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Standardization of Traditional Herbal Formulation (Kajal), Used for Eye Care in Chitrakoot region, Madhya Pradesh

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Kajal is a folklore herbal formulation that has been commonly used for eye care in all communities of Indian families since ancient times. It is also known as surma or kohl. There are several Muslim and Hindu countries like India, Egypt, Rome, China, Japan, South Asia, the Middle East, and Africa where the application of Kajal is a common practice and also used in many types of cosmetics formulation for beauty, prevention, and treatment of numerous eye diseases. In the Chitrakoot region, Kajal was also applied to newborn babies as well as small children and people for a multipurpose such as an antiseptic, and astringent, to improve eye health and traditions with religious and therapeutic significance. Tribal women prepare different types of Kajal with the help of herbal plants. One is known as a Belha Kajal in the local language. The present study was carried out with the aim of documentation of folklore practices, and preparation of herbal Kajal used by the villagers of the Chitrakoot region. For standardization of Herbal Kajal was performed based on various selected parameters like botanical identification, physicochemical parameters, heavy metal screening, HPTLC fingerprint profile, and antimicrobial activities as per the standard procedure of Ayurvedic Pharmacopeia of India.

Keywords: Traditional knowledge, Physicochemical, Belha Kajal, Standardization.

Introduction

Kajal is the most popular herbal formulation used by Indian families since the Vedic period to maintain a healthy eye. In ancient Ayurvedic books *viz*. Sushrut Samhita, Charak Samhita, Astanghriday, Bhav Prakash Nighuntu, Nayan Drastam, and Ras Tarang have reported that several single medicinal plants and compound formulations are used to treat various eye diseases such as Timir (cataract), Adhimanth (glaucoma) and abhishyand (conjunctivitis) as well as its beauty in the forms of extract (water extract), arkas (aqueous distillation) and semisolid paste (Kajal-collerium)[1-2]. There is a blind belief and traditional behavior of Indian families that Kajal (collerium) is medically beneficial for the good health of the eye and is ultimately promoted to its uses. People especially women have been faith that herbal Kajal increases eye size, improves the eye's vision, protects the eyes against diseases

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(*viz.* Chalazion, trachoma, blepharitis, pterygium, and conjunctivitis), and keeps the eyes cool and clean.[3-4]

Perhaps Kajal is the first cosmetic product used by most people to make the eye look attractive including tradition and beautification. The usage of Kajal can date its elegance and its therapeutic benefits to more than 5000 years[5-6]. Several ethnomedicinal plants are used for eye disorders, either in single or compound formulations reported in the ancient Indian Ayurvedic books. Their uses and treatment have also been described. Use of the various plants in different dosage forms like fresh extract, arkas (aqueous distillate), Kajal (collerium), and washing with different extracts have also been prescribed. Triphala is a polyherbal formulation and mixture of three fruits Aonla (*Phyllanthus emblica)*, Baheda (*Terminalia bellirica*), and Haritaki (*Terminalia chebula*) with an equal ratio of 1:1:1 and is widely used for all eye diseases as well as stomach diseases[7-9]. Similarly rose water extract is used to cure constipation, inflammation, and swelling and for easing throat infections traditionally[10].

India has a vast storehouse of traditional medicinal knowledge available in the form of home remedies and local health traditions. The people living in remote areas who are untouched by modern civilization use plants for their basic health care needs[11]. Chitrakoot is one such area situated in the northern region of Satna District of Madhya Pradesh, India. It lies between $80^0$52’ to 80^0 73’ in latitude and $25^0$10’ to $25^0$52’ longitude, covering an area of 1584sq km. It is surrounded by lush green hills of the legendary Vindhyachal range and is inhabited by various scheduled caste and scheduled tribe communities like Mawasi, Khairwar, Kol, Gond, etc. Other traditional societies have rich traditional knowledge and practices and are real propagators of these traditions[12]**.** The young generation due to urbanization and fast-changing trends in their lifestyle does not want to follow in the footsteps of their forefathers. As a consequence, this knowledge is getting eroded. These rich traditions of India that continue to provide the health care needs of the vast majority of people, need to be protected before it is lost forever. In this concern, work has been undertaken to document and standardize traditional medicinal knowledge with special reference to herbal Kajal, a formulation used in almost all households all over India for centuries.

During the survey, it was observed and documented that women of the Chitrakoot region prepared several types of Kajal from different methods and different ingredients. After analysis, we have innovated and suitable formulation which is known as Belha Kajal in the regional language of Chitrakoot, Madhya Pradesh, and selected it for standardization and further promotion. This Kajal is made at home by the old age of women. Belha Kajal used children, youngsters as well as old people. Belha Kajal is prepared with 16 ingredients (such as Amarud- (*Psidium guajava* L.) leaf, Jamun- [*Syzygium cumini* (L.) Skeels]-leaf, Karounda- (*Carissa carandas* L.)- leaf, Bilva- [*Aegle marmelos* (L.) Correa.], Ber- (*Ziziphus mauritiana*

ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 11 Iss 11, 2022

Lam.)- leaf, Kaitha- (*Limonia acidissima* L.)- leaf, Nimbu- [*Citrus lemon* (L.) Osbeck] and Cow ghee etc.). Formulated medicated Kajal as a cosmeceutical product to combat eye infections and beautification was thought of as an innovative approach as Kajal is most important in eye makeup but still, the medicinal use of Kajal is limited. Looking at that the present study aimed to prepare a contemporary formulation from the herbal plants called the Belha Kajal and standardize it in terms of identification, physicochemical evaluation, heavy metals tests, phytochemical tests, HPTLC fingerprints profile, and antimicrobial analysis for toxicity and safety usage.

Material and methods:

Systematic survey and documentation of folk-lore/traditional knowledge

For the present study, a systematic survey and documentation of traditional knowledge with special reference to Kajal used for eye care by rural people in the Chitrakoot region, Madhya Pradesh was carried out in 14 villages *viz.* Chauraha, Kelhoura, Dalela, Kanpur, Barua, Bhargawan, Bundelapur, Chandai, Devlaha, Tagi, Hiraundi, Kailashpur, Koldari and Parewa,. These villages are located within a radius of 50 km around Chitrakoot town. A total of 450 women were interviewed in 78 intensive field visits during 2019-2020, covering almost all the seasons. The 45-80 years traditional healers, local vaidya's, especially experienced women who have been actively engaged in the formulation and preparation of Kajal were interviewed and their prior informed consent was recorded. Detailed information regarding the formulation and uses of the Kajal such as plant (ingredients) name, parts used, the ratio of ingredients, mode of preparation, mode of application, doses, duration, benefits age groups, etc have been recorded with the help of standard questionnaires. The information was cross-checked on different field visits to the same localities or in the other localities. The voucher specimen was also collected, identified with the help of local floras, prepared the herbarium, and preserved in a research laboratory, Arogyadham, Deendayal Research Institute, Chitrakoot, dist. Satna (M.P.) (Fig.1).

Collection of herbal plant materials-

The fresh herbal ingredients of the Kajal formulation were collected from the herbal harden, Arogyadham Chitrakoot, district Satna (M.P.) viz. Amarud- (Psidium guajava L., Myrtaceae) leaves; Jamun- [*Syzygium cumini* (L.) Skeels, Myrtaceae] leaves; Karounda (*Carissa carandas* L., Apocynaceae) leaves; Bilva- [*Aegle marmelos* (L.) Correa, Rutaceae) leaves; Ber- (*Ziziphus mauritiana* Lam., Rhamnaceae) leaves; Kaitha- (*Limonia acidissima* L., Rutaceae) leaves; Nimbu- *Citrus lemon* (L.) Osbeck, Rutaceae] leaves, Aonla- (*Phyllanthus emblica* L., Phyllanthaceae) leaves; Sitaphala- (*Annona squamosa* L., Annonaceae) leaves; Chichida- *Achyranthes aspera* L., Amaranthaceae) fruits and Bilva- [*Aegle marmelos* (L.) Correa, Rutaceae] fruit rind. While Ajowan – [*Trachyspermum ammi* (L.) Sprague, Apiaceae] fruits, Laung- [*Syzygium aromaticum (*L.) Merr. & L.M. Perry, Myrtaceae] flower buds, Elachi- [*Elettaria cardamomum* (L.) Maton, Zingiberaceae] fruits, Supari- (*Areca catechu* L., Arecaceae) fruits purchased from Chitrakoot market, and pure cow ghee purchased from a farmer of Mohkamgarh village. All samples were identified and authenticated by a Senior Scientist of Deendayal Research Institute, Chitrakoot, Satna M.P. Materials were used for the formulation of herbal Kajal and further studies. Formulations composition of the herbal compound formulation of Kajal is given in (Table -1).

Method for preparation of Kajal formulation

For formulation and preparation of the Belha Kajal collected ingredients leaves (Amarud, Jamun, Karounda, Bilva, Ber, Kaitha, Nimbu, Awala, Sitaphala) and other ingredients parts (Chichida, Ajowan, Laung, Elachi, Supari), and then the fruit rind of the Bilva taken as per given quantity in Table1 formulation composition. The hollow (fruit rind of Bilva) then put all the ingredients in it and tied it with the help of a clean cloth and dig a pit in the ground under the Bilva tree and leave it for 12 - 15 days, after take those leaves with other ingredients out in a fool or brass utensils (Petal Kopari on the surface side), along with celery and pure cow ghee was mixed in and weighed (rub with Petal Lota) very well about 1-2 hours for 3 days till become free from granules and make a past**e**[13]**.** The prepared paste is known as Belha Kajal. Store it in air-tight containers for further analysis.

Macroscopic study

Organoleptic characters or macroscopic of the Kajal sample like colour and odour were evaluated.

Preparation of slides for microscopic study of Kajal

About 2 gm of sample was washed separately thoroughly with potable water and poured out of the water without loss of material. Mounted a small portion of glycerin was used to all characters of the samples separately, a small quantity of samples was cleared by heating with chloral hydrate solution, washed and mounted in glycerin, treated a few mg with iodine solution, and mounted in glycerin, another small quantity of samples stained with sudan red solution and mounted with glycerin, all mounted slides were seen under a microscope at 40 x 10x magnification of the Trinocular Research Microscope **separately**[14,15, 16].

Physico-chemical tests

Physico-chemical parameters of the Kajal include raw materials such as moisture content (loss on drying at 105^0C), water-soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and *pH* (10 %) aqueous solution, acid value, viscosity, saponification value, and ester value were Performed separately[17-18].

ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 11 Iss 11, 2022

Preliminary phyto-chemical analysis

Preliminary phyto-chemical tests of Kajal were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, carbohydrates, flavonoids, protein, resins, saponin, tannin, and steroids[19, 20].

Screening of heavy metals tests

Heavy metals are toxic and generally occur throughout the earth in plants. The four main types of heavy metals harmful to us are Pb, Cd, As, and Hg. These heavy metals were detected through an Atomic Absorption Spectrophotometer as per standard method [21].

High-performance thin layer chromatography (HPTLC) fingerprint profile

For High-performance thin layer chromatography, 5 gm of Kajal including ingredients powdered samples were extracted with 100 ml of methanol overnight, filtered, and concentrated. A ferulic acid standard marker was used for the identification of Ferulic acid active phytochemical, in the Kajal sample. Similarly, Quercetin standard and Stearic acid standards were used for the estimation of Quercetin and Stearic acid active phytochemicals in the Kajal. For the preparation of standard marker working solutions, 10mg of Ferulic acid, Quercetin, and Stearic acid were dissolved in a 10 ml volumetric flask and made up the volume with methanol separately. Then 1 ml from stock solution to a 10 ml volumetric flask and made up the volume with methanol separately. Standard markers were applied by spotting extracted samples on pre-coated silica-gel aluminium plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The sample with ingredients and standard marker, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from the left margin of the plate, and 10 mm part. Plates were developed using a mobile phase consisting of *toluene: ethyl acetate* (7:3v/v). Linear ascending development was carried out in a 20x20 cm twin through a glass chamber equilibrated with a mobile phase. The optimized chamber saturation time for the mobile phase was 30 min. at room temperature. The length of the chromatogram run was 8 cm. 20 ml of the mobile phase. After the development, a thin layer of chromatography plate was dried with the help of a Hot Air Oven instrument. The peak area for samples and standard were recorded with the camera photo documentation system Camag Reprostar 3. Visualization of spots was made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at 254nm and 366nm after derivatization with Win cat software and R_f values were noted [22, 23].

Microbial limit tests

Formulated Kajal was performed to check the quality with the microbiological limit tests for the estimation of the number of total microbial count (TBC), Yeast & Mould count, enumeration of *Staphylococcus aureus*/gm, enumeration of *Salmonella sp*./gm, enumeration of

Pseudomonas aeruginosa/gm and enumeration of *Escherichia coli* through cylindrical plate count method using specified agar media and enrichment media from Himedia, Pvt. Ltd. Mumbai. Finally, the growth was observed by colony counter, counted per volume plated and the result was expressed as cfu/g (Colony forming unit per gram of the sample).

The following tests were carried out as per [24-25]

Result &Discussion

Macroscopic characters

The formulated herbal Belha Kajal color is black, with a characteristic odour, smooth in texture, and non-gritty with a semisolid consistency.

Powder microscopic characters

Various types of covering and glandular trichomes (Amrud); Crystals fibres, Striated walled parenchymatous cells (Jamun); Parenchymatous cells filled with prismatic crystals of calcium oxalate, Lower epidermis with paracytic stomata (Karounda); Parenchyma embedded with crystals and starch grain, Oil cells (Bilva leaf); Epidermal cells with papilose attached with palisade cells, Collenchymatous cells (Ber leaf); Covering trichomes with pits, Parenchymatous cells embedded with yellow contents, Lower epidermis with crystals and stomata (Kaitha leaf); Covering trichomes (Nimbu leaf); Pitted covering trichome, Parenchyma embedded with crystals (Sitaphal leaf); Striated walled parenchymatous cells (Awala leave),Fragment of perisperm filled with starch grains, masses of aleurone grains, fragments of cotyledon with lower epidermis and underlined cells (Chichida fruit); Epidermis with papillose and glandular out growths with cuticular striations radiating from base (Ajowan); fragments of aril cells, thin walled perisperm cells packed with minute rounded polyhedral starch grains and containing small prismatic crystals of calcium-oxalate, dark brown coloured schlerenchyma of testa (Ela); small rounded triangular tetrahedral pollen grains 15µ-20µ, elongated parenchymatous cells containing rosette crystals of calcium-oxalate, fragments of epidermal cells with anomocytic stomata in surface view, shizolysigenous oil cells (Lavanga). (Fig. 2(2A-2F).

Physico-chemical tests

The physico-chemical parameters such as Loss on drying at 105^{0}C, extractive values (water soluble extractive and alcohol soluble extractive), and ash values (total ash value and acid insoluble ash value) were performed Kajal with its used ingredients in triplicate separately and obtained result expressed as an average value separately. Kajal and cow ghee physicochemical parameters like *pH* (10 %) aqueous solution, acid value, saponification value, and ester value were performed separately. Physico-chemical results are given in (Tables 2 & 2a respectively).

ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 11 Iss 11, 2022

Preliminary phyto-chemical investigation

Qualitative phytochemical tests were performed in water and ethanol extracts. Alkaloids, flavonoids, resin and saponin, and tannin were present in the Kajal.

Heavy metals tests

Heavy metals (Pb, Cd, As, and Hg) tests were performed and found under limits as prescribed in WHO guidelines, and results are given in (Table 3).

HPTLC (high-performance thin-layer chromatography) fingerprints profile

High-performance thin layer chromatography (HPTLC) study of the methanolic extracts in two spots of the Kajal sample extract with ingredients and Ferulic acid, Quercetin, and Stearic acid standard marker spots applied in precoated TLC plate. Applied 8 µl of the test solution as well as a standard marker as 6 mm bands and developed the plate in a solvent system *toluene: ethyl acetate* (7: 3 v/v) to a distance of 8 cm. dried the developed plate at room temperature and examined. Derivatized the plate using 5% *Methanolic-sulphuric acid* reagent and dried at 105^0C in a Hot air oven, till the bands were visible. Major spots R_f values with colour were recorded before derivatization at 254 nm and 366 nm. Major spots of R_f values at 254nm before derivatization are 0.24 black, with Ferulic acid, 0.38 black with Quercetin, standard, and at 366nm major spots R_f values are 0.24 sky blue with Ferulic acid, and 0.40 yellow with Quercetin standard. Chromatograms profile is given (Fig.3, 4 & 5).

Microbiological limit tests

Microbiological analysis of pathogenic bacteria *viz. Salmonella* sp., *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were done and found absent in Kajal where total microbial plate count (TBC) was found 65cfu/g, yeast & moulds were found 45 cfu/g. The microbiological profile of the Kajal sample was found satisfactory under prescribed limits in WHO/ Ayurvedic Pharmacopoeia of India guidelines *viz.* for *Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* limits absent, while for total microbial plate count (TBC) 10^5cfu/g and for yeast & moulds 10^3cfu/g.

Discussion

As we know there are different types of eye cosmetics including mascara, eyeliners, kohl, eye shadow, and kajal have been used for a long time to attractiveness and beauty of eyes, improve personal appearance, attract the interest of others, and treatment of various eye diseases[26]. So, the task was undertaken to document the folklore medicinal knowledge used for eye care and eye cosmetics in the Chitrakoot region of Madhya Pradesh.

During the field visits and documentation of folklore medicinal knowledge, it was observed that different communities of villagers used different formulations of Kajal to maintain the good health of their children and own eyes as well as eye cosmetics, but the basic ingredients were the same. So, after analysis of documented data, we have selected the most common effective multi-herbal formulation is called Belha Kajal in the local language. Belha Kajal is formulated with 16 single ingredients which were collected from Arogyadham and purchased from the market, as mentioned in (Table 1).

The Kajal was standardized as per the guideline of Ayurvedic Pharmacopoeia of India, Ministry of AYUSH, Government of India. The standardization was subjected to various analytical techniques like organoleptic tests, macroscopic and microscopic identification tests, physicochemical tests, heavy metals tests, microbiological tests, phytochemical analysis, and TLC profile tests were performed and established the distinguished anatomical characters for Kajal, results are depicted as (Fig.5), and these specific anatomical characters may be helpful for identification of ingredients in the Belha Kajal herbal formulation[27-28]. Qualitative phyto-chemical analysis was performed in water and ethanol extracts. Several phytochemical-like alkaloids, tannin, flavonoids, protein, resin, and saponin were present in the sample, which could make the formulation useful for potential and preventive eye healthcare needs[29]. Awala, Beil, and Ber are the most important ingredients of the formulation linked with flavonoids, and tannins. These are also used in all eye problems[30]. Amrud leaves improve eyesight and slow down the appearance of cataracts, Quercetin, reduces allergic response, sting, and itch, Similarly, Karounda leaves are used to treat various eye diseases, hair care wounds indigestion lowering blood sugar level, Ajowan is beneficial as an anti-oxidant, antifungal and antiulcer. It was reported that vitamin E is very useful for the eye's health and found in the fresh herbs, formulated herbal kajal is mostly fresh herbs. Jamun and Sitaphal have strong antibacterial activities. These help keep the eye clear of foreign diseases. This allows the lens and eye muscle to be reinforced and calm and manages to improve the eye vision[31]. Bronze utensils were typically used for the final preparation and reproduction of herbal Belha Kajal. Over-rub the enlarged and stressed blood vessels and thereby enhance optimal eye health. Lavanga, Ela, and Chichida are the primary components of the Kajal, these reduce mild eye eruptions and promote eye protection[32]. Another ingredient used in the processing of multi-herbal Kajal is pure cow ghee, it is also known as a vehicle for drugs and is considered an Ayurvedic medicine, and it works as promising, due to its ability to easily cross the blood-brain barrier. Pure cow ghee along with various herbs like triphala[33] have been used for the treatment of different eye disorders. 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[34]. Results are given in Table **2A.** Cow ghee is also a way to keep the eyes clear of diseases and healthy.

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Detection of heavy metals (Pb, Cd, As & Hg) tests were performed and found below limits as per WHO guidelines. It means formulated Kajal is safe and free from toxic metals. Microbiological analysis of pathogenic bacteria, *viz. Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were done and found absent in Kajal, where total microbial plate count (TBC) was found 65cfu/g, yeast & moulds were found at 45 cfu/g. The microbiological profile of the Kajal was found satisfactory under prescribed limits *viz.* for *Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* limits were absent, where for total microbial plate count (TPC) 10^5cfu/g and for yeast & moulds 10^3cfu/g given in Ayurvedic Pharmacopoeia of India[35]. These findings indicate that the formulated Belha Kajal (a folklore eye care formulation) is a very good eye care herbal cosmetic used by villagers of the Chitrakoot region to maintain eye health[36]. HPTLC chromatograms of the herbal Belha Kajal formulations with ingredients and standard markers like ferulic acid, quercetin, and stearic acid were performed. The R_f values and colour of the resolved bands are noted (Table 3A, 3B & 4). Developed chromatograms indicate the presence of Ferulic acid and Quercetin standards in the formulated Kajal. Quantification of marker compounds i.e., ferulic acid, quercetin was done in the kajal formulation with its ingredients, and ferulic acid was found to be in Kajal (**0.0082 ±0.002**), Amrud (0.01691 ±0.001), Jamul (0.2061 ±0.002), Karunda (0.4317+0.002), Bilva (0.1957 ±0.002), Ber (0.0359 ±0.001), Kaitha (0.4226 ±0.002), Nimbu (0.9647±0.002), Sitaphal (0.0928 ±0.001), Amla (0.1075 ±0.001), Chichira (0.1978 ±0.002), Ajwain (0.3503 ±0.001), Loung (0.1126 ±0.001), Ela (0.0575 ±0.002), and Supari (0.0314 ±0.003).

Similarly, Quercetin was found in Kajal (0.1449 ±0.002), Amrud (ND), Jamul (ND), Karunda (0.0965 ±0.002), Bilva (0.3065 ±0.002), Ber (ND), Kaitha (ND), Nimbu (0.2197 ±0.001), Sitaphal (0.0122 ±0.001), Amla (0.1075 ±0.001), Chichira (0.0675 ±0.001), Ajwain (ND), Loung (0.1805 ±0.001), Ela (ND), and Supari (0.0331 ±0.001).

People have been using the herbal Kajal formulation from ancient times, for improving eye vision, and cure various eye diseases viz. bacterial keratitis, conjunctivitis, cataracts, glaucoma as well as eye decorating purposes in India, Egypt, Rome, Japan, China, Afghanistan, and Pakistan mainly Hindu and Muslim[37,38,39]. Nowadays, lead toxication is the major issue[40] with the uses of Kajal formulation, but no report has reported lead in the toxification of eyes of children and women especially in India. The reason there are different types of methods adopted by villagers for the preparation and formulation of Kajal such as using herbs, the number of ingredients, ghee, and oils, these procedures are also described in Ayurvedic, Unani, Siddha, and Greko-Arabic systems of medicine. Therefore, we attempted to rule out the presence of lead and other toxic metals and prove its potential as an antimicrobial against pathogens responsible for eye infections as well as eye care. This study, confirms the batch-to-batch consistency of the finished products and can serve as the quality standards for the manufacturer of the same formulation in the future. These findings are indications of multi herbal formulations preparation of Kajal that it is a very good folklore eye care and beauty cosmetic used by villagers of the Chitrakoot region.

Conclusion

From the present study, survey, documentation, formulation, and standardization of Belha Kajal were done by involving various parameters such as documents of traditional medicinal eye care knowledge, selection of formulations, and its importance as a child as well as human eye care health used by villagers of Chitrakoot region, Satna district of Madhya Pradesh. The results of the present investigation like organoleptic study, macroscopic and powder microscopic identification characteristics, physicochemical parameters, phytochemical analysis, and HPTLC fingerprints profile found similar characteristics as reported for all single drugs in the official Pharmacopeia. Screening of heavy metals results found under limits as prescribed by WHO guidelines, it showed product is free from toxic metals. Thereafter microbiological tests showed the absence of pathogenic bacteria *viz. Salmonella* sp., *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, while total microbial plate count (TBC) and Yeast & Moulds were found within limits. These results indicated the safety of the Belha Kajal. These studies concluded that the formulated multi-herbal Belha Kajal is a way to hold the eyes free from diseases and make them healthy. Also, it is safe and can be used as one of the herbal eye cosmetic products.

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Ethical issues

There is none to be applied

Conflict of interest

None

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 Journal Volume 11 Iss 11, 2022

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Table 1 Formulation composition of Kajal

S. No	Common Ingredient Name	Botanical name	Part used	Quantity
1	Amarud	*Psidium guajava* L. (Myrtaceae)	Leaf	1 part
2	Jamun	*Syzygium cumini* (L.) Skeels (Myrtaceae)	Leaf	1 part
3	Karounda	*Carissa carandas* L. (Apocynaceae)	Leaf	1 part
4	Bilva	*Aegle marmelos* (L.) Correa (Rutaceae)	Leaf	1 part
5	Ber	*Zizyphus mauritiana* Lam. (Rhamnaceae)	Leaf	1 part
6	Kaitha	*Limonia acidissima* L. (Rutaceae)	Leaf	1 part
7	Nimbu	*Citrus lemon* (L.) Osbeck (Rutaceae)	Leaf	1 part
8	Sitaphala	*Annona squamosa* L. (Annonaceae)	Leaf	1 part
9	Aona	*Phyllanthus emblica* (Phyllanthaceae)	Leaf	1 part
10	Chichida	*Achyranthes aspera* L. (Amaranthaceae)	Fruit	
11	Ajowan	*Trachyspermum ammi* (L.) Sprague (Apiaceae)	Fruit	1/10part
12	Laung	*Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae)	Flower bud	1/10part
13	Elachi	*Elettaria cardamomum* (L.) Maton (Zingiberaceae)	Seed	1/10part
14	Supari	*Areca catechu* L. (Arecaceae)	Fruit	1/10part
15	Bilva	*Aegle marmelos* (L.) Correa (Rutaceae)	Fruit rind	
16	Cow ghee	Clarified butter	Ghee	

ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 11 Iss 11, 2022

Table 2: Physico-chemical analysis results of Kajal and its ingredients

Name of Curna	**LOD (% w/w)**	**Mean (%)**	**Total ash (% w/w)**	**Mean (%)**	**AI ash (% w/w)**	**Mean (%)**	**ASE (% w/w)**	**Mean (%)**	**WSE (% w/w)**	**Mean (%)**
Kajal (formulation)	5.22 5.30 5.12	**5.2**	6.95 6.90 6.91	**6.9**	2.50 2.80 2.72	**2.5**	15.81 15.80 15.72	**15.7**	42.8 42.9 43.8	**43.2**
Amarud (leaf)	6.8 6.5 6.7	**6.6**	8.77 8.72 8.32	**8.60**	3.45 3.23 3.12	**3.26**	19.85 19.67 19.91	**19.81**	22.51 22.34 22.19	**22.34**
Jamun (leaf)	6.96 6.18 6.72	**6.62**	7.80 7.43 7.25	**7.49**	3.43 3.98 3.67	**3.69**	19.13 19.56 19.98	**19.55**	18.85 18.45 18.54	**18.61**
Karounda (leaf)	6.62 6.43 6.49	**6.51**	11.26 11.09 11.22	**11.19**	1.32 1.43 1.56	**1.43**	12.83 12.52 12.23	**12.52**	23.43 23.19 23.22	**23.28**
Bilva (leaf)	4.50 4.90 4.80	**4.73**	8.44 8.68 8.75	**8.62**	1.62 1.31 1.25	**1.39**	17.00 17.49 17.32		38.89 38.78 38.78	**38.81**
Ber (leaf)	6.23 6.56 6.73	**6.50**	8.73 8.92 8.67	**8.77**	1.87 1.88 1.89	**1.88**	12.78 12.34 12.69	**12.63**	19.33 19.66 19.23	**19.40**
Kaitha (leaf)	7.67 7.15 7.45	**7.42**	12.33 12.30 12.69	**12.44**	1.43 1.46 1.79	**1.56**	17.85 17.98 17.68	**17.83**	36.23 36.13 36.45	**36.27**
Nimbu (leaf)	7.92 7.56 7.68	**7.72**	9.76 9.54 9.43	**9.57**	1.38 1.67 1.82	**1.62**	13.11 13.32 13.63	**13.35**	29.39 29.32 29.21	**29.30**
Sitaphala (leaf)	6.37 6.78 6.39	**6.51**	8.35 8.21 8.14	**8.23**	1.23 1.36 1.29	**1.29**	15.08 15.10 15.23	**15.13**	26.43 26.12 26.23	**26.26**
Awala (leaf)	5.09 5.18 5.00	**5.0**	7.00 6.98 6.94	**6.9**	2.17 2.10 2.18	**2.1**	15.56 15.20 15.45	**15.4**	44.3 43.8 43.9	**44.0**
Chichida (Fruit)	7.35		6.43		1.76		12.48		15.9	
Ajowan (fruit)	8.40 8.48 8.60	**8.49**	5.61 5.44 5.64	**5.56**	0.19 0.11 0.12	**0.14**	4.16 4.20 4.32	**4.22**	18.76 18.40 19.20	**18.78**

IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 11 Iss 11, 2022

Lavanga (flower bud)	3.40 3.10 3.18	**3.23**	5.40 5.30 5.38	**5.36**	0.67 0.68 0.65	**0.66**	14.12 14.18 14.20	**14.1 6**	15.4 15.8 15.6	
Elã (fruit)	6.12 6.45 6.48	**6.35**	4.10 4.18 4.19	**4.1**	3.61 3.62 3.61	**3.61**	10.38 10.36 10.37	**10.3 7**	15.10 15.11 15.12	**15.11**
Supari (fruit)	5.56 5.46 5.32	**5.44**	4.90 4.76 4.88	4.84	0.43 0.41 0.39	0.41	23.45 23.23 23.12	23.2 6	19.67 19.65 19.47	19.59

Table 2A: Physico-chemical analysis results of Kajal and cow ghee

Tests	**Kajal**	**Cow ghee**
pH (10 %) aqueous solution	7.2	7.1
Acid value	1.4318	1.3968
Saponification value	231.24	234.09
Ester value	229.8082	232.6932

Table: 3 Quantification of marker compound in Kajal formulation with its ingredients

S/No	**Formulation/ Single Ingredient**	**Ferulic acid (% dry weight)**	**Quercetin (% dry weight)**
1.	Amrud	0.01691 ±0.001	ND
2.	Jamul	0.2061 ±0.002	ND
3.	Karunda	0.4317 ±0.002	0.0965 ±0.002
4.	Bilva	0.1957 ±0.002	0.3065 ±0.002
5.	Ber	0.0359 ±0.001	ND
6.	Kaitha	0.4226 ±0.002	ND
7.	Nimbu	0.9647 ±0.002	0.2197 ±0.001
8.	**Kajal**	**0.0082 ±0.002**	**0.1449 ±0.002**
9.	Sitaphal	0.0928 ±0.001	0.0122 ±0.001
10.	Amla	0.1075 ±0.001	0.0121 ±0.002
11.	Chichira	0.1978 ±0.002	0.0675 ±0.001
12.	Ajwain	0.3503 ±0.001	ND
13.	Loung	0.1126 ±0.001	0.1805 ±0.001
14.	Ela	0.0575 ±0.002	ND
15.	Supari	0.0314 ±0.003	0.0331 ±0.001

n=3, ±SD

ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 11 Iss 11, 2022

Fig.1 Documentation of folklore knowledge and traditional preparation of Kajal

IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
Research Paper Journal Volume 11 Iss 11, 2022

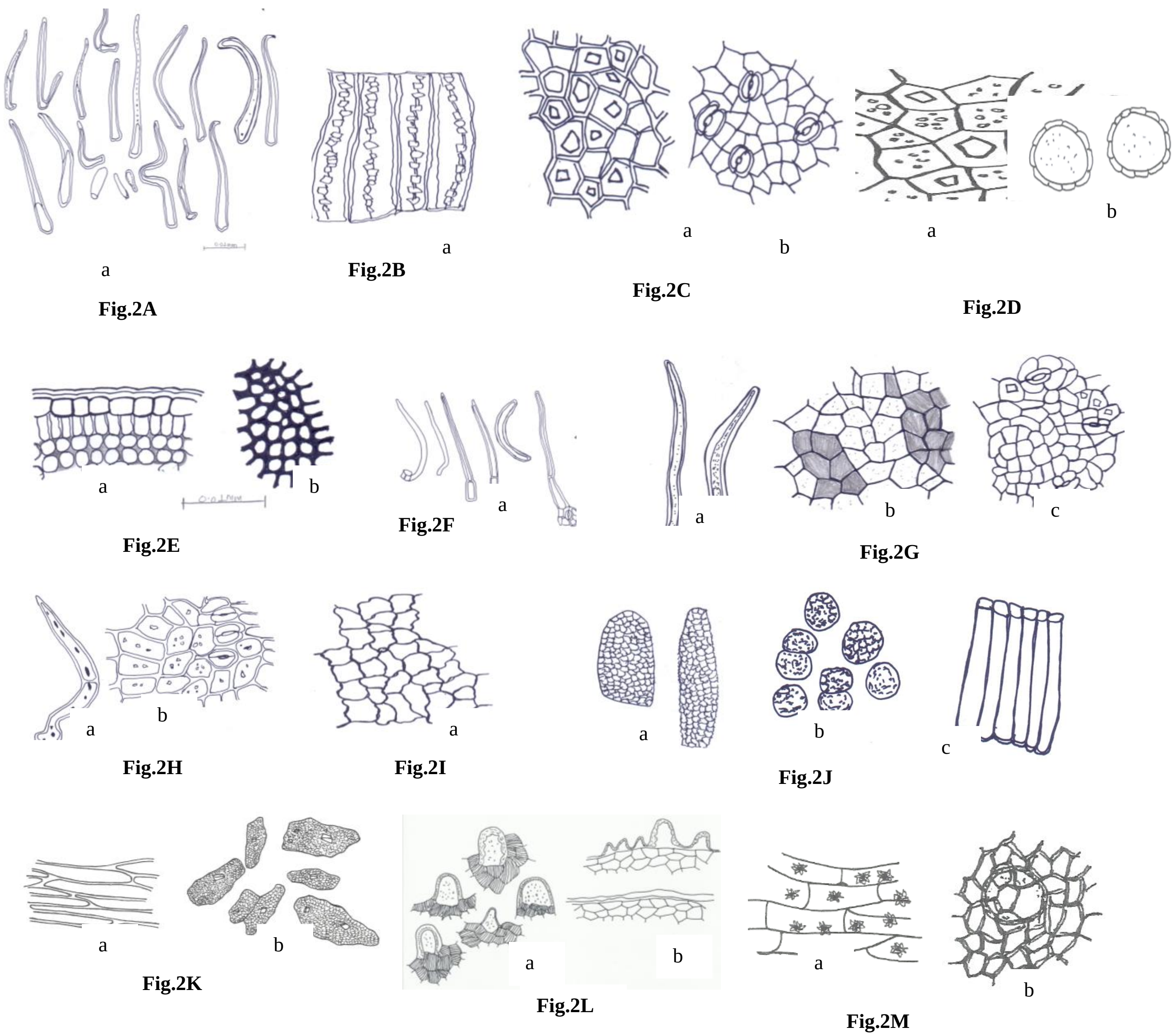

Fig.2- Powder microscopy of Kajal (2A) Amrud (a) Various types of covering and glandular trichomes (2B) Jamun (a) Crystals fibres (2C) Karaunda (a) Parenchymatous cells filled with prismatic crystals of calcium oxalate (b) Lower epidermis with paracytic stomata (2D) Bilva (a) Parenchyma embedded with crystals and starch grain (b) Oil cells (2E) Ber (a) Epidermal cells with papilose attached with palisade cells (b) Collenchymatous cells (2F) Nimbu (a) Covering trichomes (2G) Kaitha (a) Covering trichomes with pits (b) Parenchymatous cells Embedded with yellow contents (c) Lower epidermis with crystals and stomata (2H) Sitaphal (a) Pitted covering trichome (b) Parenchyma embedded with crystals (2I) Awala (a) Striated walled parenchymatous cells (2J) Chichida (a) Fragment of perisperm filled with starch grains (b) Masses of aleurone grains (c) Fragments of cotyledon with lower epidermis and underlined cells (2K) Ela (a) Aril cells (b) Parenchymatous cells of the perisperm with starch grains and prism (2L) Ajowan (a) Glandular trichomes with striated cuticle (b) Epidermis with papillose and glandular out growth (2M) Lavang (a) Elongated parenchyma with rosette crystals (b) Fragments of shizolysigenous oil cells

ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 11 Iss 11, 2022

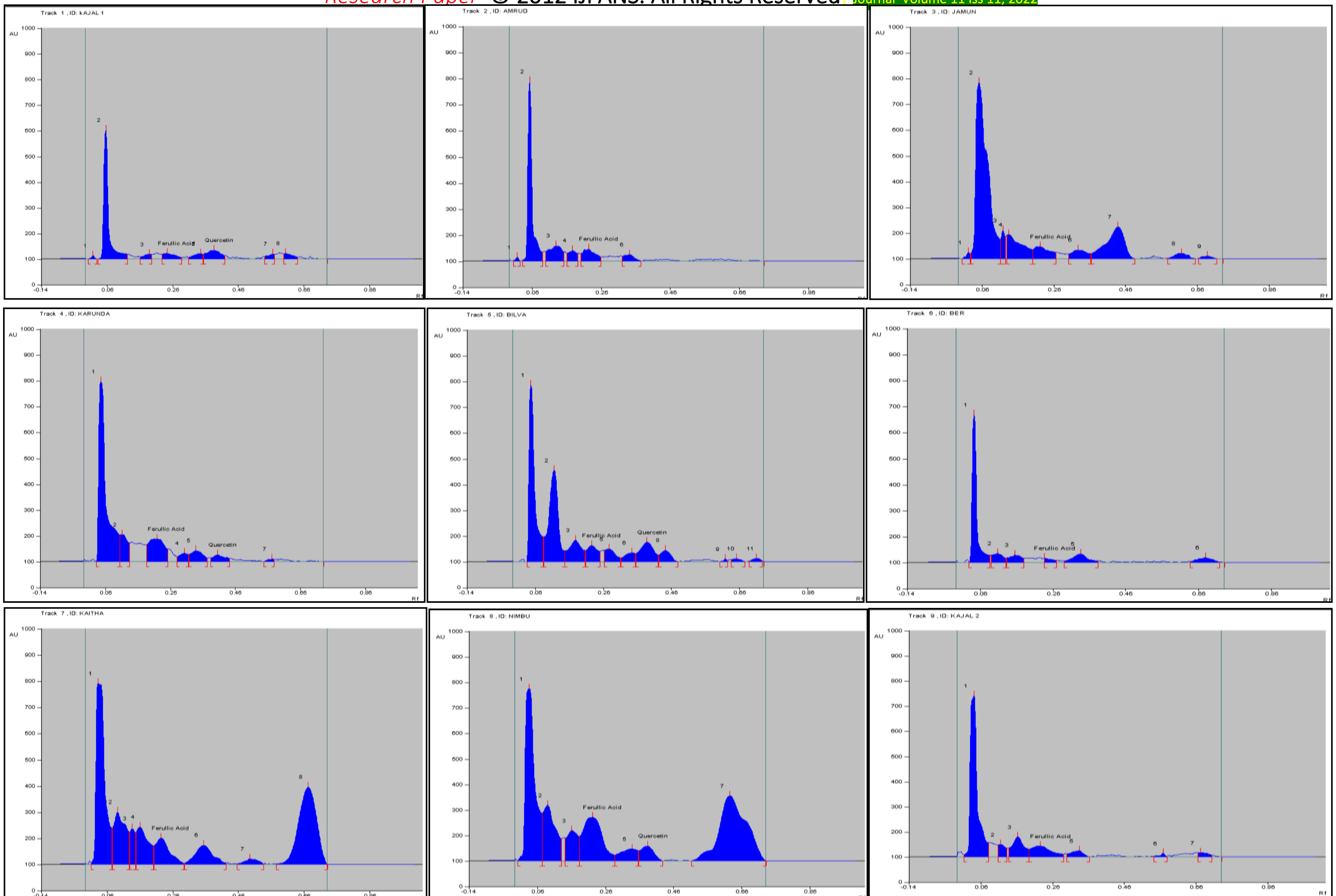

Fig: 3A HPTLC profiling of Kajal formulation with its ingredients.

Where Track 1: test solution of *Kajal-1;* Track 2: test solution of Amrud; Track 3: test solution of Jamun; Track 4: test solution of Karounda; Track 5: test solution of Bilva; Track 6: test solution of Ber; Track 7: test solution of Kaitha; Track 8: test solution of Nimbu; Track 9: test solution of Kajal -2

IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876

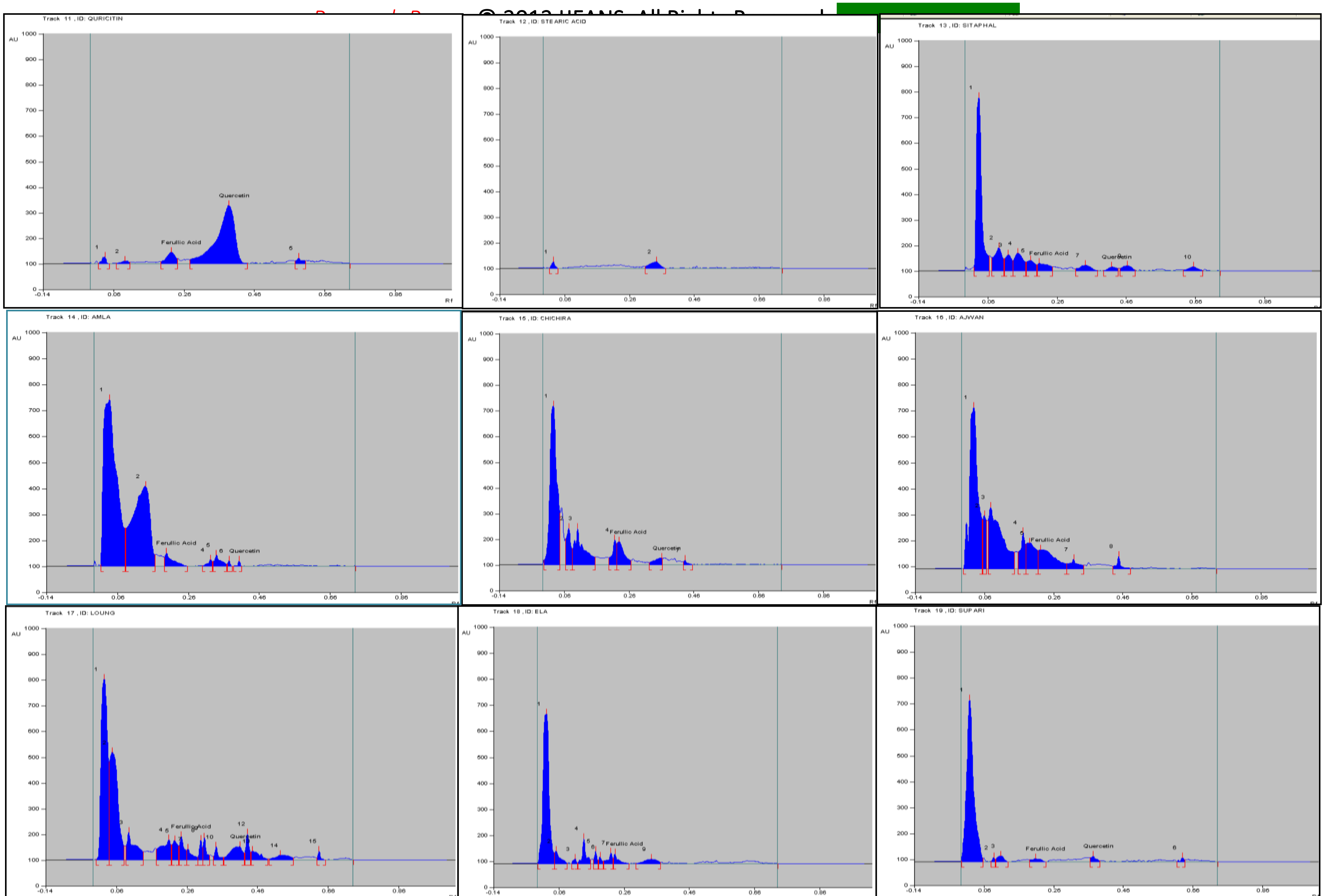

Fig: 3A TLC profile (A) and 3D densitometric analysis of Kajal formulation with its ingredients.

Where Track 10: Ferulic acid standard; Track 11: Quercetin standard; Track 12: Stearic acid standard; Track 13: test solution of Sitaphal; Track 14: test solution of Awala; Track 15: test solution of Chichida; Track 16: test solution of Ajowan; Track 17: test solution of Lavanga; Track 18: test solution of Ela; Track 19: test solution of Supari.

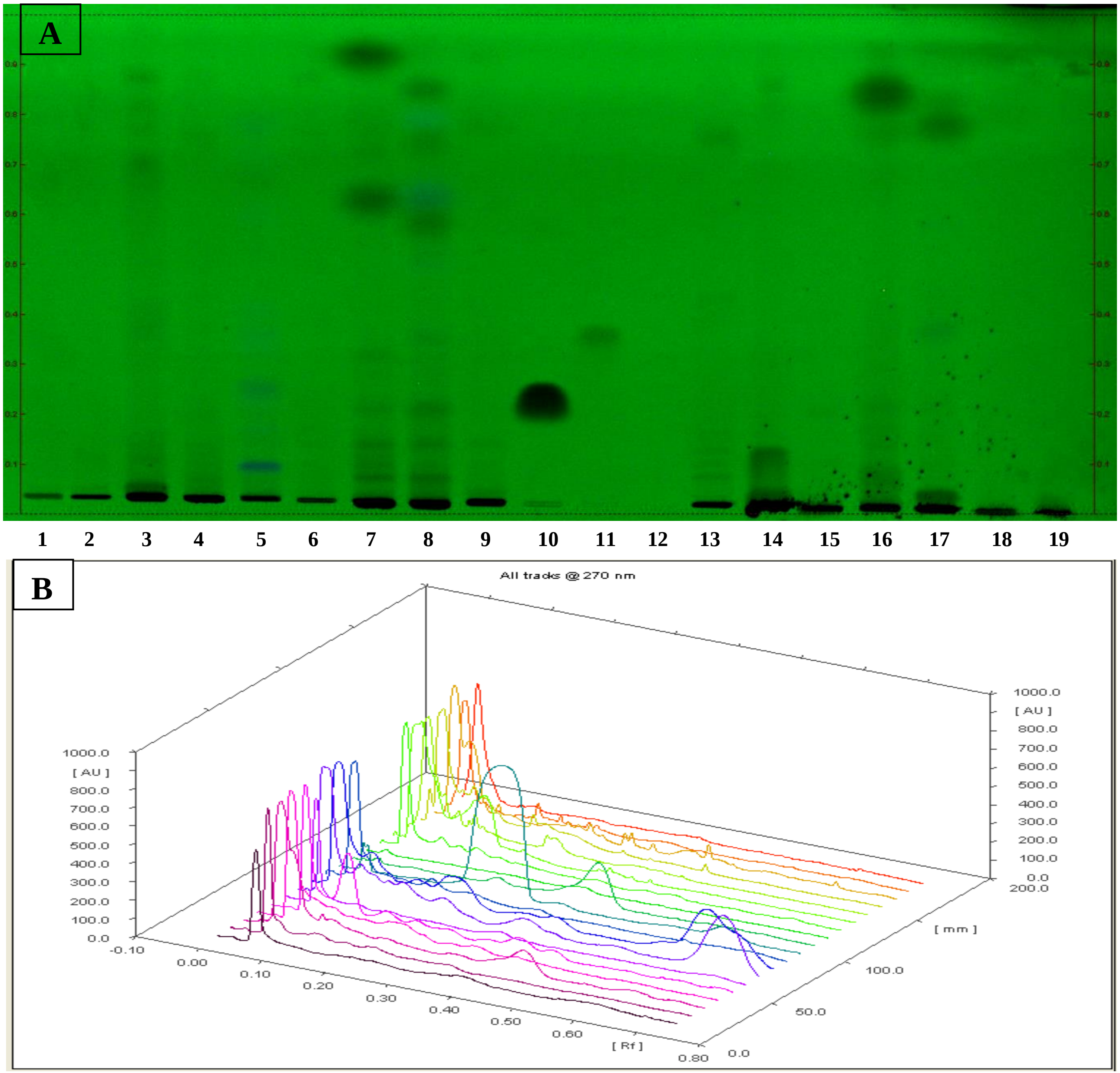

Fig. 4: HPTLC profile (A) and 3D densitometric analysis of Kajal formulation with its ingredients.

Where Track 1: test solution of ***Kajal-1****;* **Track 2:** test solution of Amrud; **Track 3:** test solution of Jamun; **Track 4:** test solution of Karounda; **Track 5:** test solution of Bilva; **Track 6:** test solution of Ber; **Track 7:** test solution of Kaitha; **Track 8:** test solution of Nimbu; **Track 9:** test solution of Kajal -2; **Track 10:**Ferulic acid standard; **Track 11:**Quercetin standard; **Track 12:**Stearic acid standard; **Track 13:** test solution of Sitaphal; **Track 14:** test solution of Awala; **Track 15:** test solution of Chichida; **Track 16:** test solution of Ajowan; **Track 17:** test solution of Lavanga; **Track 18:** test solution of Ela; **Track 19:** test solution of Supari.