt. Ltd, Mumbai, India. Marketed formulations of Kajal's were procured from local market of Kasegaon, Sangli, India.

2.2 Synthesis of carbon soot particles

Initially, Copper plate was put in to container filled with 200 mL alcohol and ultrasonicated (Sonicator, Model-EI-6LHSP) at 20 kHz and 20W for 30 min, followed by ultrasonication using deionised water for 15 min, at same volume and frequency conditions. Subsequently, same copper plate was used for the deposition of soot particles.

Simultaneously, the fresh *Aloe vera* leaf was taken and the mucilage separated using knife was applied on copper plate. The *aloe vera* mucilage was also spreaded on plate (inner side) made by the copper (Cu). The mucilage applied copper plate was kept in sunlight for few hours for drying purpose. Further, cow ghee filled lamp was placed below the stand. The cow ghee soot particles were collected on a copper substrate placed above the flame for 1h. Collection of soot particles has been carried out. In the stipulated time duration, the emanated soot particles from the lamp containing cow ghee were collected in a layered pattern over the copper plate. The collected soot was scraped off and collected in an air tight glass container and stored properly until further use.

2.3 Characterization of Soot by SEM, TEM, FTIR, XRD and EDAX

The morphology and particle size of the cow ghee soot were evaluated by SEM (JSM-6700F) and TEM (FEI Tecnai TF20 operated at 200 kV). The cow ghee soot samples were prepared by introducing a drop of cow ghee soot solution ($100 \mu\text{g mL}^{-1}$) in alcohol on a 200 mesh copper grid (C coated). The functional group(s) were confirmed using Fourier transformation infrared spectrophotometer (FT-IR, Bruker, Model – Alpha, PN: 1005151/06, SN: 200767) at the wavelength range $400\text{--}4000 \text{ cm}^{-1}$. EDX were examined by Hitachi S-4700 SEM to confirm the elements present in the prepared carbon soot with their percentage. The X-ray diffraction pattern of cow ghee soot particles was recorded using a Bruker D2 Phaser Cu-K alpha 1.540\AA .

2.4 Extraction of ghee from milk

Direct cream method, as reported by Ahmad and Saleem (2019) [14], was followed with slight modification for the desi cow ghee making (Khillari cattle family member of *bos indicus*). Fresh milk sample from 5 cows was collected from dairies located in the vicinity of Atpadi, Sangli district, India. The milk was collected and mixed in presence of author, so as to ensure purity. Cream separator was used to get fresh cream from desi cow milk. The temperature range for the separation of ghee from cream or butter was reported from 105 to 120⁰C), Therefore the collected 500 g of cream sample was heated in an open pan (115 ⁰C) with continuous stirring until it melts. Indram Tech Thermocouple was used to confirm the temperature of molten cream. After continued heating for 10–15min, ghee got fully separated from the other cream residues due to differences in density of fat and non-fat segments [15].

2.5 Standardization of Cow ghee

Physicochemical properties of the cow ghee namely melting point, solidification point, and specific gravity were studied.

Acid value - 100 milligram of ghee sample was dissolved in pure alcohol as per procedure mentioned by Bhinge *et. al.* 2017 [16]. Then the above ghee sample was heated on a hot plate for 5 to 6 min. One to two drops of phenolphthalein was mixed as an indicator in resultant solution and then titrated with 0.1N potassium hydroxide solution until faintly pink color developed. The acid value was calculated as per formula given mentioned by Bhinge *et. al.* 2017 [16].

Peroxide value - One gram of ghee sample was weighed in a 100 mL flask, 6mL of acetic acid and chloroform solution were mixed. Then 0.1mL of KI solution was dropped with continuous stirring. Then 6 mL of deionized water transferred. Finally the resultant ghee solution was then titrated with 0.1M sodium thiosulfate solution. End point of titration was noted with yellow color to colorless. Lastly 0.1 mL of 1% starch added in above titrated solution. Then after again titration procedure was repeatedly continued with constant shaking to get free iodine from chloroform layer noted with blue color disappearance. Peroxide value was calculated as per formula mentioned in Bhinge *et. al.* 2017 [16].

Total fatty matter - One gram of ghee sample was weighed transferred in conical flask containing 22 mL of 1:1 diluted HCl. The above resultant mixture was heated in water bath till to get the clear solution. Then the resultant solution was drained in 100 mL separating funnel and kept aside for separating the layer. 25 mL of petroleum ether was measured and transferred in the

funnel. The organic phase was separated after shaking of the funnel. The resultant layer (aqueous) was partitioned thrice with 25 mL of pet. ether solvent. Then obtained organic layers were evaporated to get sample extract. The sample extract was filtered using water and sodium sulfate. Finally resultant mixture was filtered again. The collected extract was cleaned and dried. The content was quantified Total fatty matters was calculated as per formula mentioned in Bhinge *et. al.* 2017 [16].

Iodine value - It was estimated as per Wij's method with slight modification, the total number iodine in g absorbed by the 100g of the Cow ghee (BIS, 1981) [17]. Oxidative parameter like peroxide value of desi ghee was estimated as per the procedure given in BIS, 1981 with slight modification.

The saponification value- It determines the mg of KOH necessary to neutralize the free acids and saponify the esters contained in 1.0 g of the substance. Whereas, remaining matter was considered as "Unsaponifiable Matter" and was soluble in ordinary fatty solvents. The anisidine value is defined "as 100 times the optical density measured in a 1 cm cell of a solution containing 1 g of the substance to be analyzed in 100 mL of a mixture of solvents and reagents". Total oxidation value is defined by the formula: 2*Peroxide value + The anisidine value.

2.6 Preparation of carbon based Kajal formulation

Two gram of cow ghee soot was taken in sterilized copper container aseptically. And then, 4-6 drops of prepared cow ghee (liquid) was added and mixed well. Adequate ghee was dropped and levigated till semisolid consistency was attained. Care was taken while adding sufficient cow ghee, which will avoid formation of smudgy and thick carbon based kajal.

2.7 Evaluation of prepared carbon based Kajal and marketed kajal formulations

2.7.1 Evaluation of Physical parameter

All the marketed formulations of Kajal and prepared Kajal were evaluated for physical parameters namely color, odor, texture and consistency.

2.7.2 Determination pH of Formulations

The pHs of the marketed as well as prepared carbon based kajal were determined by pH meter (Systronics digital-DI-707). 1 gram of kajal of all samples were weighed and dispersed in 25 mL

of DMSO and kept for 120 min. The assessment of pH of all Kajal formulations was recorded three times and average value was taken.

2.7.3 Spreadability

A special apparatus designed by scientist Mutimer et al., (1956) was used for determining spreadability of the marketed and prepared Kajal. A surplus amount of Kajal samples were sandwiched between two glass slides and a 500 g weight was kept on upper slide for 5 minutes to compress samples of Kajal for uniform thickness. The 50 gm was added to the pan tide with the thread. The exact time required to separate out the two slides from each other was taken as a measure of spreadability. Smaller the time taken for separation better is the spreadability. It was determined by using the formula:

$$S = M.L/t$$

Where, M indicate the weight in gm tied to the upper glass slide; L denote the length in cm moved on the glass slide and t is time to required to separate the slide in second. [18].

2.7.4 Ocular Irritation of carbon based Kajal Formulation

3 Albino rabbits (2–3 kg) were selected for the ocular irritation studies. Rabbits were placed in a Rajarambapu College of Pharmacy, Kasegaon animal house under observation with proper diet feeding and ad libitum access to water as per the guideline laid by the Institutional Animal Ethical committee (IAEC). Also the guidelines laid by the IAEC and OECD 405 were strictly followed during the experiments as per the protocol (RCP/P-07/18-19). Ocular irritation study of prepared Carbon based Kajal formulation was executed according to the Draize technique [19]. Prepared Kajal formulation (60 µL) was applied to the left eye (lower eyelid) of each rabbit, whereas control was considered as untreated right eye of each rabbit. The prepared carbon based Kajal formulation was applied daily two times for a period of 15 days. The treated rabbits were under observation to note the selected parameters namely redness, swelling and watering of the eye.

2.7.5 Antimicrobial activity

In vitro antimicrobial activity of prepared carbon based kajal and a marketed kajal formulation was performed using the Agar Well Diffusion technique [20-21]. The sterile agar was inoculated

with the bacterial cultures separately (*S. aureus*, *P. aeruginosa* and *E. coli*) for 48 h, at 37⁰C. Antimicrobial activities were tested on nutrient medium against aforesaid organisms responsible for routine eye infections [22]. Bores were made in the wells by using a sterile borer, and all marketed and prepared formulations (5 mg/mL solution was prepared by dissolving the test sample of Kajal in DMSO) and 80 µl volume were placed into bore. All Plates were kept for two and half hours in the refrigerator to enable prediffusion of the formulations into the agar (temperature condition). Subsequently, the plates were incubated for the night at 37⁰C for 24 h. The antimicrobial potential was determined by measuring the diameter of zone of inhibition [16].

2.7.6 Statistical analysis

All the values were proposed as mean ± standard deviation (S.D.) in each group. Statistical investigation was performed using student t-test. A value of P < 0.0001, 0.05, 0.01, or 0.001 was considered statistically significant.

3. RESULTS

3.1 Microstructure of soot particles

The synthesis of nano sized cow ghee soot particles prepared by combustion of cow ghee in the absence of a precursor was confirmed using SEM and TEM images, as depicted in **Fig. 1**. In SEM and TEM image showed the fragile network of soot particles, seem interconnected with weak Van Der Waals force. Soot particles showed an average size of 20–50 nm. However, not all carbon soot particles could be confirmed as carbon nanospheres. From EDX spectra, it is revealed that the cow ghee soot have the weight percentage of carbon and copper as 79.20 and 17.10 % respectively. According to EDX result of cow ghee soot confirmed the absence of Lead (**Fig. 2A**).

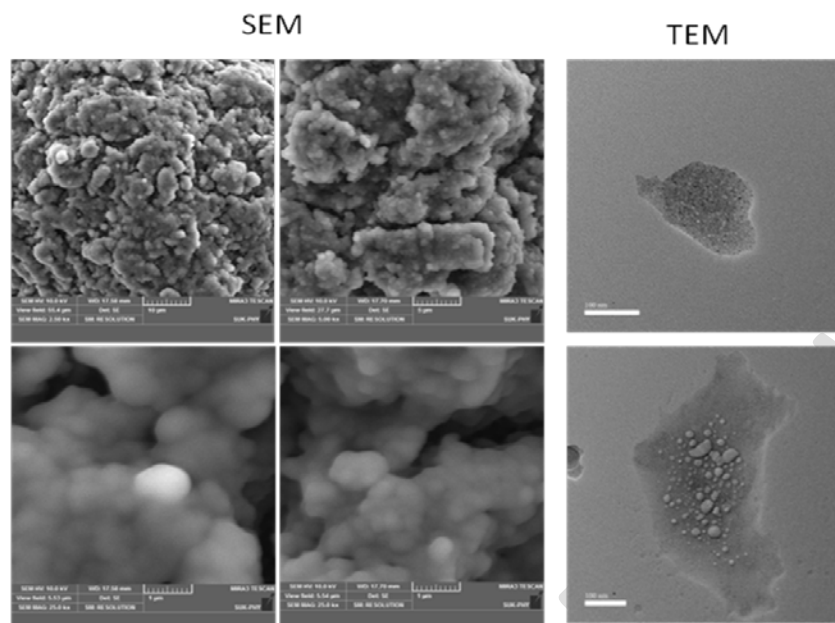


Fig. 1. SEM and TEM images of Prepared Carbon Soot

3. 2 FTIR Analysis of soot particles and Carbon containing Kajal Formulation

The functional groups present in the soot was confirmed as reported by Ntaote Ezekiel (2012) and Sahoo and Kandasubramanian (2014) [23-24]. Particularly, C–H_{stretching} bands were observed between the range 3200 and 2750 cm^{-1} . The peaks at 2871.23 and 2822.82 cm^{-1} correspond to the C–H_{stretching} for sp^3 bonded C element. IR absorption of the double-bonded is containing various functional groups observing between 1890 to 1440 cm^{-1} . Carbonyl group (C=O_{stretching}) band noted at 1694.49 cm^{-1} . The FTIR spectrum of ghee soot particles exposed the characteristic (C–C_{aromatic}) stretching peak at 1596.76 cm^{-1} . The results are depicted in (Fig.2B).

Especially, C–H_{stretching} bands were observed between the range 3100 and 2755 cm^{-1} . The peaks at 2914.27 and 2845.84 cm^{-1} correspond to the C–H_{stretching} for sp^3 bonded C element. IR absorption of the double-bonded is containing various functional groups observing between 1800 to 1540 cm^{-1} . Carbonyl group (C=O_{stretching}) band noted at 1734.81 cm^{-1} . The FTIR spectrum of ghee soot particles exposed the characteristic (C–C_{aromatic}) stretching peak at 1582.48 cm^{-1} (Fig.2C).

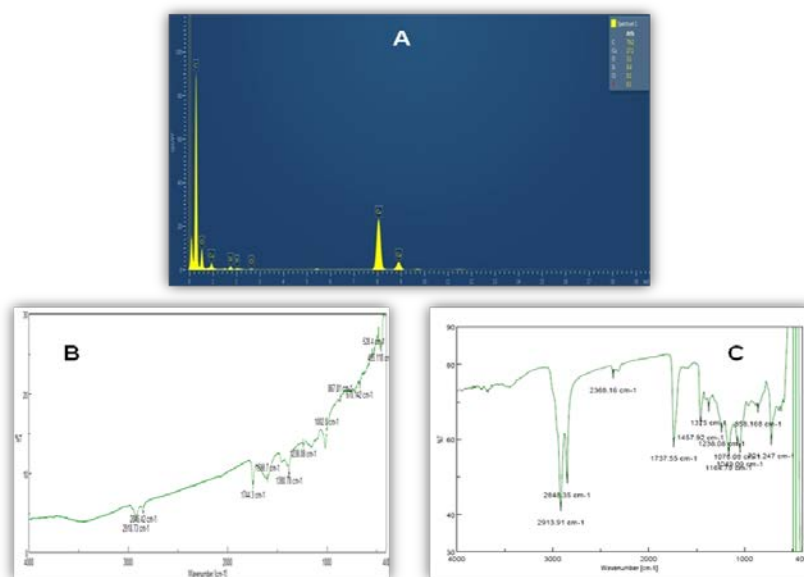


Fig. 2. (A) EDAX image of Prepared Carbon Soot; (B) FTIR of Prepared Carbon Soot, (C) FTIR of Prepared Carbon Based Kajal Formulation

3.3 Diffraction study of ghee soot particles

The Diffraction results of carbon soot have been in good agreement with earlier reports by Chen et al., and Wilson et al. [25, 26]. The maximum amount of amorphous carbon present in the ghee were confirmed by the high intensity peak at 25.20 (2θ), corresponding to graphite (002). 2θ broad band was observed in the range of 20–30° also exhibited the presence of amorphous carbon and graphite [24-26]. Whereas, the presence of diamond shaped carbon particles was also confirmed from the observed 2θ value at 43.2 in the diffractogram (**Fig.3**). The 2θ broad band which was observed in the range of 40–50° also exhibited the presence of diamond shaped carbon.

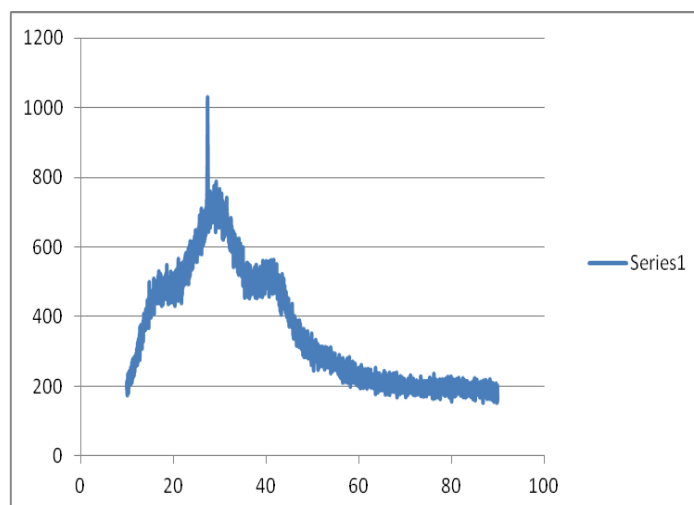


Fig. 3. XRD image of Prepared Carbon Soot

3.4 Standardization of Cow ghee

Calculated data of Acid Value, Ester Value and Peroxide Value values of prepared ghee sample were found to be 0.3546 ± 0.0154 , 250.15 ± 0.1547 and 7.25 ± 0.3546 respectively. Whereas Iodine value, Saponification value and Unsaponifiable Matter (%) and data in prepared cow ghee sample were observed to be 37.54 ± 0.5641 , 224.5 ± 0.14 and 0.9054 ± 0.1245 , respectively. In the prepared cow ghee sample, Anisidine and Total Oxidation Value were found to be 9.25 ± 0.4578 and 23.7546 ± 0.7525 respectively. Whereas the prepared cow ghee sample contain negligible quantity of heavy metals. Specific gravity, Melting Point and Solidification temperature of the prepared cow ghee sample were noted within range as per the report of BIS (1981). The cow ghee sample was found to be within the range for all above mentioned parameters (**Table 1**).

Table 1. Parameters for characterization of freshly prepared cow ghee

Parameters	Result
Specific gravity (g cc ⁻¹)	0.9425 ± 0.0054
Melting Point (°C)	36.20-36.60
Acid Value	0.3546 ± 0.0154
Ester Value	250.15 ± 0.1547
Peroxide Value	7.25 ± 0.3546
Iodine Value	37.54 ± 0.5641
Saponification value	224.5 ± 0.14
Unsaponifiable Matter* (%)	0.9054 ± 0.1245
Solidification temperature (°C)	20.25-21.97
Anisidine value*	9.25 ± 0.4578
Heavy metals* (%)	0.0002 ± 0.0001
Total Oxidation Value	23.7546 ± 0.7525

3.5 Physical evaluation of prepared Carbon Based Kajal Formulation

Prepared and marketed Kajal formulations were evaluated for different physical parameters namely color, odor, texture and consistency. From the results all evaluated formulations showed the similar physical characteristics like color is black, characteristic odor and smooth appearance / texture and all formulations were possessing semisolid consistency. Results obtained are shown in **Table 2**.

3.6 pH determination

Prepared carbon based Kajal and the marketed Kajal formulations having the pH in the range of 6.9 -7.9, which posses satisfactory remark as per the requirement of ophthalmic product reported by Garcia-Valdecabres et al., 2004 [27]. The results are shown in **Table 2**.

3.7 Spreadability

All the formulations and prepared carbon based Kajal having optimum spreadability, in the range of 8.5 to 10.2 as represented in **Table 2**.

Table 2: Results of Physical parameters of prepared and marketed formulations

Sr. No	Formulations	Spreadability	pH
1	Kajal Formulation (Test)	8.51± 0.3010	7.06±0.1247
2	Jaai*	9.70± 0.4115	6.90±0.0816
3	Patanjali*	9.91± 0.4625	7.06±0.1247
4	Himalaya*	10.22± 0.4520	7.20±0.0816
5	Oriflam*	8.71± 0.3715	7.71±0.0816
6	AIDD's*	10.11± 0.4050	7.91±0.0816

All formulations were semisolid in nature, had black colour, characteristic odour, and smooth texture.

*indicate the formulations are Marketed formulations

3.8 Ocular Irritation of Carbon based Kajal formulations

Eye irritation parameter of the prepared carbon based Kajal formulation was characterized into 4 grades. Means, practically no irritation (score 0-3); slight irritation (score 4-6); moderate irritation (score 7-9) and severe irritation (score 10-12) [28]. The total score for eye irritation was calculated as per the report of Gan et al. (2009). The observed eye irritation score in the control was 0.25 and for Kajal formulation was noted 0.95, which indicated the prepared carbon based Kajal formulation has excellent ocular tolerance in Rabbit. Additionally, abnormal clinical signs or any ocular damage or conjunctivae were not observed. Moreover, redness of the eyes, watering of the eye or swelling was not observed for Kajal formulation treated and control treated group. Generally the noted results revealed that prepared carbon based Kajal formulation is safe for ocular application. (Fig. 4)

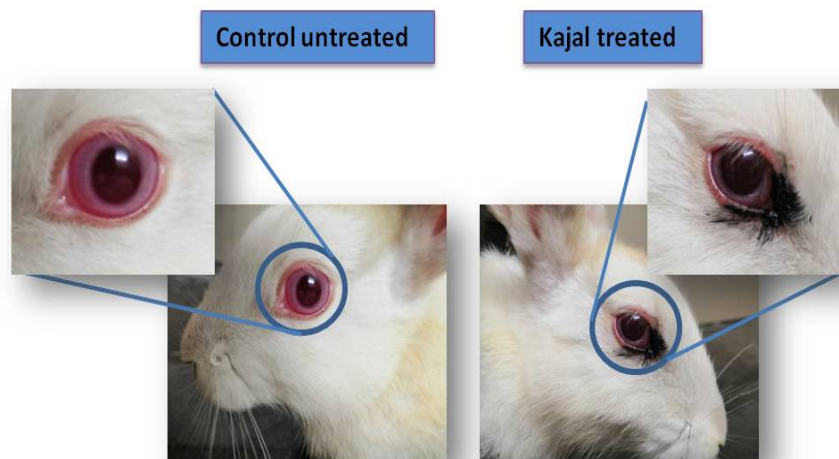


Fig.4. Ocular Irritation test of carbon based Kajal Formulation

3.9 Antimicrobial activity

From the results of antimicrobial activity, the zone of inhibition of the marketed formulation namely Jaai, Patanjali, Himalaya, Oriflam and AIDD's kajal were found to be 9.26 ± 0.38 , 14.36 ± 0.41 , 2.86 ± 0.26 , 2.83 ± 0.32 and 3.16 ± 0.06 against the *Pseudomonas aeruginosa* respectively. Whereas zone of inhibition of prepared carbon based kajal was found to be 13.05 ± 0.11 . In case of *Staphylococcus aureus* the zone of inhibition was observed to be 8.96 ± 0.16 , 7.06 ± 0.12 , 8.06 ± 0.12 , 8.08 ± 0.10 and 3.18 ± 0.061 for Jaai, Patanjali, Himalaya, Oriflam and AIDD's kajal respectively. While prepared carbon based kajal exhibited zone of inhibition 20.08 ± 0.13 . Moreover *E-coli* the zone of inhibition were observed to be 3.36 ± 0.12 , 8.01 ± 0.15 , 3.06 ± 0.12 , 3.15 ± 0.04 and 3.05 ± 0.10 , for Jaai, Patanjali, Himalaya, Oriflam, AIDD's kajal respectively and zone of inhibition of prepared carbon based kajal was found to be 22.06 ± 0.18 . Ciprofloxacin was used as standard which showed the zone of inhibition 30.15 ± 0.04 , 38.20 ± 0.03 and 38.14 ± 0.03 against the *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli* respectively. Marketed formulation of kajal showed lesser potential as compared to the carbon base proposed Kajal formulation as suggestive from the zone of inhibition measurements. The marketed formulations of Himalaya and Oriflam showed very less antimicrobial potential against *Pseudomonas aeruginosa* and *E. coli*, whereas, AIDDS exhibited very less antimicrobial potential against all the selected strains of microbes. Noteworthy to

mention was potential activity of prepared Kajal formulation against gram negative bacteria's. The carbon based kajal was formulated with an aim to serve both the purposes of an eye cosmetic and also to promote healthy eyes free from infections. The results of zone of inhibition are shown in **Table 3** and (**Fig.5 and 6**).

Table 3 Antimicrobial sensitivity results for of marketed and test formulations of Kajal.

Sr. No.	Sample name	Zone of Inhibition Diameter (mm) against the selected microorganisms		
		<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>
1	Kajal Formulation (Test)	13.05±0.11	20.08±0.13	22.06±0.18
2	Jaai ^a	9.26±0.38	8.96±0.16	3.36±0.12
3	Patanjali ^a	14.36±0.41	7.06±0.12	8.01±0.15
4	Himalaya ^a	2.86±0.26	8.06±0.12	3.06±0.12
5	Oriflam ^a	2.83±0.32	8.08±0.10	3.15±0.04
6	AIDD's ^a	3.16±0.06	3.18±0.061	3.05±0.10
7	Ciprofloxacin ^b	30.15±0.04	38.20±0.03	38.14±0.03

All formulation results are in triplicate; b indicates standard antimicrobial agent.

Zone of Inhibition (mm)

Fig. 5. Graph showing comparative antimicrobial activity of all formulations against bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli* with standard ciprofloxacin.

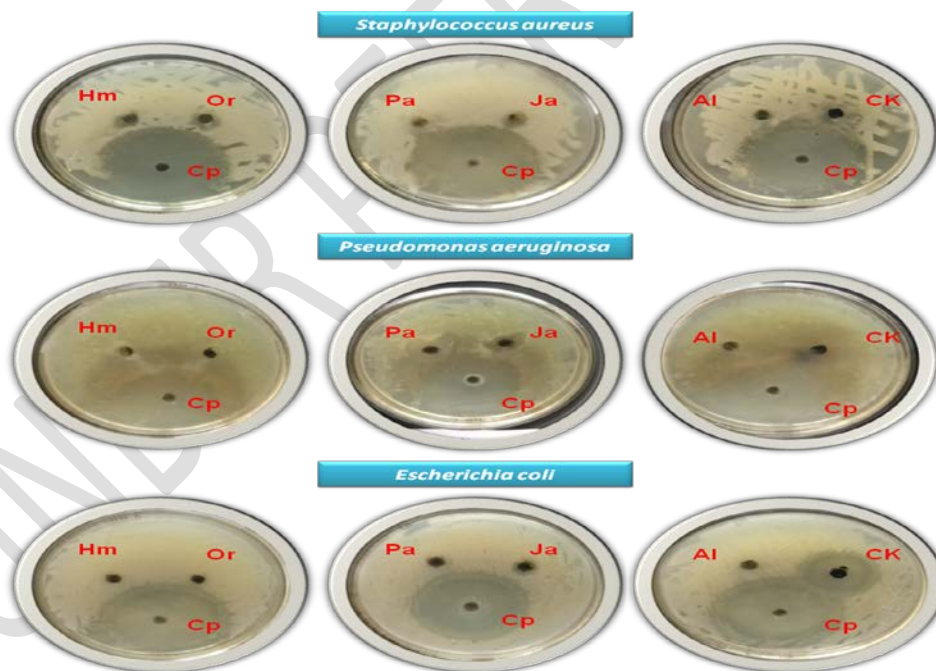


Fig. 6. Antimicrobial activity of Himalya Kajal (Hm), Oriflam Kajal (Or), Pantnjali Kajal (Pa), Jai Kajal (Ja), AIDDS Kajal (Ai), Prepared Carbon Based Kajal (Ck) and standard ciprofloxacin (Cp) against selected the micro-organisms

4. DISCUSSION

Kajal has been used for eye decorating purposes and also for defense and cure of different eye diseases like bacterial conjunctivitis, bacterial Keratitis since, ancient civilizations, mainly Hindu and Muslim religious regions, like India, Rome, Afaganisthan, Egypt, China, Japan, etc. As per reports of Unani, Ayurveda, Greko-Arabica systems of medicine literature etc, cosmetics of eye are as old as vanity [29]. Centuries ago, particularly, in the 16th era, the people were using the Kajal/kohl for improving the appearance of eye. Recently, lead toxication was the major issue with the usage of Kajal formulation as per report of Gilfillan SC (1965). Nevertheless, most of the hindu and islamic religious countries have continued application of Kajal formulations till today. Especially in India, no reports have reported lead intoxication to eyes of women and specially childrens. The reason lies in procedures adopted in making of kajal formulations, differing in Unani, Ayurveda, Greko-Arabica systems of medicine etc. As aforesaid, different techniques for the preparation of the Kajal using herbs, different oil and metals have been reported. Therefore, our attempt was to rule out presence of lead in Kajal formulation using EDX analysis, and prove its potential as antimicrobial against pathogens responsible for eye infections.

Elemental carbon has demonstrated very low toxicity, as evidenced from literature [30]. Carbon nanotube having carbon element in a uniform layer as graphite [31]. As per the report of Beg et al. (2010), CNTs could also be used for ocular purpose [32]. So use of carbon in ocular formulation becomes obvious.

As a vehicle for drugs, ghee has been considered as a promising, due to its ability to easily cross the blood brain barrier. Thus, most of the Ayurvedic formulations acting on psyche recommends its use [33]. More importantly, ghrita (ghee) along with triphala churna have been used for the treatment of eye disorders [34]. Ghrita is nothing but Ayurvedic medicine, in cow ghee form [35]. Therefore we have used freshly prepared cow ghee as a formulation base in Kajal making. As anticipated, prepared cow ghee was found to be within the limits, as specified in standardization protocols (**Table 1**).

The use of copper by human civilization dates back to between the 5000 and 6000 years. The use of copper and its advantages have been known to the people since long time, however, have currently received renewed attention. Copper is the most prominent agent used for prevention of microbial infection, and its use has been continued until the advent of commercially available

antibiotics in 1932 [36]. Therefore we preferred use of sterile copper vessel for the deposition of carbon soot, which was subsequently used for Kajal formulation. The EDX results revealed that the Kajal formulation comprised of 17.3% of elemental copper and the rest part as carbon (**Fig. 2C**). Most probably, the antimicrobial activity of the kajal formulation would be due to the presence of copper metal in kajal formulation. The soot recovered from burning, the leaves, fruits, flowers, roots etc. of *Opuntia monacantha*, *Solanum incanum*, *Sida tenuicarpa*, *Zea mays*, *A. oppositifolia*, *F. wakefieldii*, *P. barbatus*, *R. natalensis* etc. were used for the purpose of curing the liver disease, peptic ulcer, spleen disorder, oral candidiasis etc. in Sankuru, Elgeyo Marakwet, Kenya, as reported by Kigen et al. (2017) [37]. Similarly, as mentioned in traditional literature of Ayurveda, Chinese, Unani medicinal system, the soot prepared from plant materials have been used for curing the diseases. The use of herb for the preparation of soot is a very common practice in Asian countries, especially in India. Also, *Aloe vera* mucilage and extract have potent antimicrobial and antiviral properties [38]. Therefore *aloe vera* mucilage was applied on the copper plate to get the soot. Carbon soot was obtained from burning of cow ghee flame and its deposition on the copper plate layered with sun dried *aloe vera* mucilage. As the prepared kajal formulations contain cow ghee and carbon soot, characteristic IR peaks were observed at 1737.55 cm^{-1} (strong peak), 1078.08 cm^{-1} (strong peak) of C=O and C-O saturated fatty acid respectively (**Fig. 2A** and **2B**). From the IR of soot and kajal formulation, no unfavorable interactions were noted in carbon soot and cow ghee.

The non-irritancy of ophthalmic preparations, is a prerequisite, and was assessed by ocular irritation study after the application of the prepared Kajal formulation to rabbit. The score of the irritation like was observed to be almost same for applied kajal formulation and control treated eye of the rabbit.

Further, the study of the antimicrobial potential of the prepared Kajal formulation was assessed against the microbial strains namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E coli*, because, they are commonest cause of various eye infections. Especially, it is surprising finding that, against both gram negative bacteria's (*Pseudomonas aeruginosa*, and *E coli*), it has got excellent activity. Numerous branded Kajal preparations are available in the asian market and the popularity of Kajal preparations is ever increasing as a cosmetic formulation. As per Ayurveda literature, Kajal formulation is not restricted to only beautification, but also for the protection of the eyes from the bacterial infections etc. The results were compared with different

marketed Kajal preparations popularly used in the Asian countries. The marketed preparations might have been devoid of cow ghee as a base, or containing some other oleaginous substances used as base. The prepared Kajal formulations were found to be within the limit for the various physical parameters namely spreadability and pH. The antimicrobial potential of the prepared Kajal formulation showed the excellent zone of inhibition than the selected marketed formulations and comparable results with the standard.

5. CONCLUSION

Carbon based kajal was formulated and comparatively evaluated with similar marketed branded formulations. The physical evaluation was suggestive of a cosmetically appealing and acceptable product with significant antimicrobial activity, revealing its importance towards health and protection of eyes, compared to all marketed formulations. As, the use of kajal in numerous countries has been banned in the view of lead toxicity, test formulated kajal was devoid of same, thus, can be used safely in women and children Here we suggest, the studied carbon based kajal prepared from cow ghee could be useful from cosmetic point, and as an antimicrobial protecting women and children's from eye infections. Antimicrobial activity of prepared Kajal formulation against gram negative bacteria's, its use as an appealing ophthalmic base, has a potential to open up a new avenue for future research on ophthalmic.

Ethical Approval

The protocol (Protocol Number – RCP/P-07/18-19) was approved by the Institutional Animal Ethical Committee (IAEC), Rajarambapu College of Pharmacy, Kasegaon (Registration Number – 1290/PO/RE/S/09/CPCSEA)

CONFLICT OF INTEREST

No conflict of interest was declared by the authors

ABBRIATION

SEM – Scanning electron microscopy; TEM – Transmission electron microscopy; FT-IR – Fourier Transform Infra red spectroscopy; XRD – X-ray diffraction; EDX – Energy

dispersive X-ray spectroscopy; mL – milliliter; OECD – Organization for economic co-operation development; DMSO – Dimethyl sulphoxide

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