

## PHARMACEUTICO ANALYTICAL STUDY AND CLINICAL EVALUATION OF RAKTACHANDANAADI LEPA IN VYANGA WITH SPECIAL REFERENCE TO HYPERPIGMENTATION ON FACE

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### Abstract

Nowadays, face value is also important for self-confidence and making once successful. Vyanga is one of the Kshudra roga produced due to vitiation of Vata and Pitta dosha, along with the involvement of Rakta dhatu in Twacha of Mukha Pradesha. It is analogous to all the hyper-pigmentation disorders like Melasma, Cholasma, Freckles, Lentiges etc in Western system of Medicine. In today's world, Ayurvedic medicines are gaining popularity in field of cosmetology and dermatology. So, though there are lot many treatment modalities and cosmetics for these disorders, there is a need of standardization of Ayurvedic classical medicines. Thus, in the present study Rakchandannadi Lepa mentioned by Sharangadhara as Mukhakantikara and Vyangahara Lepa was selected. The main aims and objectives of the studies are Pharmaceutical study, Physico-chemical Analysis, Clinical evaluation of Raktachandanaadi lepa in Vyanga. Tyrosinase Enzyme Inhibitory activity test was conducted. A single group clinical study with pre and post test design was done. In tyrosinase inhibition assay, the Aqueous extract of Lepa churna showed 57.31% inhibition of tyrosinase at 800ug/ml with IC50 of 445.2µg/mL compared to the positive control (Kojic acid). The clinical study has shown statistically highly significant reduction in the objective parameters with the p value less than 0.05. Hence, it was inferred that the selected Raktachandanaadi lepa Inhibits the activity of Melanin Pigment and there by curing Vyanga.

**Keywords:** Vyanga; Hyperpigmentation on face; Raktachandanaadi lepa; Tyrosinase Inhibitory Assay.

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## **INTRODUCTION**

Ayurveda the science of life that has its roots from vedic period. It mainly focuses on maintainance of Health, rather than treating disease. The main aim of Ayurveda is maintainence of health and eradication of the diseases.<sup>[1]</sup>

“Face is the index of mind”. Today’s society places a high value on appearance. The beauty and attraction of the individual is reflected by skin’s health.<sup>[2]</sup> The concept of beauty has prevailed since mankind earliest ages and but in today’s world also beauty is one of the most valued and desirable attributes. The objective nature of human beauty is timeless and Ayurveda stands supreme as for as this concept is concerned.

In present scenario, skin conditions constitute major percentage of the outpatient attendance in any hospitals, among which hyper-pigmentation on face is the one. Though it’s not a life threatening disease, it significantly affects a person’s overall emotional well-being and can contribute to decrease in social functioning, self-esteem and productivity at work place or school/college.

Hyper-pigmentation (Melanosis or Melanoderma) is a group of disorders characterized by abnormally darker skin that results from increase in the concentration of pigment, which occurs either due to increase in the number of melanocytes or increase in activity of melanocytes.<sup>[3]</sup>

Hyper-pigmentation most commonly results from exposure to sunlight, due to air pollution, different cosmetic using habits, stressful life, dietary changes, inclination towards junk or fast food, sleeping habits in day to day life.

In these disorders there is formation of sharply demarcated light to dark brown macules of different size and shapes affecting the sun exposed areas particularly face.<sup>[3]</sup>

A Hyperpigmentation disorder has been correlated condition in Ayurveda literature known as Vyanga. According to Ayurveda ‘Vyanga’ is one of the diseases mentioned under kshudra roga, presenting the signs like Niruja (painless), Tanu (light), or Shyava Varnayukta Mandala (blackish discoloration) on Mukha Pradesh (face), as described by Acharya Shusrutha.<sup>[4]</sup> Vyanga is considered as swatantra vyadhi in the Ayurvedic literature. Vyanga is a disease of skin in which there is production of painless, thin black patches on the face decreasing complexion and luster of the skin.

Though there are many treatment modalities for Hyperpigmentation, they are usually not satisfactory as it leads to various side effects such as Contact Dermatitis and Complete Depigmentation. Every fine morning, all across India we witness advertisements claiming fairness, glow, pimple free skin, and many more clearly violating norms of Drugs and Magic Remedies Objectionable Act, 1954.<sup>[5]</sup> It is more pain full when all these unethical, immoral, and illegal activities are under the umbrella of Ayurvedic medicines.

The herbal medicines (phyto-chemicals) have regained their popularity for the treatment in the field of cosmetology with their efficacy and safety aspect being supported by controlled clinical studies.

Thus, to overcome the adverse side effects of modern cosmetics, there is need for the research of Ayurvedic formulations which can be used for external application, effective as modern cosmetics, with least

adverse effects that can be applied in day to day practice.

Acharya Charaka has broadly classified Chikitsa under three main headings, namely Anta Parimarjana, Bhahir Parimarjana and Shastra Pranidhana.<sup>[6]</sup> Lepa Kalpana is the Upakalpana, which comes under Bhahir Parimarjana Chikitsa. The external applications of medicines in the form of fine paste is called as Lepa.

Raktachandanadi lepa is a formulation mentioned in Sharangadhara Samhitha<sup>[7]</sup> and Bhaishajya Ratnavali.<sup>[8]</sup>

It consists of seven Varnya Dravyas. Classics say that the fine powders of these drugs, made into paste using water and applied over Mukha pradesha, enhances Mukha-kanti and relieves Vyanga.<sup>[7]</sup>

In the present study Raktachandanadi lepa was selected, having the ingredients predominantly with tikta, madhura, kashaya rasa, predominant sheeta veerya, katu vipaka, guru and ruksha in nature with alleviating action on all three doshas.

Also the Analytical study and Pharmacological profiles of Raktachandanadi lepa has been reviewed for its Tyrosinase enzyme inhibition activity.

Hence the present study was undertaken to evaluate the efficacy of Raktachandanadi lepa in the management of Vyanga with special reference to Hyperpigmentation on face with the hope of quicker action. Pharmaceutico Analytical study was carried out to ensure the Quality Assurance and Quality control.

## MATERIALS AND METHODS

### Selection of drug

The selected drug Raktachandanaadi Lepa is a poly herbal formulation in the form of churna exclusively to increase Mukha Kanti and cures Vyanga. Seven drugs are described in Raktachandanaadi Lepa of Sharangdhara Samhita are taken in equal quantity and combined well. The patients were advised to apply the Lepa on affected part.

### Procurement of the drug

Ingredients of study drug Raktachandanaadi Lepa include seven herbal drugs namely Rakta Chandana (*Pterocarpus santalins*), Manjishtha (*Rubia cordifolia*), Lodhra (*Symplocos racemosa*), Kushtha (*Saussurea lappa*), Priyangu (*Callicarpa macrophylla*), Vatankura (leaf bud of *Ficus bengalensis*), Masoor dal (*Lens culinaris*) were first identified and authenticated from expert botanists at FRLHT, Yelahanka Bengaluru. The raw materials were collected from authorized dealers of raw drugs.

### Method of preparation of lepa

Churnikarana of all the above mentioned drugs were done at Rasa Shala of Ramakrishna Ayurvedic Medical College. Vastragaalita churna of all the drugs were taken in equal quantity in the and mixed thoroughly. About 50gms of above obtained churna was packed in air tight plastic zip-lock covers.

### Analytical study

Analytical study of the prepared medicine is done to standardize the formulation and check its efficacy.<sup>[8]</sup>

**Table 1: Subjective Parameter**

Sl. No.	Parameter	Before Treatment	Review	After Treatment
			15days	30days
1	Neerujam	Yes / No	Yes / No	Yes / No
2	Shyavam	Yes / No	Yes / No	Yes / No
3	Tanu	Yes / No	Yes / No	Yes / No
4	Mukhamaagatya Mandala	Yes / No	Yes / No	Yes / No

**Table 2: Objective Parameter**

Sl.No.	Parameter	Score		
		Before Treatment	Review (15days)	After Treatment 30 days
1	Size			
2	Colour			

**Table 3: Result Assessment**

Sl.No.	Parameter	Before Treatment	After Treatment	% of improvement
1	Size			
2	Colour			

**Table 4: Distribution of patients according to Gender**

Gender	No. of Subjects	Percentage
Female	23	76.66%
Male	7	23.33%
Total	30	100%

**Table 5: Distribution of patients according to marital status**

Marital Status	No. of Subjects	Percentage
Married	23	76.66%
Unmarried	7	23.33%
Total	30	100%

**Table 6: Distribution of patients according to occupation**

Occupation	No. of Subjects	Percentage
Agriculture	1	3.33%
Business	1	3.33%
Worker	10	33.33%
House maker	10	33.33%
Student	5	16.66%
Teacher	3	10%
Total	30	100%

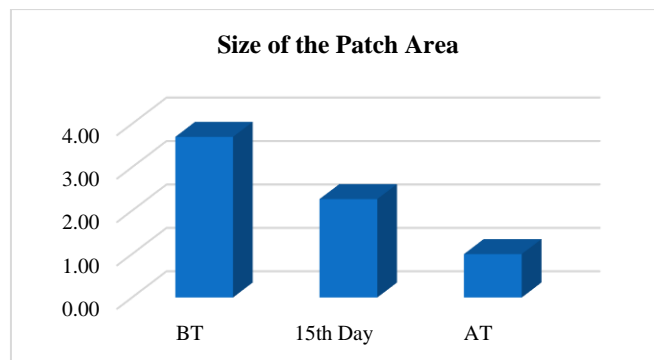
**Table 7: Showing patients Deha Prakruti**

Deha Prakruthi	No. of Subjects	Percentage
Vata Pittaja	16	53.33%
Vata Kaphaja	9	30%
Kapha Pittaja	5	16.66%
Total	30	100%

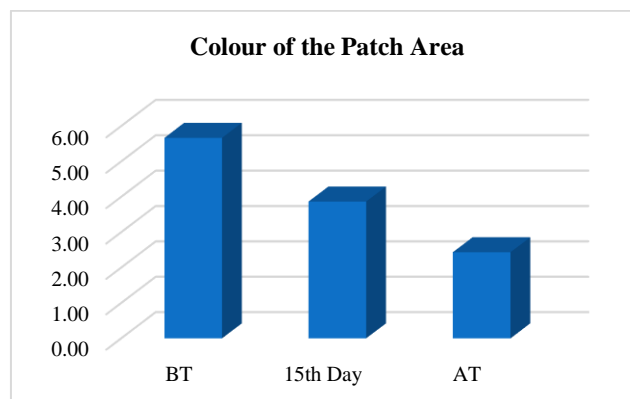
**Table 8: Parameters and results**

Sl.No.	Parameters	Results	Protocol
1.	Particle size	100% of the material passes through 425 micron sieve.	API PART 2
2.	Loss on ignition	93.52%	API PART 2
3.	pH (10.0% aqueous solution)	4.99	API PART 2
4.	Volatile oil, v/W	0.99%	API PART 2
5.	Total Ash, w/w	6.36%	API PART 2
6.	Acid – Insoluble Ash, w/w	1.45%	API PART 2
7.	Water – Insoluble Ash, w/w	2.22%	API PART 2
8.	Loss on Drying at 105 deg C, w/w	9.42%	API PART 2
9.	Total Bacterial Count / g	Less than 10 CFU	IP-2018
10.	Total Yeasts & Moulds Count / g	Less than 10 CFU	IP-2018

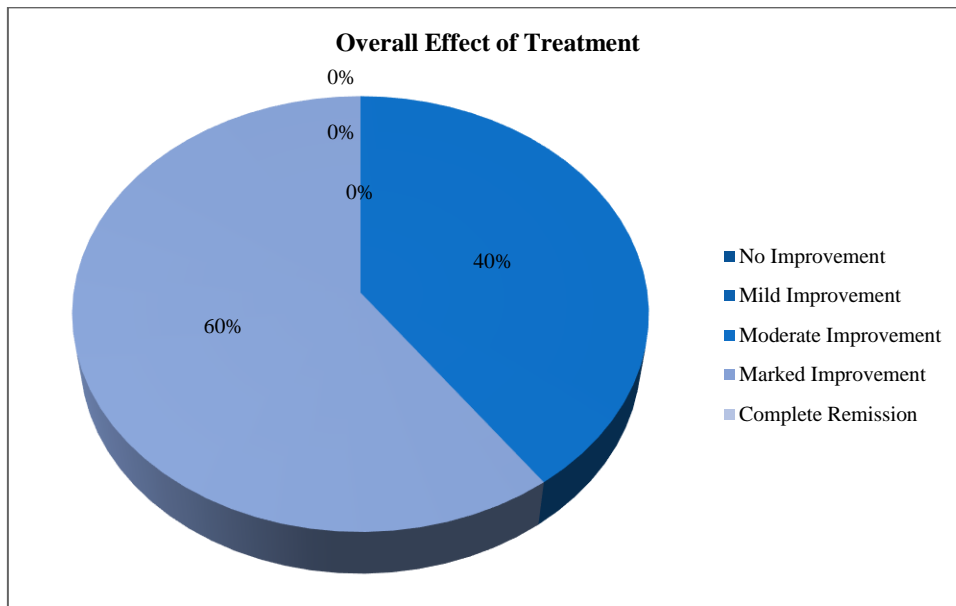
**Figure 1: Showing effect of treatment on Size of the patch Area**



**Figure 2: Showing effect of treatment on Colour of the patch Area**



**Figure 3: Showing patient wise relief score in all symptoms and its percentage**



In the present study, sample of Raktachandanaadi lepa prepared was given for laboratory analysis at Merieux Nutrisciences Bangalore Private Limited, Rajajinagar, Bengaluru.

Tyrosinase Inhibitory Activity<sup>[9][10]</sup> was also conducted at Skanda life Sciences Pvt. Ltd, Bengaluru to evaluate the action of Raktachandanaadi lepa on Melanin Pigment.

### Study design

A randomized, uncontrolled clinical trial study.

### Study population

Patients were selected from the OPD and IPD of Ramakrishna Ayurvedic Medical College, Bengaluru, Medical camps and other sources who were suffering from Vyanga (hyperpigmentation on face).

### Sampling

Incidental selection and purposive sampling technique was employed.

### Study sample

The patients were various area of Bengaluru and towns surrounding Bengaluru city suffering from hyperpigmentation on face (vyanga).

### Sample size

The study was completed in a total of 30 subjects.

Subjects of all gender, irrespective of socio-economic status and religion, having the signs and symptoms of Vyanga with special reference to Hyperpigmentation on face fulfilling the inclusion criteria were registered for the study.

### Inclusion Criteria

- Patients between the age group of 18 – 60 yrs (of either gender, religion, occupation, socio-economic status).
- Patient representing signs and symptoms of Hyperpigmentation (Vyanga) who were willing to participate in study.

### Exclusion Criteria

- Patients having systemic and endocrinal diseases, like Addison's diseases cushing syndrome etc.
- Hyperpigmentation since birth.
- Hyperpigmentation caused by tumor like malignant melanoma.
- Patients with drug induced chloasma.
- Patients who were not willing to participate.

### Ethical consideration

Ethical clearance was obtained from the institutional ethical committee of Ramakrishna Ayurveda Medical college and Hospital, Yelahanka, Bengaluru, held on 19-05-2017.

### Criteria of withdrawal

- Increase symptoms like Redness of the skin, Kandu (itching), Excessive burning sensation.
- Patients who are not willing to continue.

### Diagnostic Criteria

Patients are diagnosed as per clinical features mentioned in classics.

- Neeruja i.e. painless patches.
- Tanu i.e. Light brown pigmentation on facial skin.
- Shyava i.e. Dark brown pigmentation on facial skin.
- Mukhamaagatyamandala i.e. patches over face.

### Technique of data collection

The patients suffering from Vyanga with clinical manifestations of round shaped lesions, Hyper pigmentation, Kandu and Daha were included in the study with due consent of the patient.

The sample size was decided based on the prevalence of Vyanga and willingness to participate.

The patients were thoroughly interrogated; history and facts were noted in a specialized structured clinical proforma based on Ayurvedic classical frame work incorporating physical status examination. General information about the patient and their family, chief complaints to know manifestations of disease, history of skin disease, factors relieving disease, Family History, Personal History to get information on Diet, Appetite, Nidra, Vyasana, allergies if any were noted.

Examination included number of facial patches or macules, size, vaivarnya, Kandu, daha, etc. Size and Colour of the patch area before treatment was noted. Examination also included Nadi, Mala, Mutra, Dashavidha Pariksha and all the Systemic Examination.

The patients were asked to apply Lepa on affected area with water. The feedback obtained from the patients which included graded responses. The effect of trial drug was noted on 15<sup>th</sup> and 30<sup>th</sup> day.

### Treatment methodology and schedule

The patients as per inclusion criteria were randomly selected. Methodology of the treatment for the patients as follows.

### Intervention

- Local application of Raktachandanaadi lepa mixed with water, was done over the affected area, once a day for 30 days.
- When the lepa starts drying, the patient is asked to wash it with cold water.

**Duration of Intervention:** 30 days



## Rules of Application of Lepa

- The sufficient quantity of Raktachandanaadi churna was told to be mixed in water, triturated well and kept for 5mins. It was told to be instant use and for single use only.
- Patient was asked to wash the face with mild soap, before the application in order to keep the affected area clean and hygienic.
- Lepa was told apply against the hair follicular direction on the affected area to facilitate quicker and better absorption.
- Generally thickness of Varnya lepa is ½ angula (approximately 0.97 cm), as it is difficult to measure the thickness of lepa every time, patient was asked to apply the lepa as thick as possible.
- Lepa are removed soon after they dry over the place of application. This would generally take 40-50 mins.
- Therapeutically Lepa are effective in wet state and they turn non potent and skin irritating when dry.
- Previous Lepa should never be covered with fresh one.
- Application of Lepa during night hours is prohibited.
- Patient was asked not to speak, smile, laugh, do any sort of facial movements during the time of application.
- Exposure to sunlight, wind, artificial air such as Fan, air condition etc was prohibited during the time of application.
- During the period of 30 days intervention, patient was asked not to get exposed to sunlight and ultraviolet rays. Patient was asked to cover the face (affected area) with the cloth in order to prevent direct sunlight.

The patients were clearly explained regarding above rules, clearly told about the study in the language which they would understand and consent form was got signed by the patient.

## Assessment Criteria

The subjective and objective parameters of baseline data to pre and post medication were compared for assessment of the results.

### Subjective parameter

The signs and symptoms of Vyanga as well as Hyperpigmentation explained in Ayurvedic and Modern texts will be subjective parameters.

1. Neerujam (Painless lesion)
2. Shyavam (Brownish pigmentation)
3. Tanu (Light Pigmentation)
4. Mukhamaagatya Mandala (Hyperpigmented patch over face).

### Objective parameter

#### 1. Size

Size	Score
0-1 cm	1
1-3 cm	2
3-6 cm	3
>6 cm	4

In case of multiple patches or lesions, size of largest lesion is taken into consideration.

#### 2. Colour

Colour	Score
Very fair	1
Fair	2
Medium	3
Olive	4
Brown	5
Dark brown	6

Standard graded scale (Fitzpatrick scale) is used to assess the change in colour of the skin in affected area before and after treatment.





### Assessment Schedule

Assessment of was done as per the following schedule

- 1<sup>st</sup> assessment – 1<sup>st</sup> day (before treatment)
- 2<sup>nd</sup> assessment – 15<sup>th</sup> day (during treatment)
- 3<sup>rd</sup> assessment – 30<sup>th</sup> day (after treatment)

Likewise, data was collected of all the 30 patients.

### Assessment Chart

Assessment was made by Subjective parameter and Objective parameter. (Table 1 and 2) Finally over all result was calculated by taking average of all parameter results. (Table 3)

### Adverse effect evaluation criteria

Evaluation and reporting of adverse effect was done as per the guidelines of national pharmacovigilance program for Ayurveda, Siddha and Unani (ASU) drugs.

### Data analysis

The results were analyzed statistically by using descriptive statistics. Statistical evaluation of the data obtained was done using means, standard deviation, standard error, percentage and paired 't' test using Service product for statistical solution (SPSS) for windows software. (Table 4 to Table 7)

## RESULTS

A laboratory study of the prepared Raktachandanaadi lepa was conducted to standardize the preparation.

The preparation which was prepared in first batch was given for Analytical study in Merieux Nutrisciences Bangalore Private Limited, Rajajinagar, Bengaluru. (Table 8)

### Result of tyrosinase inhibitory activity

In mushroom tyrosine inhibition assay, the positive control (Kojic acid) showed mushroom tyrosinase inhibition with an IC<sub>50</sub> value of 58.63 µg/ml.

The test sample showed 57.31% inhibition of mushroom tyrosinase at 800ug/ml with IC<sub>50</sub> of 445.2µg/mL. (Figure 1, 2 and 3)

## DISCUSSION

### Discussion on Analytical study and its results

- The 100% of the materials of the lepa passes through the 425-micron sieve. This facilitates the easy absorption of the lepa in the skin.
- The pH of the Lepa was 4.99, which shows that the nature of Lepa is slightly acidic in nature. The melanin pigment is always bound to Protein. As pH decreases the polymerization of the melanin pigment with Protein also decreases and this leads to decrease in the activity of them melanin pigment.
- The loss on ignition of the compound is 93.53% and the total ash remaining is 6.36%.
- Acid soluble ash in 1.45% and water-soluble ash is 2.22%.

- Loss on drying at 105 degrees Celsius is 9.42%.
- Total Bacterial and Total yeast count is less than 10 CFU, which shows that there is a very less chances of microbial growth in the preparation
- The Mushroom Tyrosinase Inhibitory Assay showed 57.31% inhibition of mushroom tyrosinase at 800ug/ml with IC<sub>50</sub> of 445.2µg/mL, which proves that the lepa does the inhibition of the tyrosinase enzyme, there by inhibiting the melanin production.
- Thus, the lepa on external application proved to be highly effective in vyanga.

#### Discussion on Results of clinical study

In the present study the results of subjective parameters which were assessed such as Colour of the Patch Area and Size of the patch area showed statistically highly significant reduction with the p value less than 0.05.

This can be attributed chiefly to the Varnya, Vata-pitta shamak, Kapha-pittashamak, Sheeta Veerya, Katu vipaka, cooling, disinfectant and blood purifying anti-inflammatory properties of the drugs present in the intervention, decreases about Vata and Pitta in the Twacha and enhances Varna of Twacha. Therefore, the pathological disintegration - Niruja, Sahyava, Tanu, Mukhamadala i.e Size and colour of the Patch area gets decreased.

The decrease in the mean suggests significant reduction in the Vyanga. It also indicates that after each day of intervention the severity of the condition is getting relieved, which can be attributed to the Varnya, Vata-pitta shamak, Kapha-pittashamak, Sheeta Veerya, Katu vipaka, cooling, disinfectant and blood purifying

anti-inflammatory properties of the drugs present in the intervention. Hence, it was inferred that the intervention selected for the present study has shown combined effect in the management of Vyanga vis-à-vis Hyperpigmentation on Face.

#### CONCLUSION

On the basis of concepts, analysis of Analytical study and clinical observations made in this study, the following conclusions were drawn:

Vyanga is one of Kshudra Roga produced due to vitiation of Vata and Pitta dosha with the involvement of Rakta dhatu. It is characterized by symptoms like Niruja (painless), Tanu(thin), Shyava Varnayukta Mandala (bluish – black lesion/patch) on Mukha Pradesha (face).

It is analogous to Hyperpigmentation on face leading to Melasma/ cholasma, Freckles and lentiges as described in the Western system of Medicine.

External application of Raktachandanaadi lepa proves to be highly beneficial in treatment of Vyanga.

An attempt was made in the present study to re-establish a classical treatment in management of Vyanga, instead of lot many Cosmetics and chemical treatments. Though Vyanga is a Kshudra roga , it effects the social wellbeing of the patients. So , in todays world there is a need of standardized and proven ayurvedic treatments for the management of such simple diseases.

Mushroom tyrosinase enzyme activity Assay was carried out to prove the inhibitory action of the Aqueous extract of the Raktachandanaadi lepa.

Raktachandanaadi Lepa was specifically selected considering its guna, karma, indications, phyto chemical constituents and properties which are beneficial in subsiding Vyanga and increase varna of the Twacha. The study was conducted in a single group, consisting of 30 subjects.

The results of objective parameters which were assessed such as the colour and size of the patch area of Vyanga showed statistically highly significant reduction with the p value 0.05.

In mushroom tyrosine inhibition assay, the positive control (Kojic acid) showed mushroom tyrosinase inhibition with an IC<sub>50</sub> value of 58.63 µg/ml. The Aqueous extract of Lepa churna showed 57.31% inhibition of mushroom tyrosinase at 800ug/ml with IC<sub>50</sub> of 445.2µg/mL. Which proves that the lepa churna inhibits the tyrosinase enzyme activity, thereby decreasing the production of melanin Pigment

By this it was interpreted that application of Raktachandanaadi Lepa showed effect in the management of Vyanga with special reference to hyperpigmentation on face.

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