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ORA- Analytical Study

Pharmaceutical and Analytical Study of *Yashtimadhu Anjana* prepared with various wick materials using FTIR and XRD.

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ABSTRACT:

Background: Conversion of herbal drugs in soot/carbon can be done and brought in therapeutic practice. Based on this innovative concept, *Yashtimadhu Anjana* was prepared with 3 different techniques. **Aim:** Pharmaceutical and analytical characterization of end product studied for concluding suitable material for soot preparation. **Objective**: To evaluate pharmaceutical and analytical characterization of 3 different methods of preparation of *Yashtimadhu Anjana* and conclude the suitable material for soot preparation **Material and Methods**: For soot preparation 3 substrates were used viz. cotton cloth, surgical cotton and surgical gauze. Precise weighing was done for unbleached standardized cotton cloth (20 cm²). After that, identical masses of surgical gauze and surgical cotton wick were made for comparison. All 3 wicks soaked with GG *(Glycyrrhiza glabra)* decoction and dried for 7 times & burnt in cow ghee to create black soot. Obtained product subjected for pharmaceutical and analytical analysis according to the Ayurvedic Pharmacopoeia of India. **Results**: Soot obtained from cotton cloth 10.23gms, surgical cotton 12.89gms and surgical gauze 9.18gms. FT-IR of cotton cloth showed minor specific bands which may be due to environmental exposure or surface impurities. Surgical cotton soot showed minor chemical modifications which may occur during processing. Whereas surgical gauze does not show any distinct peaks and closely resembles that of purified cellulose. XRD analysis verified, all soot samples were primarily amorphous carbon. **Conclusion**: Though cotton cloth and surgical cotton produced more soot however surgical gauze may be appropriate for therapeutic purposes. Despite lower yield, surgical gauze may be determined to be suitable substrate because of relatively cleaner spectral profile as per FTIR findings, and all three soot are basically amorphous in nature which was confirmed by XRD analysis.

KEYWORDS: Collyrium, FT-IR (Fourier Transform Infrared Spectroscopy), *Glycyrrhiza glabra,* soot, XRD (Powder X-ray Diffraction).

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1. INTRODUCTION

Classical method of application of *Anjana* mimics like use of current day eyeliners where cosmetics are applied over eyelid margin. All synthetic chemical-based cosmetics have sparked worries about long-term safety issues like contamination, ocular irritation and infections. [1] Regarding eye cosmetics, a number of reports have found that commercial Kajal's contain heavy metals like lead, cadmium and arsenic, increasing the risk of lead toxicity with prolonged usage. [2]

As a result, cosmeceuticals made from herbs are thought to be safer substitutes. Recent years have seen the successful development of herbal cosmetics with enhanced efficacy, stability, color, retention, and fragrance. [3] In 2025, the global market for natural and organic personal care products was estimated to be worth US$79.7 billion, by 2035 it is expected to have grown to US$144 billion. [4]

According to Ayurveda, *Yashtimadhu* i.e., *Glycyrrhiza glabra* (GG) has a variety of therapeutic benefits. It has been shown to improve ocular health in conditions like conjunctival hyperemia, dry eye disease, and glucose-induced cataracts. [5–7] By considering cosmetic importance of GG, *Charaka* categorized this drug under *Varnyamahakashaya* (a group of ten drugs promoting complexion). [8] It is also referred to as liquorice, is frequently used in contemporary cosmeceuticals. Pharmacological studies have proved the anti-inflammatory, anti-sensitizing and skin lightening properties of liquorice by which its use in anti-aging formulations, cleansers, toners, sunscreens and makeup products is justified. [9]

Kajal (i.e. carbon soot) (also known as Kohl or Surma) is traditionally made by burning a wick submerged in herbal extract or by directly burning with the wick dipped in plane/ medicated oil/Ghee. [10-12] According to reports, these carbon-based Kajal have antimicrobial qualities. [13-15]

While making herbal kajal extra medicinal ingredients are frequently added for supporting eye health. Due to which ocular benefit of particular bioactive ingredient is unknown and during incineration alteration of properties of active ingredients may occur.

Cotton cloth, surgical cotton and surgical gauze wick, were the three substrates used in this study to prepare the carbon soot of GG. Fourier-transform infrared spectroscopy (FT-IR) and powder X-ray diffraction (XRD) were used to characterize the soot. In order to support the logical development of safe and efficient herbal-based ocular cosmetics, these analyses were used to identify the chemical functional groups and crystallographic features of soot derived from GG.

Aim and objectives

I. To prepare soot using 3 different cellulose materials.
II. To evaluate obtained 3 different soot using sophisticated advanced instruments like XRD, FTIR.

2. MATERIALS AND METHODS

The entire methodology of the study was divided into,

a. Pharmaceutical part
b. Analytical part

Pharmaceutical part

Pharmaceutical preparation of the black soot was done in the GMP-certified KLE Ayurveda Pharmacy Belagavi.

Collection of study drug and authentication:

Root of GG was procured from GMP Certified KLE Ayurveda Pharmacy Belagavi and authenticated by the AYUSH-approved Drug Testing Laboratory for ASU Drugs at Central Research Facility, Shri B M Kankanawadi Ayurveda Mahavidyalaya, Belagavi voucher specimen no. CRF/Auth/537/2024.

Preparation of GG decoction, 3 different wick, 3 different soot was self-prepared under supervision of experts in GMP certified KLE Ayurveda Pharmacy Belagavi.

Preparation of GG decoction:

Authenticated raw drug was made into coarse powder. Later SOP (Standard operating procedures) of Ayurvedic *Kashaya Kalpana* (decoctions), was followed to prepare *Kashaya* in a

1:16 (drug & water) proportion. [16] In an open stainless-steel vessel with 16 parts (2400mL) of regular potable water, one part (150gm) of the GG coarse powder was boiled in medium flame until 1/8th reduction (300 ml) and it is divided in three different vessels of 100ml each for staining of 3 different wick materials.

Wick preparation procedure:

Precise weighing was done for unbleached standardized cotton cloth (20 cm^2). After that, identical masses of surgical gauze and surgical cotton wick were made for comparison. Saturation of each material was done by immersing in fresh *Kashaya* separately. After squeezing out extra liquid, the materials were left to dry in shade, for 7 times each materials is immersed, squeezed and shade dried. Each of the three dried medicated materials were rolled into a wick shape at the end of the 7th cycle and stored in air tight container for further usage.

Preparation of black soot:

A rolled wick is made out of afore said materials was placed in a mud lamp of 150ml capacity and filled with cow ghee till its brim. All wicks ignited and covered with a copper plate with doom shaped inverted margins of blue flame i.e. just touching tip of fire flame. Preferably diameter of copper plate should be double of that of mud lamp. This setting is done at room temperature (25^{0} C, humidity 40 %) in a room devoid of heavy breeze and moving of people around so that the flame burn in a constant non-flickering way. The setup was maintained till black layer of soot particles deposited on inverted surface of copper plate. Once the combustion fuel is exhausted and wick burnt completely, allow the copper plate for self-cooling. Later careful scraping of soot of all the three samples was done separately with a soft clean and sterile bristle brush with all aseptic measures like wearing hand glows, face and head mask and stored in a sterile clean air tight container for further usage. During this procedure mud lamp was refilled frequently till 1 litre of cow ghee get burnt completely. All the three soots are prepared only in single batch and labelled (*Yashtimadhu* Anjana prepared out of cotton cloth batch no.1, surgical cotton batch no.1surgical gauze batch.no1)

Analytical part

Analysis of GG and its decoction:

Basic physicochemical parameters such as moisture content, total ash, water soluble extractive values, alcohol extractive values, TLC (Thin layer chromatography), etc were tested for the raw drug *Yastimadhu* at AYUSH approved Drug Testing Laboratory for ASU Drugs at Central Research Facility, Shri B M Kankanawadi Ayurveda Mahavidyalaya, Belagavi. In accordance with SOP described in API (Ayurveda pharmacopeia of India) *Yashtimadhu kashaya* physicochemical analysis was done for evaluation of organoleptic properties, p^{H} value, specific gravity, and total solids content. [17-18]

Fourier Transform Infrared Spectroscopy (FT-IR) [19]

FT-IR was done in KLE college of Pharmacy, Belagavi. The analysis was conducted using IRAffinity-1 model, manufactured by SHIMADZU Corporation, Japan.

KBr mode utilized 64 scans with a resolution of 4 cm^{-1} in operating instrument across the 4000–400 cm^{-1} range. Before sample analysis, self-diagnostic validation check was done using a built-in polystyrene film (standard) which confirmed wavenumber accuracy within ±0.1 cm^{-1}.

Mixture was prepared consisting of 10% sample soot particles and 90% KBr for analysis. Mixture was triturated thoroughly and pallet formation, using IRAffinity-1 instrument analysis was done. All samples were recorded under identical experimental conditions to ensure reproducibility of the results. It is observed that no observable shift noted indicating measurement consistency.

Powder X-ray Diffraction (XRD). [20]

The analysis was performed at Department of Science and

Technology, Delhi. Sophisticated Analytical Instrument Facility (SAIF) Dharwad Karnataka University, Dharwad. utilizing Rigaku Smart Lab SE.

Analysis system is fully automated computerized under SAXS (small angle X-ray scattering) capabilities and X ray had a source i.e. 3 kW Cu anode with a ceramic X-ray tube and a Lynx Eye detector that used 250 silicon slip detector technology. In addition, Ni filter is used as beta filter by system to improve analysis quality.

Instrument calibration for instrumental broadening and peak positions accuracy was done using a NIST SRM 640 (Silicon powder[standard]) (National institute of standards and technology standard reference material 640), confirming a peak position accuracy within ±0.01^0 (2θ).

To ensure stability samples were mounted on zero-background sample holder and secured using PMMA (poly methyl methacrylate) sample holder, the diffraction patterns obtained were consistent with no observable peak shift during measurements.

3. RESULTS

The black soot preparation process differed in yields and losses at various stages, *rasapanchaka* of *Yashtimadhu*, macroscopic description and ayurvedic parameters as shown in (Table 1). Prepared soot was not at all dissolvable in any organic solvent. Very little quantity was dissolved in HCl. But We cannot run the Microbial test in HCl. Hence, microbial limit test was not conducted. Organoleptic analysis, physicochemical, microbial limit (qualitative and quantitative) and TLC results of GG showed in (Table 2), analytical assessment of *Yashtimadhu kashaya* results showed in (Table 3). While (Table 4) illustrates the phytochemical analysis results for the aqueous and alcohol extract of GG. The decoction of GG produced 230 mL from an initial 1600 ml of water, giving a yield of 14.375% and a loss of 85.625%. This decoction produced 100 ml of wick material, giving a yield of 43.478% and a loss of 56.522%. Finally, 10 g of black soot was produced from 100 ml of decoction, giving a yield of 10% and a loss of 90%. The physicochemical analysis of the GG decoction showed that it has a specific gravity of 1.027, pH of 5.56, and total solids content of 8.694%. (Table 5) (A, B and C) cotton cloth, surgical cotton and surgical gauze respectively show angle and other details of XRD. Fig 1 (A,B and C) cotton cloth, surgical cotton and surgical gauze respectively show the peaks of the components from XRD. Fig 2(A,B and C) cotton cloth, surgical cotton and surgical gauze respectively display the absorption bands generated from FT-IR analysis of black soot, FTIR peak results showed in (Table 6).

Table 1: *Rasapanchaka* of *Yashtimadhu*, weight details of soot, macroscopic description and ayurvedic parameters of soot.

Rasa panchaka of Yashtimadhu (As per API)			
Rasa	*Madhura*		
Guna	*Guru, Snigdha*		
Veerya	*Sheeta*		
Vipaka	*Madhura*		
Karma	*Balya, chakshushya, drushya, varnya, vata-pittajit, raktaprasadana.*		
Weight of obtained soot with 3 different wicks used in preparation, Macroscopic description and Ayurvedic parameter documentation.			
	Cotton cloth	**Surgical Cotton**	**Surgical gauze**
Initial weight of material	6.02 gm	6.02 gm	6.02 gm
Stained weight of material	7.6 gm	8.2 gm	7.2 gm
Quantity of ghee used	1 lit	1 lit	1 lit

Weight of soot	10.23 gm	12.89 gm	9.18 gm
Form	Fine powder	Fine powder	Fine powder
Color	Blackish	Blackish	Blackish
Odour	Aromatic	Odourless	Aromatic
Rupa	*Krishna varna* (Jet Black)	*Krishna varna* (Jet Black)	*Krishna varna* (Jet Black)
Rasa	*Kashaya* (Astringent)	*Kashaya* (Astringent)	*Kashaya* (Astringent)
Gandha	*Lakshana Gandha* (characteristic)	*Nirgandha* (no odour)	*Lakshana Gandha* (characteristic)
Sparsha	*Ati sukshma* (extra fine) and *slakshna* (smooth) when rubbed between the fingers	Ati sukshma (extra fine) and *slakshna* (smooth) when rubbed between the fingers	Ati sukshma (extra fine) and *slakshna* (smooth) when rubbed between the fingers

Table 2: Analytical assessment of course powder of GG used for *Kashaya* preparation.

Sl.	Analysis	Obtained Report	API limits
	Organoleptic and Physicochemical		
01.	Form	*Bharad*	-
	Color	Brownish yellow	Yellowish brown/Dark brown
	Taste	sweet	Sweetish
	odour	Aromatic, Characteristic	Characteristic
02.	Moisture content	5.812%	Not more than 12%
03.	Foreign matter	Nil	Not more than 2%
04.	Ash value	5.369%	<10%
05.	Acid insoluble ash	0.591%	<2.5%
06.	Water soluble extractive value	16.347%	>20%
07.	Alcohol soluble extractive value	8.679%	>10%
	Microbial limit(Qualitative) and (Quantitative)		
	Organisms	**Limits**	**Results**
08.	E coli	Absent/100ml	Absent
	S aureus	Absent/100ml	Absent
	P aeruginosa	Absent/100ml	Absent
	S abony	Absent/100ml	Absent
09.	Total bacterial count	30-300 cfu/ml	8 cfu/ml
10.	Total fungal count	10-100 cfu/ml	No growth
	TLC		
	TLC (Alcohol Extract)	Rf Values	
11.	Mobile phase- toluen:Ethyl acetate	Short wave : 0.52, 0.61, 0.70	
	Ratio- 7:3	Long wave : 0.30, 0.38,0.48, 0.55, 0.61, 0.65, 0.82	
		Day light : 0.52, 0.61, 0.70	

*Cfu- colony forming unit , †TLC- Thin layer chromatography, ‡Rf- Refractive index

Table 3: Analytical assessment of *Yashtimadhu Kashaya*

	Organoleptic characters		
Sl.no	**Test**	**Result**	**API**
01.	Form	*Kashaya*	Not available
02.	Color	Dark brownish	Not available
03.	Odour	Characteristic	Not available
04.	Taste	Sweetish	Not available
	Physicochemical analysis		
05.	Specific gravity	1.027	Not available
06.	pH	5.56	Not available
07.	Total solids	8.694%	Not available

Table 4: Phytochemical analysis of GG

Sl.	Tests	Aqueous Extract	Alcoholic Extract
01.	Carbohydrates	Positive	Positive
02.	Reducing sugar	Positive	Negative
03.	Monosaccharides	Positive	Negative
04.	Pentose sugar	Negative	Negative
05.	Non reducing sugar	Negative	Negative
06.	Hexose sugar	Negative	Positive
07.	Proteins	Negative	Negative
08.	Amino acids	Negative	Negative
09.	Steroids	Negative	Negative
10.	Flavonoids	Positive	Positive
11.	Alkaloids	Negative	Negative
12.	Tannins	Positive	Positive
13.	Cardiac glycosides	Negative	Negative
14.	Anthraquinone glycosides	Negative	Negative
15.	Saponin glycosides	Positive	Negative

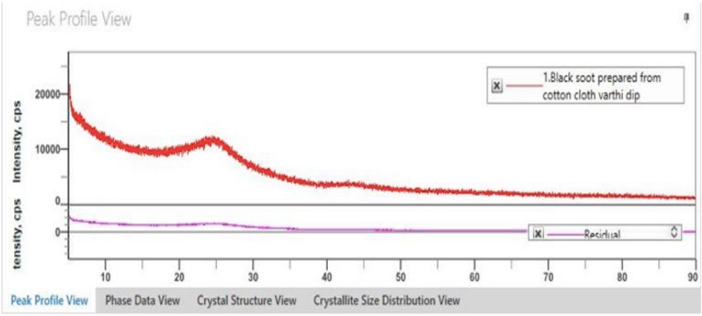

Figure 1A: XRD - Cotton Cloth-Peak profile view

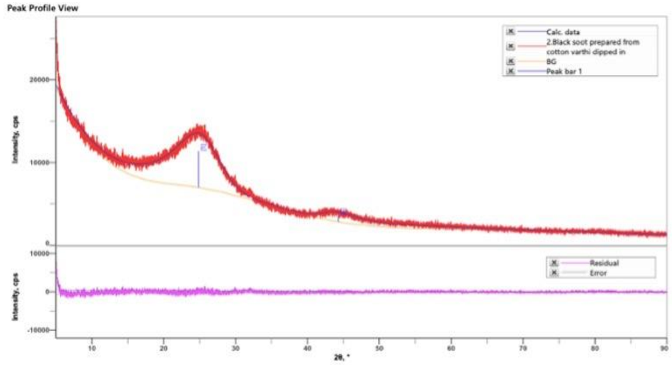

Figure 1B: XRD - Surgical Cotton Peak Profile view

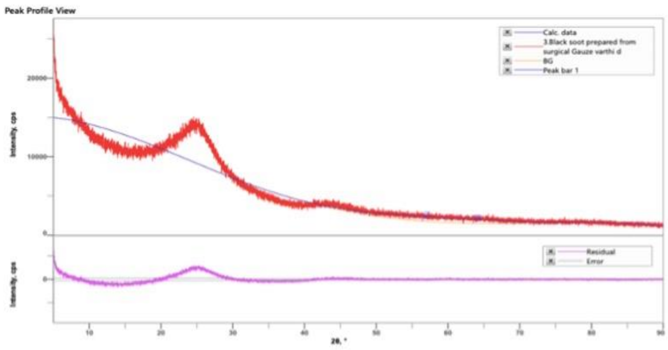

Figure 1C: XRD - Surgical Gauze Peak Profile view

Table 5(A,B,C): XRD results of black soot particles.

Table 5 (A): XRD results of black soot particles from cotton cloth.

Sl.	2 theta/2θ	FWHM	d spacing	Relative intensity
01.	Not detected	Not detected	Not detected	Not detected

Table 5 (B): XRD results of black soot particles from surgical cotton.

Sl.	2 theta/2θ	FWHM	d spacing	Relative intensity
01.	24.84286	7.8857	3.58110 Å	11.1577 %
02.	44.27854	7.8780	2.04399 Å	13.9681 %

Table 5 (C): XRD results of black soot particles from surgical Gauze.

Sl.	2 theta/2θ	FWHM	d spacing	Relative intensity
01.	56.41204	12.7548	1.62976 Å	17.4629 %
02.	63.46843	9.6067	1.46450 Å	10.2262 %

Note: *2θ- Diffraction angle , †FWHM- Full-width at half maximum, ‡d spacing- Distance.

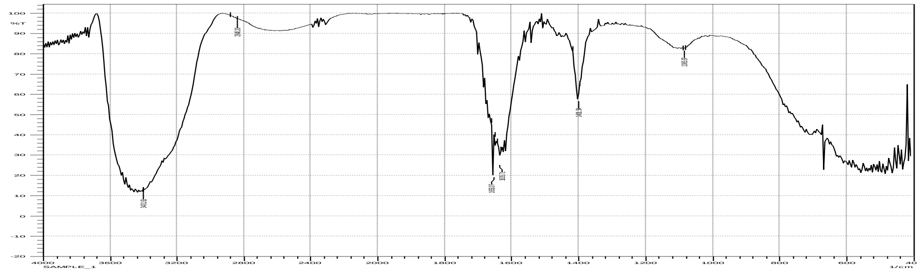

Figure 2A: FTIR graph of Cotton Cloth.

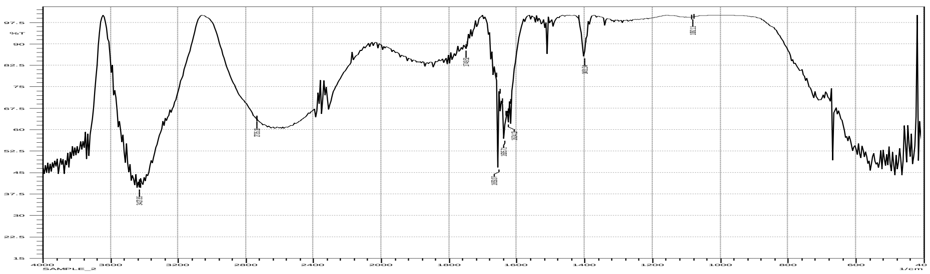

Figure 2B: FTIR graph of Surgical Cotton.

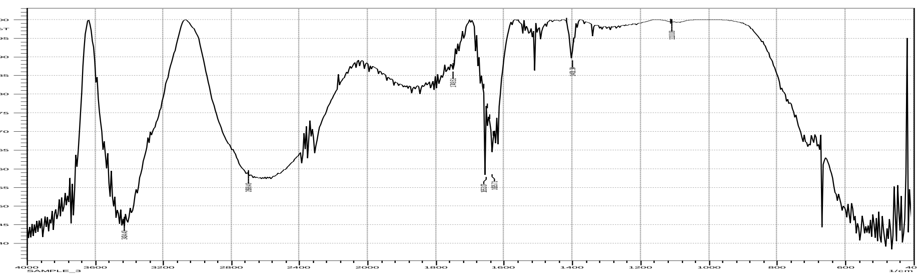

Figure 2C: FTIR graph of Surgical Gauze.

Table 6: FTIR Peak Results. [21]

Functional Group	Stretch	Cotton Cloth	Surgical Cotton	Surgical Gauze
O-H / N-H	Broad Stretch	3401.61 cm^{-1}	3427.65 cm^{-1}	3434.40 cm^{-1}
C-H (Alkane)	Symmetric Stretch	2840.30 cm^{-1}	-	-
C-H (Aldehyde)	Sharp Stretch	-	2735.18 cm^{-1}	2699.49 cm^{-1}
C=O (Carbonyl)	Strong Stretch	-	1749.51 cm^{-1}	1749.51 cm^{-1}
C=O / C=C	Amide I	1653.07 cm^{-1}	1653.07 cm^{-1}	1653.07 cm^{-1}
C=C (Alkene)	Medium Stretch	1635.71 cm^{-1}	1635.71 cm^{-1}	1635.71 cm^{-1}
C-H (Alkane)	Bending	1401.34 cm^{-1}	1401.34 cm^{-1}	1401.34 cm^{-1}
C-O (Alkoxy)	Strong Stretch	1085.97 cm^{-1}	1082.11 cm^{-1}	1110.08 cm^{-1}

4. DISCUSSION

Traditionally particular medicinal ingredient is taken and carbonizing is done to get dark pigment in preparing Ayurvedic *Anjana* that thought for supporting eye health and to have calming properties. The purpose of this study was to describe the soot particles characteristics with respect to safety and efficacy that are produced when prepared with plant-based materials, which are rich in inorganic compounds. To determine the actual source of therapeutic benefit i.e., from burnt soot or from other active ingredients, proper examination of carbonized particles is necessary.

The combustion substrate is important variable in this concern. As we working with root in our samples, it only seemed logical for us to extend our study to include standardized cellulose samples so that we might understand the impact of material structure and purity on the final soot.

For comparative study this 3 materials were selected as mentioned below.

Cotton Cloth- Having variable weaving patterns and possible chemical processing in manufacture.

Surgical Cotton- It is natural, minimally processed with its own organic impurities.

Surgical Gauze- Highly purified and standardized cellulose form, providing few baseline contaminants.

This study assess whether the physical and chemical properties of the carbonized material itself impact its final properties or due to pharmacologically active plants. However, *Yashtimadhu* is *Netrya,* maintaining good vision is emphasized in Ayurvedic *Nitya Anjana* practice continues to be the main focus of the study. [22]

In particular, the *Sushruta Samhita* describes a type of *Prasadana Anjana* (vision-enhancing substance) that requires substances with a *Madhura Rasa* (sweet taste), *Madhura vipaka* (post-digestive effect), *Sheeta Virya* (cold potency), and *Snigdha Guna* (unctuous). [23] *Yashtimadhu*, which is traditionally known as *Chakshushya* (Beneficial for occular health), [24] perfectly embodies these qualities. Based on these guidelines, in depth investigation was done on *Glycyrrhiza glabra* and it has passed all the tested analytical parameters and proved for its quality and purity. (table-2 and table-4) thus it is selected for further study. *Kashaya* prepared out of GG was having 5.56 p^{H}, specific gravity 1.027 with total solids 8.694%. (Table 3) This *Kashaya* was instantly used for soaking and drying of three different wick materials.

Weight of obtained soot from 3 different supporting material was analysed. The data reveals a direct correlation between structure of material and its absorptive capacity. As surgical cotton is loose and non-woven the highest uptake of *Kashaya* occurred due to superior capillary action which led to highest mass of soot collected and highest post saturated weight. In case of surgical gauze and cotton cloth woven structure showed weaker capillary action and less porosity due to less absorption of solids surgical gauze was least affective resulting in lower weight increase and soot yield as shown in (Table-1). All the sample of soot were done in a single batch hence scalability and reproducibility cannot be discussed. For *rasa* assessment all the 3 samples were tested for *rasa* and charred taste was appreciated and according to Ayurveda it may be considered as *Kashaya rasa*.

Fig - 1A, 1B, 1C All three are carbon-based black soot that has been produced by the burning of an organic substrate that has been treated with *Yashtimadhu Kashaya.*

The XRD pattern of these three carbonaceous soots' shows a variation in structure and purity; this can be directly linked to the combustion and their individual organic components. As indicated by the strong broad peak at 20-30° 2θ, all three are essentially amorphous carbons but cotton soot is unique as it shows a strong characteristic peak indicative of partial graphitization. The strong, sharp and definitive peak at ~26.5° indicates presence of crystalline graphite regions, which indicates higher local temperature or time of combustion process; this allows carbon atoms to arrange themselves in a long range ordered lattice. Whereas cotton cloth and surgical gauze soot not showed any peak for graphite instead their patterns give highly disordered and turbostratic carbons that has short range nano scale order.

Table 5 (A, B and C) explains XRD results of black soot particles, Table 5A shows XRD result of Soot particles from cotton cloth where 2θ, FWHM, d spacing and Relative intensity is not detected which shows the soot from cotton cloth have not enough crystalline structure to produce readable diffraction pattern that suggest soot here is entirely amorphous carbon with negligible crystallinity. Table 5B gives result of XRD of black soot particles from surgical cotton has two diffraction peaks at 2θ ≈ 24.84° and 44.28° and d-spacing of 3.58 Å and 2.04 Å respectively, peak observed near 24.84° is assigned to (002) reflection of graphitic carbon, showed the presence of partially ordered turbostratic structures. Using Scherrer equation calculated average crystallite size is approximately 1.05 nm, suggests formation of nanocrystalline domains, [25] and broad FWHM value indicates poor crystallinity. Table 5C shows XRD result of soot particles from surgical gauze shows diffraction peaks at higher angles 2θ ≈ 56.41° and 63.47° and smaller interplanar spacings 1.63 Å and 1.46 Å, indicates increased structural disorder, crystallite size was comparatively lower ~0.75 nm. Reflects high degree lattice distortion and reduced ordering showed comparatively greater peak intensities, although the noticeable peak broadening suggests low crystallinity. [26-27]

Overall, the comparison shows that while gauze soot has extremely disordered nanocrystalline carbon structures and cotton cloth soot is completely amorphous, surgical cotton soot has comparatively better graphitic ordering.

Cotton Cloth : Fig 2A and Table-6 explain FTIR result of cotton cloth, at 1085 cm^{-1} C-O stretch observed this may be from cellulose or another material, 1653 cm^{-1} is usually associated with absorbed water (H-O-H bending)in cellulose material, 2840 cm^{-1} Aliphatic C-H stretching vibration, 3401 cm^{-1} broad absorption band corresponding to O-H stretching vibrations, which are commonly present in other organic material and polysaccharides. [28-31]

Arise of minor specific bands may be due to environmental exposure or surface impurities and in general the spectral features are consistent with cellulose based material.

Surgical Cotton: In Fig 2B and Table-6, Peak at 1749 cm^{-1} may be due to carbonyl groups that can arise from mild oxidation of cellulose during processing or bleaching are from residual natural waxes and esters, 2735 cm^{-1} peak may represent overlapping weak signals.

A broad O-H stretch (3427 cm^{-1}) is characteristic of hydrogen bonded hydroxyl groups in cellulose. C-O stretch and ether linkages typical of cellulose structures are further confirmed in peak 1082 cm^{-1}. Peak at 1635 cm^{-1} may indicate minor structural variation or absorbed moisture in cellulose. [28-32]

Above finding suggest surgical cotton may have possible minor chemical modification during processing and it largely retains its cellulose structure.

Surgical Gauze: Fig 2C and Table-6 a prominent broad O-H stretch (3434 cm^{-1}) is highly characteristic of the O-H stretch and due to strong hydrogen bonding interactions. C-O stretch (1110 cm^{-1}) this is a highly reliable indicator of the C-O bonds and C-O-C ether linkages in cellulose which confirms polysaccharide framework. Carbonyl stretch (1749 cm^{-1}) a tiny peak here can occasionally be assigned to the natural waxes present in very minute amounts in cotton or during processing. Its natural hydroxyl network makes it highly absorbent, ideal for holding phytoconstituents in the GG *Kashaya* staining. [28-32]

In general, surgical gauze spectrum does not show distinct peaks corresponding to synthetic polymers or proteinaceous materials and it closely resembles that of purified cellulose.

On comparison of FTIR findings of 3 different soot's it was noted that N-H/O-H groups were present approximately 30%, 35% and 40%, whereas carbonyl groups (C=O) 5%, 30% and 35% and Aliphatic groups (C-H) 35%, 20% and 15% and C-O groups about 30%, 25% and 30% were detected in cotton cloth, surgical cotton and surgical gauze respectively. These detected functional groups with their collective concentrations in the soot of particular wick material may involve in the pharmacological activities. [33-34]

Limitations

In this study large scale manufacturing is not done. ICPMS, LCMS etc., were not done and Invitro therapeutic assessment limits the current study. Future studies should include large scale manufacturing, Method standardization, Therapeutic validation and Statistical significance.

5. CONCLUSION

Current conclusion drawn based on analytical findings of raw drug and XRD, FTIR results of three different soot. FTIR analysis of surgical gauze shows spectral characteristics consistent with cellulose including prominent C-O and O-H functional groups with no evidence of significant synthetic or proteinaceous contaminants. Minor carbonyl absorption may be attributed to trace oxidation or natural constituents commonly present in processed cotton. In comparison to cotton cloth and surgical cotton, surgical gauze exhibited relatively cleaner spectral profile which suggests a higher degree of chemical consistency with purified cellulose. Hence spectroscopic perspective of surgical gauze shows it is suitable as a wick material for soot preparation. However, to establish safety or biocompatibility, further physicochemical and toxicological studies are recommended. XRD analysis indicated that although the three substrates primarily produced amorphous carbon soot, the structure was different, the soot of surgical gauze was the purest amorphous carbon without crystalline phases, cotton soot partially graphitized, and the soot of cotton cloth was highly disordered with mineral impurities. The purity of the combustion substrate is of utmost importance and recommends surgical gauze for soot preparation. It also proves that a pharmaceutically inert, pure source of cellulose serves as an efficient vehicle of the *Kashaya* and thus proves that the therapeutic effect arises from the resorbed plant phytochemicals and not from the properties of carbon.

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