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Instrumental Characterization of Black Soot of *Glycyrrhiza glabra*: A Colouring Pigment for Eye Cosmetics

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ABSTRACT

Background: *Añjanaṃ* (collyrium) in Ayurveda is predominantly centered around its role in maintaining eye health rather than cosmetic use. This aligns with the traditional kohl/kajal application, prepared using herbs rich in anti-microbial properties, thus offering ocular benefits. Such kajals incorporate burnt black soot from plants to achieve the desired dark color. Analyzing this carbonized form is essential to confirm its therapeutic contribution among other ingredients. In this study, black soot particles obtained from *Glycyrrhiza glabra* (GG), a potent Ayurvedic herb known for promoting vision and addressing conditions such as dry eye disease, Cataract and conjunctivitis, were examined. **Materials and Methods:** The traditional method of preparing black soot was employed, involving scraping it from a copper plate after burning a wick dipped in GG's decoction seven times. **Results:** Physico-chemical analysis of the raw GG and its decoction met the API (Ayurvedic Pharmacopoeia of India) standards. The GG decoction pH, total solids and specific gravity were 5.3, 14.636% and 1.048 respectively. FT-IR analysis revealed four bands corresponding to GG's methylene and methane groups, while XRD indicated the presence of cupric and cuprous oxide. The SEM-EDX results showed that the black soot contained 68.29% carbon, 14.62% oxygen, and 17.09% copper. **Conclusion:** These findings suggest that even in its carbon form, black soot derived from GG using traditional methods retains active ingredients from the combustion of plant biomass. This underscores its potential therapeutic benefits in addition to its cosmetic purposes. The use of black soot from herbs in kajals can offer both cosmetic enhancement and ocular health advantages.

Keywords: Black soot, FT-IR, Herbal cosmetic, Kajal, SEM-EDX, XRD, *Yashtimadhu*.

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INTRODUCTION

Herbal cosmetics lately have gained great momentum in terms of acceptance by large population and safety in comparison to cosmetics prepared out of synthetic chemicals. Preference towards synthetic cosmetics raised concerns about its safety over prolonged use along with other infections, eye irritation and contaminations.[1] With respect of eye cosmetics reports are additionally suggestive of branded kajals containing lead and arsenic that can potentially increase the fear of lead toxicity upon chronic use.[2] Hence we may say that due to the above discussed reasons choice of using herbal based cosmeceuticals exists in an upper hand. In the present era, herbal cosmetics have been effectively developed to preserve their colour, scent, elegance, and efficacy, offering various benefits.[3] In 2016, the worldwide market for natural and organic personal care products was estimated to be worth around US$ 11 billion. Over the next six years, this market projected to grow substantially, with anticipated revenues reaching US$ 22 billion by 2022.[4]

The classical texts of Ayurveda attributes *Glycyrrhiza glabra* (GG) to provide beneficial effects to eye justified by its use in diseases like dry eye disease, glucose induced cataracts, conjunctival hyperaemia etc.[5-7] The cosmetic appraise for this drug in Ayurveda too is not limited. This drug is said to possess complexion enhancing properties justified by its position in *Varṇyamahākaṣāyaḥ* (group of ten drugs useful in increasing complexion) in Charaka Samhita of Ayurveda.[8] Known as licorice in English, this herb is widely used in the field of modern cosmeceuticals. Licorice extracts have also been historically used to treat various health conditions, including digestive and respiratory disorders, epilepsy, and skin-related issues like psoriasis and hyper pigmentation. They offer skin-whitening, anti-sensitizing, and anti-inflammatory properties, making them valuable additions to cosmetic formulations, especially for sunscreens, anti-aging products, and skincare items like cleansers, toners, and makeup products. These research confirm the promising potential of licorice extracts for cosmeceuticals formulations.[9]

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Traditionally, kohl, kajal, or surma is created by blending carbon from herbs, obtained by either direct burning of the substance or burning a wick soaked in the herb's extract.[10-12] These carbon-based kajals have been reported to possess antimicrobial properties.[13-15] In the preparation of herbal kajals, therapeutic substances that effect ophthalmic health are often included along with the synthesized carbon particles. However, determining the true efficacy and the specific component responsible for such effects becomes a concern. The carbon form of drugs contains incinerated drug particles, which might alter their original state and potentially reduce their effectiveness. To investigate this, the current study focuses on preparing carbon soot of GG and analyzing it using instrumental methods like Fourier Transform Infrared Spectroscopy (FT-IR), Powder X-ray Diffraction (Powder XRD), and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX).

MATERIALS AND METHODS

The entire methodology of the study was divided into,

- Pharmaceutical part
- Analytical part

Pharmaceutical part

Pharmaceutical preparation of the black soot was done in the laboratory of Department of Rasashastra and Bhaishajyakalpana of KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka.

Collection of study drug and authentication

Root of GG was procured from GMP Certified Ayurvedic pharmacy and made authenticated and tested its physico-chemical properties by the Drug Testing Laboratory of ASU Drugs at KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka.

Preparation of GG decoction

Initially coarse powder of GG was made by pounding it in a stone mortar and pestle and was passed through sieve number ten. Later the coarse powder of GG was subjected to decoction preparation following the standard operative methods of Ayurvedic *kaṣāyakalpanā* (decoctions).[16] For the same one part (100 g) of the drug was made boiled in an open stainless-steel vessel containing 16 parts (1600 mL) of normal potable water. Keeping the flame level of LPG cylinder set up moderate, boiling was continued until 1/8th reduction (200 mL). Later the contents were filtered using a dry cotton cloth.

Preparation of wick

Unbleached cotton cloth of size 20 cm^2 was cut. In the prepared decoction the cloth was dipped, squeezed and dried in shade. This dipping and drying process was repeated for totally seven consecutive times. Cloth after seventh time of dipping and drying process was made rolled in shape of a wick.

Preparation of black soot

A mud lamp filled with cow ghee up till the brim was taken. Wick prepared from the above method was placed in lamp, moistening its tip with little ghee smeared on it. Lamp with ghee was ignited and allowed to burn with a steady and non-flickering flame. A copper plate with diameter larger than the lamp was chosen and placed inverted exactly over the tip of burning flame with maximum gap of one finger thickness between the flame and plate. The set up was left undisturbed till black layer of soot particles in seen deposited in the inverted surface of copper plate completely. Level of ghee in lamp was also fuelled repeatedly in between to ensure continuous burning. Later upon self-cooling, the carbon soot particles from the plate surface were carefully scraped with a soft bristle brush and stored in air tight boxes for further use.

Analytical part

Analysis of GG and its decoction

Raw drug GG was tested for their basic physico-chemical parameters like loss on drying, total ash, water soluble extractive values etc., along with physico-chemical analysis of GG decoction which involved the assessment of organoleptic characteristics, specific gravity, pH levels, and total solids content at Central Research Facility of KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka. All these tests were conducted following the Standard Operating Procedures (SOP) outlined in the Ayurvedic Pharmacopoeia of India, ensuring consistency and conformity with established guidelines.[17,18]

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR was done in School of Advanced Sciences, Vellore Institute of Technology, Vellore, Tamil Nadu. The analysis was conducted using a Nicolet iS50 model, manufactured by Thermo Scientific in the USA. The instrument utilized 64 scans with a resolution of 4 cm^{-1}, operating in KBr mode. For the analysis, a mixture was prepared consisting of 95% KBr and 5% sample soot particles. This mixture was thoroughly triturated and then subjected to the analysis using the Nicolet iS50 instrument.

Powder X-ray Diffraction (XRD)

The analysis was performed at the School of Advanced Sciences, Vellore Institute of Technology, Vellore, Tamil Nadu, utilizing a Bruker D8 Advance model from Germany. This X-ray analysis system featured a 2.2 kW Cu anode with a ceramic X-ray tube as the source and employed a Lynx Eye detector, which utilized silicon slip detector technology for precise measurements. Additionally, a Ni filter was used as a beta filter to enhance the quality of the analysis. The sample was held in place using both a zero-background sample holder and a PMMA sample holder, ensuring accurate and comprehensive data collection.

Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX)

The SEM-EDX analysis was conducted at the Sophisticated Analytical Instruments Facility situated within the Sophisticated Test and Instrumentation Centre in Cochin, Kerala. The instrumental setup employed for this examination featured the Jeol 6390LA/ OXFORD XMX N model. It offered a wide-ranging accelerating voltage, spanning from 0.5 to 30 kV, providing flexibility in the analysis process. The filament used in this setup was composed of tungsten, ensuring efficient electron emission for precise imaging. The system allowed for substantial magnification, offering capabilities of up to 300,000 times to closely examine the samples. Moreover, the EDAX system boasted impressive analytical prowess, with a resolution of 136 eV and a generously sized detector area covering 30 mm^2. This combination of equipment facilitated highly detailed imaging and accurate elemental analysis of the samples, contributing to a thorough understanding of their composition and characteristics.

RESULTS

The organoleptic character assessment of GG revealed that its part used is the root. It has a yellowish-brown colour, a sweetish taste, and a faint yet characteristic odour. The process of black soot preparation resulted in different yields and losses at various stages. Table 1 shows the results of physico-chemical analyses performed for the root of GG, whereas Table 2 shoes the results of phytochemical analysis of GG's water and alcohol extract. The GG decoction yielded 230 mL from an initial 1600 mL of water, corresponding to a yield of 14.375% and a loss of 85.625%. From this decoction, 100 mL of wick material was obtained, with a yield of 43.478% and a loss of 56.522%. Finally, 8 g of black soot was obtained from 100 mL of decoction, with a yield of 8% and a loss of 92%. The organoleptic assessment of GG decoction revealed that it is a liquid with a dark brownish colour, a sweetish taste, and a faint yet characteristic odour. The physicochemical analysis of GG decoction showed that it has a specific gravity of 1.048, a pH of 5.3, and a total solids content of 14.636%. Table 3 gives angle and other information on XRD. Figures from 1(a) to 1(e) show SEM images at different magnifications. Graph 1 shows the absorption bands obtained upon FT-IR analysis of black soot. Graph 2 provides the peaks of components from XRD. Peaks of different components from EDX are shown in Graph 3. Table 4 gives elemental concentration of different elements found in EDX.

DISCUSSION

The primary objective of this study was to characterize the soot particles derived from the combustion of plant-based materials, which often contain organic components. Traditionally, the process of preparing eye cosmetics like kajals involves the use of medicinal substances known to promote healthy vision. These kajals typically incorporate the carbonized form of certain medicinal compounds to achieve the desired darkness and provide soothing effects to the eyes, with other potent ingredients. However, to understand the active therapeutic component in such formulations, it becomes crucial to comprehensively analyze them.

The colorant in herbal kajals essentially consists of the charred remains of plant materials. The heat involved in carbonization can potentially alter or denature the original state of the phytoconstituents present in these medicinal substances. Consequently, it raises questions about whether the therapeutic benefits of these formulations stem solely from other active ingredients or also from the burnt soot itself. Therefore, this study logically delved into the analyis of the soot particles produced from the combustion of plant-derived products.

In the context of Ayurveda, the use of *Añjanaṃ* (a form of ocular therapy), is emphasized in daily routines to maintain good vision.[19] Further elaborating, the Susruta Samhita classifies *Añjanaṃ* into various types, with the *prasādana* type, which enhances healthy vision, involving the use of drugs possessing specific characteristics such as a sweet taste (*madhurarasaḥ*), cold potency (*śītavīryam*), and unctuous nature (*snigdhaguṇaḥ*).[20] *Glycyrrhiza glabra* (GG), also known as *Yaṣṭimadhu*, aligns with these criteria and is traditionally considered to be a promoter of vision (*cakṣuṣyam*).[21] Based on these principles we selected GG for our present study.

We obtained GG in the form of coarse powder from a GMP certified pharmacy for the preparation of black soot. The authentication report confirms the original quality of the GG obtained. For preparation of back soot, to maximize the extraction of active constituents from the drug used, the regular method of preparing decoction from Ayurveda was employed. Due to maximum reduction the loss percentage of 85.625% was observed. Instead of soaking unbleached cloth for 8 hr once for

Table 1: Results of physicochemical analysis of GG.

Analysis	Results	API limits
Foreign matter	Nil	Nil
Loss on drying	1.287%	---
Total ash value	8.227%	<10%
Acid insoluble ash	1.265%	<2.5%
Water soluble ash	1.119%	NA
Water soluble extractive value	34.977%	>20%
Alcohol soluble extractive value	14.348%	>10%
Microbial limit test	Lesser than limits	Complies with API

Table 2: Results of phytochemical analysis of GG.

Sl. No.	Phytochemical test	Alcoholic extract	Aqueous extract
1	Carbohydrates	+	+
2	Reducing sugar	+	-
3	Monosaccharides	-	-
4	Pentose sugar	+	-
5	Hexose sugar	-	-
6	Non-reducing polysaccharides	-	-
7	Proteins	+	+
8	Amino acids	-	-
9	Steroids	+	-
10	Cardiac glycosides	-	+
11	Saponins	+	+
12	Flavanoids	-	+
13	Alkaloids	+	+
14	Tannins	+	+
15	Anthraquinone glycosides	+	-

Table 3: XRD results of black soot particles.

Sl. No.	2 theta/2θ	FWHM	d spacing	Relative intensity
1	29.548°	0.100	3.02072 Å	11.0%
2	35.851°	0.100	2.50274 Å	28.1%
3	36.774°	0.313	2.44205 Å	100.0%
4	39.093°	0.223	2.30236 Å	21.1%
5	42.558°	0.346	2.12257 Å	40.7%
6	61.740°	0.331	1.50128 Å	15.8%
7	73.746°	0.398	1.28374 Å	11.3%

Table 4: Elemental concentration in black soot particles by EDAX.

Element	Line type	Weight %	Atomic %
C	K series	38.33	68.29
O	K series	10.93	14.62
Cu	K series	50.74	17.09
Total		100	100

all as done by Sandeep W *et al*., the method was modified to dipping and drying in sunlight for seven consecutive times.[22] This shall enable the cloth dipped in the decoction to absorb and retain more active principles, as each subsequent dipping and drying may increase the concentration sevenfold. 8% was the gain obtainable from the overall preparation of black soot. This may be because carbon particles in the form of soot are very much light in weight.

Values of physico-chemical analysis of root of GG were found to be within the limits of API. Extractive value obtained with water is higher than that of alcohol. Preliminary phytochemical analysis showed presence of carbohydrates, proteins, cardiac glycosides, saponins, flavonoids, alkaloids and tannins in the aqueous extract of GG. The above findings relate the rational sense behind using water as the medium of extraction while preparing decoction. Tannins and flavonoids are considered as subgroups of phenolic compounds indicating the presence of the same by the phytochemical analysis.[23] Usually plants like GG are attributed more for their antioxidant and free radical scavenging properties due to the presence of phenols in them. When solubility of such phenols is spoken about, in general they become soluble in water depending upon the load of hydroxyl group it contains. Furthermore, specific temperature raise in the process of extraction can help less polar phenolic compounds to dissolve in water implying the reduced polarity of water.[24] This gives a clear understanding regarding heating technique employed in the method of decoction preparation. During the dipping and drying method employed to prepare wick, approximately 14.636% of the total solids from GG decoction were made available to get adhered to the cloth. pH showed that GG decoction is a weak acid. Hence its incorporation for development of an eye cosmetic like kajal would be compatible. The specific gravity was found to be greater than one. This might be due to the maximum reduction employed for making decoction and this could have probably increased the density of the liquid by GG's active water-soluble ingredients in the form of dissolved solids.

A total of four bands were observed in the FT-IR spectrum of black soot particles. This indicates that the sample is likely a simple compound, one with a low molecular weight, or possibly a compound involving simple salts. Notably, all these bands were confined to the mid IR spectrum range (400-4000 cm^{-1}), with no presence in either the far or near IR spectral regions. Among these four bands, one was located in the fingerprint region

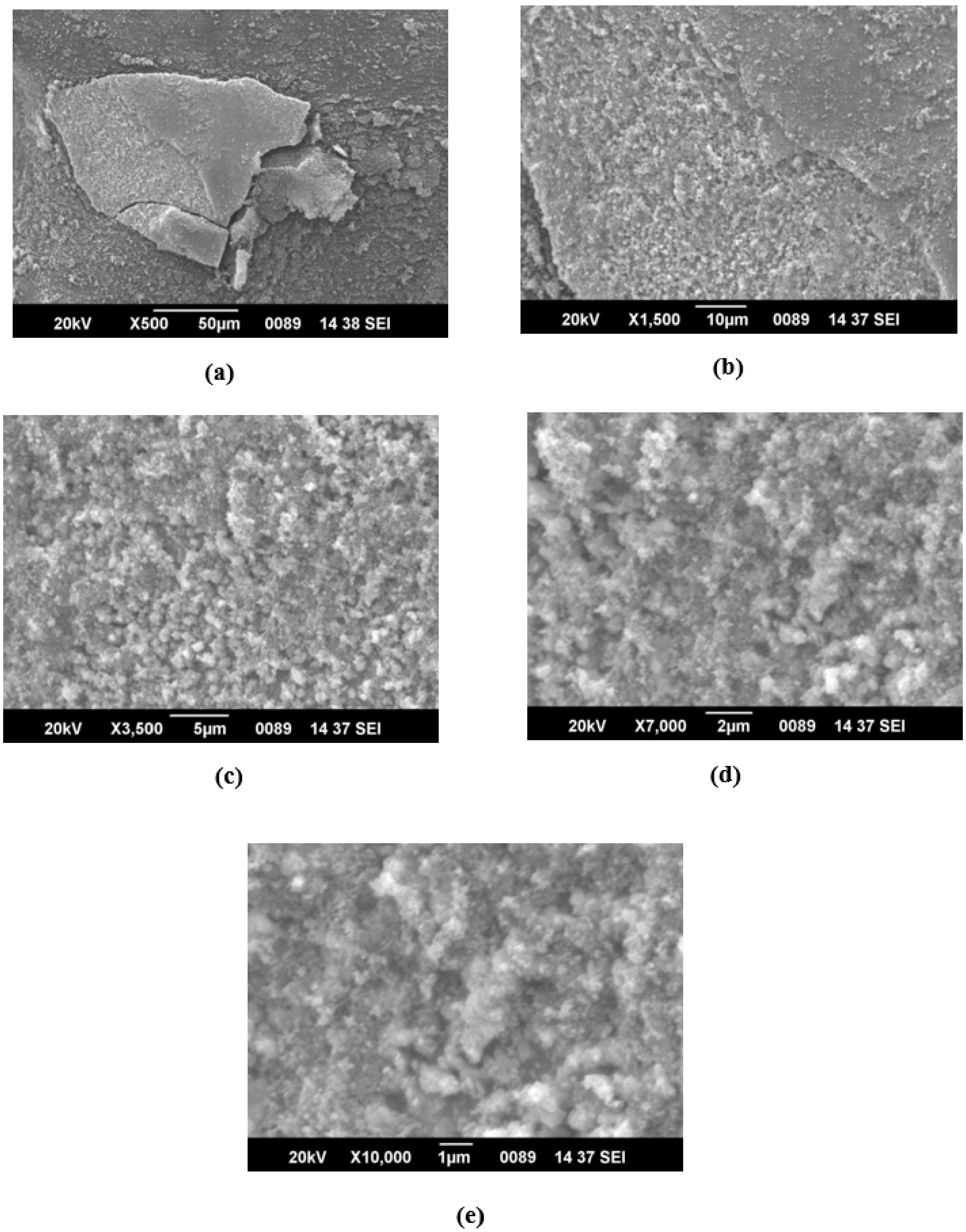
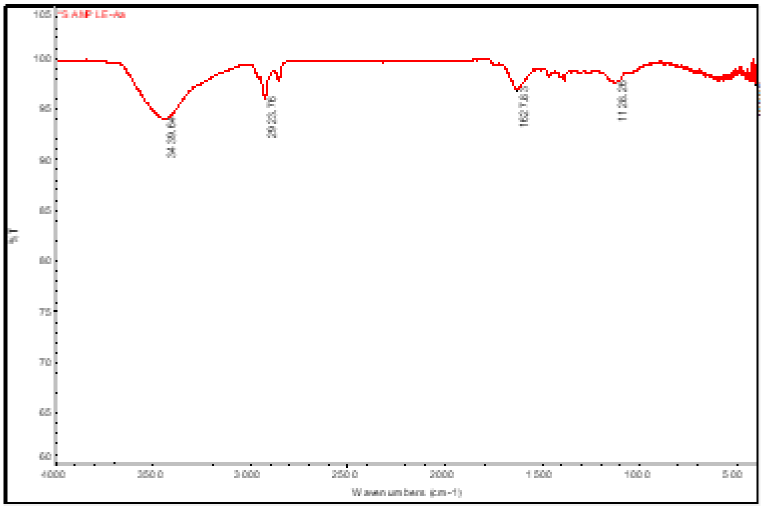
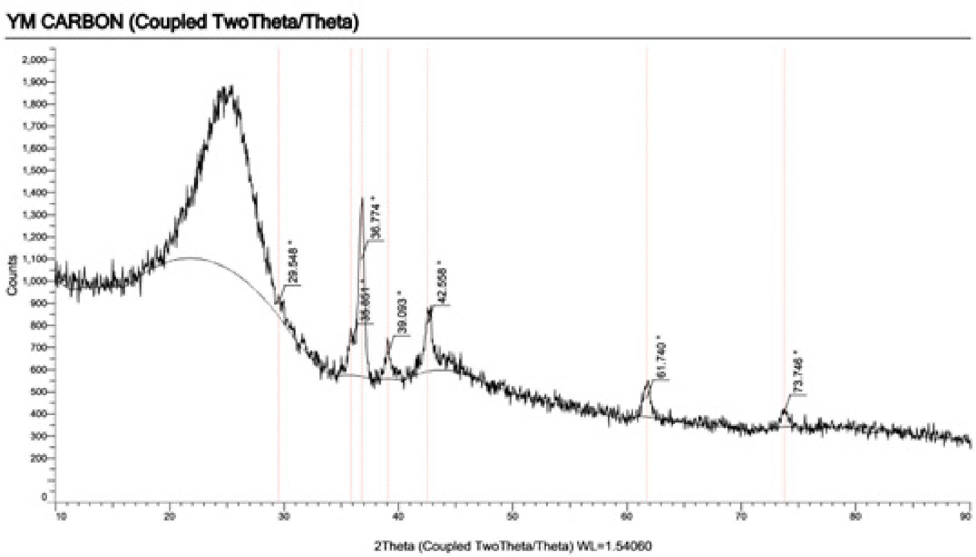


Figure 1: Scanning Electron Microscope (SEM) images in magnifications, a) x 500, b) x 1,500, c) x 3,500, d) x 7,000, e) x 10,000.



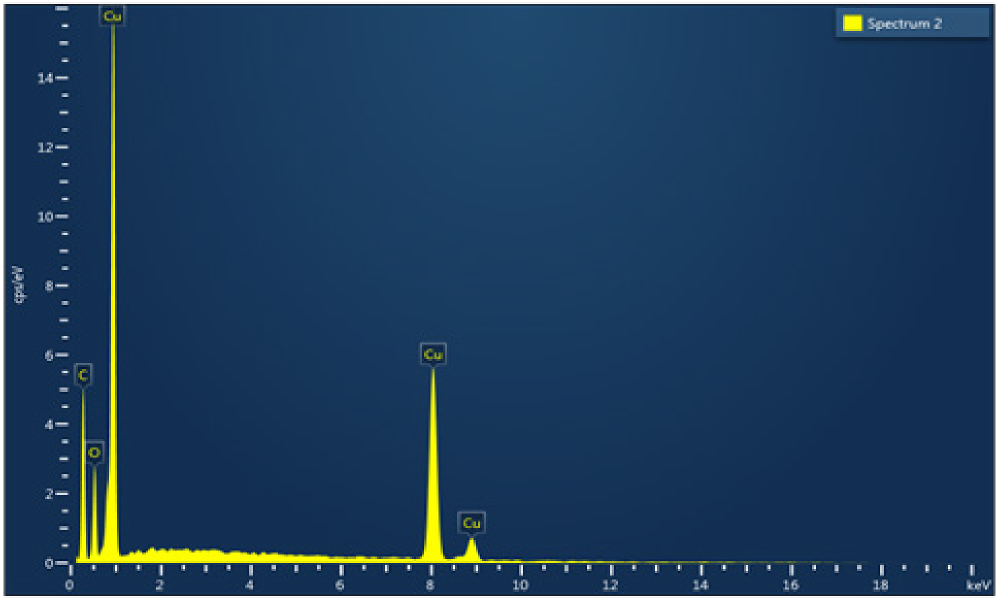
Graph 1: FT-IR absorption band of black soot particles.



Graph 2: XRD peaks of black soot particles.

(600-1500 cm^{-1}), another in the double bond region (1500-2000 cm^{-1}), and two in the single band region (2500-4000 cm^{-1}). The broad peak at 3439.64 cm^{-1}, falling between 3650 and 3250 cm^{-1}, suggests the presence of hydrogen bonding. However, the specific form of this bonding, whether ammonium or an amino group, is uncertain since there is no spectrum at 1600-1300, 1200-1000, or 800-600 cm^{-1}, and no sharp peak intensities were observed between 3670-3550 cm^{-1}, thus ruling out hydroxyl compounds or oxygen-containing groups like phenol or alcohol. The peak below 3000 cm^{-1} corresponds to single bond carbon, potentially indicating the presence of alkane-like compounds such as methyl (-CH3) and methylene (-CH2), commonly found in sugars, amino acids, proteins, and fatty acids as reported in GG water extract.[25] No specific peak for aldehyde was identified in the single bond region, and there were no observable bands in the triple bond region, confirming the absence of C≡C bonds. The IR spectrum at 1627.83 cm^{-1} may correspond to an unsaturated compound with a functional group like amide or carboxylate. Additionally, the spectrum at 1128.26 cm^{-1} suggests the possibility of the sample being a symmetric sulfonate compound (SO_2) with an alkyl-substituted ether, a large cyclic ether (C-O stretch), or even an aliphatic fluoro-compound (C-F stretch).[26]

In the Powder X-ray Diffraction analysis of the black soot particles, seven peaks were observed. Interestingly, four of these peaks coincided with those reported in previous research involving copper particles incinerated at 200°C. The resemblance in peak positions with cuprite (cuprous oxide) peaks indicates the possible oxidation of copper during soot particle preparation.[27] The heat from below, using a wick, likely reached temperatures of up to 400°C, gradually was oxidizing the copper from copper into cuprous oxide and subsequently into cupric oxide. It is important here to understand the rational use of copper in preparing black



Graph 3: Peaks of EDAX analysis of black soot particles.

soot. Copper is considered prime in maintaining retinal health of eye by acting as anti-oxidants. It is due to this reason that copper inclusion specifically for ocular health also prevents age related macular degeneration to progress rapidly.[28]

SEM analysis of the black soot particles revealed a polygonal rigid-like structure. Images (a) and (b) clearly show the oxide layer forming tiny growth over these structures. Elemental analysis indicated the presence of carbon, oxygen, and copper at concentrations of 68.29%, 14.62%, and 17.09%, respectively. The presence of copper is attributed to the use of a copper plate during wick burning, while oxygen is a result of combustion in the open atmosphere. This aligns with the XRD results. The high carbon content in the soot is consistent with plant biomass, as it primarily consists of black carbon and other organic materials. In this study, cotton cloth soaked in GG decoction can be considered as biomass. During biomass combustion, carbon within the plant reacts with oxygen, producing heat and light. Simultaneously, carbon dioxide and water are generated, leading to an increase in entropy.[29-30]

CONCLUSION

Ancient and traditional method of preparing black soot for kajal adopt unique ways to encapture a drug's active components. This method pharmaceutically provides the required colour and darkness to the product and also acts as the active pharmaceutical ingredient. Instrumentally when analyzed through FT-IR black soot particles were found to contain methylene and methane groups possibly derived from sugars, amino acids of GG aqueous extract. Presence of copper is made evident from the results of XRD and SEM-EDAX. On the whole in traditional preparation of kajals the black soot derived by burning plant-based products can also benefit eye health due to bio active components present. This can act as a therapeutically healthy substitute for artificial black colours commonly used in eye cosmetics. However, other instrumental analysis could also be performed to further analyze in depth the secondary metabolites formed during this process through chromatographic techniques.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Herbal cosmetics have gained significant popularity due to their perceived safety and effectiveness compared to synthetic alternatives, which pose risks such as eye irritation, contamination, and potential lead toxicity from prolonged use. The growing preference for herbal cosmeceuticals is reflected in the market, which was valued at approximately $11 billion in 2016 and projected to reach $22 billion by 2022.

Glycyrrhiza glabra (GG), commonly known as liquorice, is recognized in Ayurveda for its beneficial effects on eye health and complexion enhancement. Traditionally used for conditions like dry eye disease and conjunctival hyperaemia, GG is also a key ingredient in modern skincare due to its anti-inflammatory, skin-brightening, and anti-sensitizing properties.

Carbon-based kajal, prepared using herbal extracts, have been traditionally known for their antimicrobial benefits. However, the therapeutic efficacy of the carbonized form of medicinal herbs remains a concern. This study focuses on the preparation and instrumental analysis of GG-derived black soot using Fourier Transform Infrared Spectroscopy (FT-IR), Powder X-ray Diffraction (XRD), and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX). FT-IR analysis identified the presence of alkane-like compounds, while XRD revealed peaks similar to those in incinerated copper particles. SEM analysis showed polygonal structures with oxide formations, and elemental composition analysis confirmed the presence of carbon (68.29%), oxygen (14.62%), and copper (17.09%).

Traditional black soot preparation methods effectively encapsulate the active components of medicinal plants, offering both colour enhancement and therapeutic benefits. The presence of bioactive compounds in herbal kajal suggests their potential as a natural alternative to synthetic eye cosmetics. Further chromatographic studies could provide deeper insights into the secondary metabolites formed during this process.

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