Study Protocol

Evaluation of Comparative Dermal Toxicity and Efficacy of Aargwadhadi Tail and Aargwadhadi Ointment

Abstract:

Introduction: Skin diseases are among the most common of all health illness in recent years,. In Ayurveda, management of skin diseases includes internal and external administration. External administration includes various topical applications However, herbal medicines are not completely safe from adverse effects and develop irritation, rashes, redness and burning sensation on the skin as observed in recent researches.hence there is a need to search safe drug which is economical and affordable to the society. Hence present study is undertaken to study dermal toxicity profile Aa*rgwadhadi Tail* and *Aargwadhadi ointment* in an experimental animaland make available safe and efficient drug to the human being. **Aim and objective**: Pharmaceutical development, standardization and evaluation of acute, sub-acute dermal toxicity and efficacy of a Aargwadhadi tail and Aargwadhadi ointment and compare efficacy and safety of Aargwadhadi tail and Aargwadhadi ointment. **Materials and method**:Aargwadhadi Tail and Aargwadhadi Ointment will be prepared as per classical referenceand it will be converted into ointment form. Analytical study for standardization of Aargwadhadi tail and Aargwadhadi ointment will be done. Evaluation of Acute and sub-acute dermal toxicity study in experimental animals of both dosage forms as well, efficacy study of Aargwadhadi oil and Aargwadhadi ointment on animal model of vitiligo will be done. **Observations and results**: Observation will be done on the basis of assessment criteria evaluation of control group and experimental group will be noted. Results will be drawn on the basis of observations and applying suitable tests. It will be noted and presented in form of table, charts, graphs etc. **Conclusion**: Conclusion of the study will be drawn accordingly from the recorded observations, analysis of data.

Key Words: Aargwadhadi Tail, Aargwadhadi ointment, Acute & sub-acute Dermal toxicity, vitiligo

1. Introduction

Ayurveda is a complete and holistic traditional health-care system which have both preventive and therapeutic aspects. Ayurveda has eight specialized branches called as Ashtang Ayurveda. The enduring fundamentals of Ayurveda, which were said by various Acahryas are valid and still applicable because of their scientific background. In today's technology and scientific era these fundamentals must be subjected to scientific research not only to prove it but also to add something new to the existing knowledge and further in the betterment of health [1].

Acharya Charka has mentioned that any materials in the universe or even a poison can be used as a medicine when it is used properly and on the contrary improperly used medicine can act as a poison [2]. Traditionally medicinal plants have been used for many years as topical and internal preparation in the treatment of fungal and bacterial diseases. Globally, skin conditions are the fourth leading cause of nonfatal disease burden. Skin diseases are among the most common of all human health illnesses and affect almost 900 million people in the world. Five common conditions account for over 80% of all skin diseases. They are most frequently caused by *Staphylococcus aureus*, *Streptococcus pyogenes*, and coryneform bacteria. Impetigo, folliculitis, boils, and erythrasma are mostly found in the patients of skin disorders.

In recent years, there has been a considerable increase in the incidence of skin problems in the tropical and developing countries like India due to various reasons like poverty, poor sanitation, unhygienic living, and pollution [3]. Skin diseases frequently occur in children and adults can lead to disability and account for the fourth leading cause of nonfatal disease burden at the global level [4-5]. Various studies have shown that skin diseases can have a major impact on the quality of life of those affected [6]. Some skin diseases, such as skin cancer and infections are potentially life-threatening [7].

Safety is the most important consideration before administration of any herbal product in human being. In Ayurveda, management of skin diseases includes internal and external administration. External administration includes various Lepas (Paste), medicated oil, poultice, face pack, hair pack etc which are advised to apply on affected skin for varied duration [8-9]. However, herbal medicines are not completely safe from adverse effects and develop irritation, rashes, redness and burning sensation on the skin as observed in recent researches [10-12].

Though the modern medicine is having powerful antibiotics, antifungals, antihistamines, steroids etc., but better management could not be searched out till today. Moreover, topical medicines are expensive and sometimes poor people cannot afford, hence there is a need to search safe drug

which is economical and affordable to the society. The adverse reaction of modern cosmetics and higher cost of therapy are also made the society to approach ancient system of Ayurveda.

Hence present study is undertaken to study dermal toxicity profile Aa*rgwadhadi Tail* and *Aargwadhadi ointment* in an experimental animaland make available safe drug to the human being.

2.Aims and objective of study

2.1Aim of study: Evaluation of Comparative Dermal Toxicity and Efficacy of Aargwadhadi Tail and Aargwadhadi Ointment.

2.2 Objectives of the study:

1.To prepare of Aargwadhadi tail and Aargwadhadi Ointment by standard operating procedure

2.To evaluate acute dermal toxicity of *Aargwadhadi tail* and *Aargwadhadi* Ointment in experimental animal.

3.To evaluate sub-acute dermal toxicity of *Aargwadhadi Tail* and *Aargwadhadi* Ointment in experimental animal

4.To evaluate efficacy of Aargwadhadi tail and Aargwadhadi ointment in vitiligo animal model

5. To compare safety and efficacy of Aargwadhadi tail and ointment

3. Materials and Methods

Present work will be conducted under following headings.

3.1 Pharmaceutical Study

In this study Aargwadhadi Tail and Aargwadhadi Ointment will be prepared in three batches as per classical reference to establish pharmaceutical standardization. This pharmaceutical study will be done according to following steps.

- **Procurement of raw drug** – all raw drugs will be collected / procured from the field and authentic reliable sources.
- **Authentication of raw material** – all raw materials will be verified and authenticated by department of Dravyaguna of the institute. Raw drug will be standardized as per A.P.I. Table 1 show contents of Aargwadhadi tail and Aargwadhadi ointments and their quantity. Below is the flow diagram (Figure 1) of method of preparation of Aargwadhadi tail and Aargwadhadi ointment.

Table No.1 Ingredients used for *Aargwadhadi Tail* [13]

Sr no	Drug Name	Part used	Proportion
1	Aargwadh (*Cassia fistula)*	Fruit pulp	1\4th part w\w
2	Dhav (*Anogeissus latifolia)*	Bark	1\4th part w\w
3	Kushta (Saussurea lappa)	Root	1\4th part w\w
4	Hartala (Arsenic trisulfide)	-	1\4th part w\w
5	Manshila (Realgar)	-	1\4th part w\w
6	Haridra (Curcuma longa)	Rhizome	1\4thpart w\w
7	Daruharidra (Berberis aristate)	Stem	1\4th part w\w
8	Til Tail (Sesamum indicum)	-	1 part w\w
9	Water	-	4 parts w\w

Figure 1: Flow diagram of preparation of Aargwadhadi tail

All the procured and authenticated drug of *Aargwadhadi tail* will be dried and *Kalka* will be prepared

Aargwadhadi kalka(1/4Part) +*Tila Tail* (1 part) +Water *4* partwill be taken in a wide mouth steel vessel.

Above mixture will be heated on *Mandagni*(low flame-gently boiling) with frequent stirring till the *Snehasiddhi Lakshans* appears.

Tail will be allowed to get *Swang Sheeta* (self-cooled)

After cooling *Tail* will be filtered through doubled muslin cloth and stored in an air tight glass bottle

3.2 Analytical Study [14-17]

A. Analytical study of Aargwadhai Tail.

i. Organoleptic characters

- Sparsha (touch), Rupa (appearance), Gandh (odour) will be assessed

ii. Physicochemical parameters:

1. Specific Gravity
2. Refractive index at 25^0C
3. Viscosity
4. Acid value
5. Saponification value
6. Iodine value
7. Peroxide value
8. Unsaponifiable matter
9. HPTLC or GC-MS (Quantitative)

Preparation of *Aargwadhadi Ointment:*

Aargwadhadi tail will be made into ointment by following SOP. Table2. shows ingredients and quantity and below is the flow diagram (Fig. 2) of method of preparation of Aargwadhadi ointment.

Table no 2 ingredients and quantity of Aargwadhadi ointment

Sr.no	Ingredient	Proportion
1	Aargwadhadi Tail	1 part
2	White wax	1\5th part

Fig 2: Preparation of Aargwadhadi ointment

Aargwadhadi tail will be placed over mild heat until it starts foam appearing.
1\5 th part of white wax will be added to the Aargwadhadi Tail
Wax will be completely melted in oil, it will be filtered and kept in another vessel.

B. Analytical study of Aargwadhadi ointment

Organoleptic Characters: Sparsh (Touch), Roop (Colour, appearance), Gandha (Odour)

Physio-chemical Parameters such as pH, Viscosity, Homogeneity, Spreadability Test, Skin Irritation Test, TLC, Microbiologic test will be assessed.

3.3 Experimental study.

Procurement and selection of animals: Required healthy young adult (at least8-10weeks old) male\female albino rats will be selected from animal house. Total 30 Wistar strain albino rats weighing between 200-300 gm, having healthy intact skin will be taken randomly for study.

a. Inclusion criteria:

- Healthy young Wistar albino mice of commonly used laboratory strains of either sex will be considered
- Rats weighing about 200-250 gm will be included.
- Rats having healthy and intact skin
- Nulliparous.

b. Exclusion criteria:

- Pregnant and diseased mice
- Mice which are under trial of other experiment

c. Methodology of Acute Dermal Toxicity [18-19]

Table no 3: Grouping of animals and dose administration in Acute Dermal Toxicity study

Group	Drug	Dose	No. of Animals	Duration	Route
Group I	Control group- No Medicine	-	10 (5 M +5 F)	14days	-
Group II	Aargwadhadi Tail	2000 mg/kg	10 (5 M +5 F)	14 days	Topical
Group III	Aargwadhadi Ointment	2000 mg/kg	10 (5 M +5 F)	14 days	Topical

This study will be carried out according to OECD Guidelines 402. Maximum tolerated dose would be calculated by employing OECD guidelines. This dose will be applied locally only once at the first day of the study. Rats will be monitored for the duration of 24 hours, with special attention given to the first 6 hours and once daily further for a period of 14 days.(Table 3)

d] Methodology of Sub-acute dermal toxicity:[20]

Subacute dermal toxicity test will be performed according to the OECD guideline 410 for testing of *Aargwadhadi Tail* & *Aargwadhadi* ointment. The test drug will be applied daily to the skin to groups of experimental animals, one dose per group, for a period of 28 days. During the period of application of treatment, the animals will be observed daily to perceive signs of toxicity. Animals, which died during the tests, will be necropsied and at the conclusion of the tests the surviving animals will be sacrificed and necropsied. About 10 % of the body surface area will be cleared for the application of the test drug. Each group contain ten healthy rats (n=10). Based on OECD guidelines 410, at least three doses of Tail and ointment, as well as a vehicle and control groups, will be used in the study. In addition, a satellite group of 10 animals (5 animals per sex) may be treated with the high dose level for 28 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for 14 days post-treatment.(Table 4)

Table no.4: Grouping of animals for Subacute dermal toxicity study (n=10)

Group	Drug	Dose	No. of Animals	Duration	Route
Group I	Control group- No Medicine	-	10 (5 M +5 F)	28 Days	-
Group II	Aargwadhadi tail	500 mg/kg	10 (5 M +5 F)	28 Days	Topical
Group III	Aargwadhadi tail	1000 mg/kg	10 (5 M +5 F)	28 Days	Topical
Group IV	Aargwadhadi tail	2000mg/kg	10 (5 M +5 F)	28 Days	Topical
Group V	Aargwadhadi Ointment	500 mg/kg	10 (5 M +5 F)	28 Days	Topical
Group VI	Aargwadhadi Ointment	1000 mg/kg	10 (5 M +5 F)	28 Days	Topical
Group VII	Aargwadhadi Ointment	2000mg/kg	10 (5 M +5 F)	28 Days	Topical
Group VIII	No Medicine Recovery phase	-	Group IV Animal	14 Days	-
Group IX	No Medicine Recovery phase	-	Group VII Animal	14 days	-

Assessment Criteria:

1. General observation.

a) Changes in behavioral Pattern.

b) General appearance:

- CNS depression
- CNS stimulation
- Salivation
- Diarrhea
- Changes in skin color
- Hypo activity
- Passivity
- Muscle relaxation
- Muscle spasm

2. Changes in body weight (weekly)

3. Food and water consumption (weekly)

4. Fecal consistency (weekly)

5. Hematology

Qualitative urine examination will be done before exposure to the test compound and the experiment using URISTIK A 10 reagent strips at the termination of the trial.

Blood samples will be collected under light ether anesthesia, following hematological values will be analyzed.

- Packed cell Volume (PCV),
- Hemoglobin (Hb)
- Red blood cell (RBC) count,
- Total white blood cell (WBC) count,
- Differential Leucocyte Count (Polymorph and Lymphocyte ratio) and
- Prothrombin Time (PT)
- Plasma Glucose (mg/dl),
- Creatinine (mg/dl),
- SGOT (U/L) and SGPT (U/L

Blood samples will be withdrawn from the posterior vena cava of each rat and will be collected in EDTA vacuumed blood collection tubes and will gently mixed immediately to mix the blood with EDTA-anticoagulant material inside the tubes for automatic and manual hematology analyses.

6. Biochemical SGOT, SGPT, Alkaline Phosphate, Serum creatinine, Serum urea, Serum Electrolyte, Sodium and Potassium.

7. a] Histopathology study for acute dermal toxicity study-As per the need of animals will be sacrificed after 14 days by administering mild anesthesia. Liver, heart, kidney, intestine, stomach, brain will be processed for histopathological studies as per the prescribed procedures.

b] Histopathology study for subacute dermal toxicity study-

Histopathology will be done by administrating mild anesthesia. Histological examination should be performed on the preserved organs and tissues of the high dose group and the control group. Animals in the satellite group should be examined histologically with particular emphasis on those organs and tissues identified as showing effects in the other treated groups. Liver, kidney, heart, brain, intestine, lungs, stomach will be processed for histopathological studies as per prescribed procedure. Skin, liver, and kidney samples will be collected and fixed in 10% formalin for 48 hrs.

8. Rating of skin Reaction for the experiment.(Table 5-8)

Table 5.Assessment criteria (Erythema) [21]

Skin reaction	**Grade**
Slight, spotty/diffuse	**1**
Moderate uniform redness	**2**
Dark red with oedema	**3**
Fiery red with oedema or epidermal defect	**4**

Table 6. Assessment criteria [Scaling]

Skin reaction	**Grade**
Dryness shiny	**1**
Fine scale	**2**
Moderate	**3**

Severe	**4**

Table 7. Assessment criteria [Fissures]

Skin reaction	Grade
Fine cracks	**1**
Single/multiple broader fissure	**2**
Cracks with hemorrhage or exudation	**3**

Table 8. Assessment criteria [Oedema]

Skin reaction	Grade
Very slight oedema	**1**
Slight oedema	**2**
Moderate oedema	**3**
Severe oedema (raised more than 1mm)	**4**

e) Methodology of efficacy study of Aargwadhadi oil and Aargwadhadi ointment on animal model of vitiligo [22-23]

Experiment will be conducted on C57BL/6 mice of 4 weeks of age. Animals will be divided into five groups consisting of 6 mice in each group.

Dorsal region will be shaved, approximately 24 hr before. Monobenzone 40% cream will be daily applied (50 mL, for 50 days) to the dorsal region (1x1 cm^2) the same site near the tail. Cream was massaged until complete absorption using a spatula. In a different dorsal region (near the neck, 1x1 cm^2) Aargwadhadi tail or Aargwadhadi ointment as per grouping (daily, twice a day) will be applied and massaged until complete absorption. All treatments will be carried out till 65 days. 6 mm circles of ear tissue will be collected. All tail and back skin will be removed. Some samples will be stored at 80^0C for further tests. Same dorsal skin samples will be collected and placed in 10% neutral buffered formalin to histological analysis. Samples will used to evaluate de-pigmentation. Tissues will be sectioned and stained with fontana masson and then photograph in increments of 200x will be taken, analysis will be performed by counting cell per field. Ten fields from three histological sections of each group will be analyzed.(Table 9)

De-pigmentation evaluation: The extent of de-pigmentation will be analyzed by objective observation by two blinded observers.

Table no 9: Grouping of animals and dose administration in efficacy study

Group	Drug	Dose	No. of Animals	Duration	Route
Group I	Untreated group no medicine for monobenzone induced animal sample	-	6[3M+3F]	50days	-
Group II	0il Vehicle control group	2000mg/kg Til tail	6 (3 M +3F)	50 days	Topical
Group III	Ointment Vehicle control group	2000 mg/kg White paraffine cream	6(3 M +3 F)	50 days	Topical
Group IV	Experimental group	2000 mg\kg Aargwadhadi oil	6[3M+3F]	50 Days	Topical
Group V	Experimental group	2000 mg\kg Aargwadhadi ointment	6[3m+3F]	14 Days	Topical

3.4 Type of study: Pharmaceutical, Analytical, and Experimental Interventional Animal Study

3.5 Study duration- 3 months

3.6: Study centers: 1. Dattatraya Ayurved Rasashala, Mahatma Gandhi Ayurved College Hospital and Research Center, Salod (H) Wardha.

2. Animal house, Datta Meghe Pharmacy College, DMIMS(DU), Salod (H) Wardha

3. Analysis of the formulation will be done at Central Research Lab, Jawaharlal Nehru Medical College, Sawangi (Wardha).

4. According to need, study will be carried out at certified or standard institute / organization/ lab recognized or recommended by DMIMS (DU).

3.7 Observation and Results

- Observations will be noted and presented in the form of tables, chart, photographs etc.
- The data will be analyzed with application of suitable inferential statistics

3.8 Method of Data Analysis Statistical analysis: The mean and standard deviation of the treated groups will be done by applying unpaired t-test and ANOVA analysis.The data obtained will be statistically analyzed by using Statistical Package for Social Science (SPSS) software version 23. The data generated would be mentioned as Mean ± SEM difference among the groups would be assessed by employing student 't' test and ANOVA analysis.

4. Discussion

The basic Ayurvedic principles of ease of availability (natural abundance), cost effectiveness, and ease of topical application keeping in consideration the demands of current lifestyle trends and client preferences will be followed to provide a holistic remedial solution to varied issues of vitiligo and skin diseases. This formulation has been devised in accordance with the overarching guiding principles of Ayurveda that encourage individual preference and ease of preparation and application. Considering current lifestyle and trending preference amongst users, a standardized formulation in both, oil and ointment form with safety is the most important consideration before administration of any herbal product in human being. In However, herbal medicines are not completely safe from adverse effects if not used judiciously [24-25]. So acute and subacute dermal toxicity will be beneficial for providing safe product for skin disorders especially for vitiligo.

The ointment form is easier to carry and apply, and also has a better absorption [26]. The efficacy of the drug will be tested by comparing the effect of conventional Aargwadhadi oil and the Aargwadhadi ointment formulation for its efficacy in vitiligo. The dosage and frequency will be suggested at the outset and following experimental, the researcher expects to establish the efficacy, acceptability and outcome of the Aargwadhadi oil and Aargwadhadi ointment. Studies on efficacy of different Ayurvedic preparations were reported [27] but the results are not encouraging. Hence present formulation may show a ray of hope in the treatment of vitiligo.

5. Conclusion:

The present available treatment for Vitiligo does not provide complete remission of symptoms for long duration .Thus the newly developed herbal formulation will provide significant result in controlling Vitiligo and modified dosage i.e. ointment will overcome the limitation of oil and provide a cost effective cosmetic product in controlling Vitiligo.

6. **Strength of study** -. Study will create standard operating procedure about preparation and analytical studies of Aargwadhadi ointment.

2. Research formulation Aargwadhadi Tail can be converted in to new dosage form such as Cream which is convenient for external application.

7. Consent and Ethical approval

This will be taken from institutional ethical committee of Datta Meghe Institute of Medical Sciences, Sawangi, (DMIMS) Wardha and from Animal ethics committee. Study will be followed as per instructions of IAEC of DMIMS.

NOTE:

The study highlights the efficacy of "Ayurveda" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

8. References

1. Mishra LC. *Scientific basis for Ayurvedic Therapies*. CRC Press LLC, Florida, USA, p2004 no. 1-10.

2. Rathi B, Rathi R. Principals of ethical Ayurveda prescription writing in clinical practice: A literature review. J Datta Meghe Inst Med Sci Univ 2019;14:S97-102.

3. Uday Khopker (editor), Skin diseases and sexually transmitted infection, 2nd ed. 2008; Pune: Balani Book Depot Publication.p.14

4. Dalgard F, Svensson A, *Holm JO et al*. Self-reported skin morbidity in Oslo. Associations with sociodemographic factors among adults in a cross-sectional study. Br J Dermatol 2004; 151:452

5. Vos T, Flaxman AD, Naghavi M et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990: a systematic analysis for the Global Burden of Disease Study 2010.JLancet 2012; 380:2163–96.

6. Lewis V, Finlay AY. 10 years experience of the Dermatology Life Quality Index (DLQI). J Investig Dermatol Symp Proc 2004; 9:169–80.

7. Hay RJ, Johns NE, Williams HC et al. The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. J Invest Dermatol 2014; 134:1527

8. Rathi B, Khobragade P, Badwaik P, Rathi R Ethno Medicinal Documentation of Plants used in the Treatment of Skin Diseases By the Tribal's of Karanja Ghadge Tahsil of Wardha District. Journal of School of Advanced Studies, 2019; 2(1): 22-26

9. Madan, P., Rathi, B., Rathi, R., Wairagade, S., & Zade, D. (2021). Pharmaceutical Development, Standardization and Clinical Evaluation of Efficacy of a Polyherbal Hair-Pack and Hair Gel in Dandruff Control. *Journal of Pharmaceutical Research International*,2021; *33*(31B), 69-78

10. Wadnerwar N, Prasad KS. Efficacy and Adverse Effects of Gunjabeej lepa-A case series. Journal of Indian System of Medicine. 2015 Apr 1;3(2):103

11. Dweck AC. Toxicology of Essential Oils Reviewed. Personal Care 2009; 65-77.

12. Wadnerwar N, Rajput D, Deshmukh A, Gaikwad A. A Critical Review on Haratala (An Arsenical Compound). *Journal of Drug Researches in Ayurvedic Sciences,*2019: 4(3):132-137.

13. Chakradutta Chikitsasangrah of Cakrapanidutta - Edited by Dr. G. Prabhakar Rao, Chukhambha orientalalia, 3rd edition,Varanasi 2013.

14. Kumari S, Baghel DS. Pharmaceutical standardization of panchaguna taila (medicated oil) and product development as ointment, gel, cream, and physiowax. Asian Journal of Pharmaceutical and Clinical Research. 2017 Sep 1:57-62.

15. Ayurvedic Pharmacopoeia of India Editor Govt. of India, Ministry of health and Family Welfare. Dept. of Indian System of Medicine and homeopathy New Delhi (ISMH) Edition 2001, Published by the Controller of Publication Civil lines, Delhi.

16. Ayurvedic Pharmacopoeia of India part-1, Govt. of India, Ministry of health and family welfare, Delhi, 5th edition, appendix, 2007; 2.2.9: p 214

17. Ayurvedic Pharmacopoeia of India Part-II, Vol-I, appendix, Central Council of Research in Ayurveda and Siddha, New Delhi; p.113-14.

18. OECD. Test No: 402, Acute dermal toxicity fixed doseprocedure2. 402. Paris: OECD Publishing; 2017:1–3.

19. OECD. Test No: 434, Acute dermal toxicity – fixed dose procedure Section 20 to 34. 434. Paris: OECD Publishing;2004:4–5.

20. OECD: Organization for Economic Co-operation and Development Guideline for Testing of Chemicals: Repeated Dose Dermal Toxicity: 21/28- Day Study. OECD; 1981. p. 410

21. Farage MA, Maibach HI, Andersen KE, Lachapelle JM, Kern P, Ryan C, Ely J, Kanti A. Historical perspective on the use of visual grading scales in evaluating skin irritation and sensitization. Contact Dermatitis. 2011 Aug;65(2):65-75.

22. Essien KI, Harris JE. Animal models of vitiligo: Matching the model to the question. Dermatologica Sinica. 2014 Dec 1;32(4):240-7.

23. Moreira CG, Carrenho LZ, Pawloski PL, Soley BS, Cabrini DA, Otuki MF. Pre-clinical evidences of Pyrostegia venusta in the treatment of vitiligo. Journal of ethnopharmacology. 2015 Jun 20;168:315-25.

24. Dahikar GK, Rathi B, Rathi R. Exploring the therapeutic potential of *Gunja* (*Abrus Precatorius* Linn.): A classical semi-poisonous herbal drug International Journal of Botany Studies, 2020;5(6):87-94

25. Dahikar G, Rathi B. The Study Protocol of "Pharmaceutico Analytical and in Vitro Antimicrobial Activity of *Gunjadya Taila* Prepared by Using *Murchchhita* and *Amurchchhita Tila Taila Medicine & Toxicology, October-December 2020, Vol. 14, No. 4* 6675-6679

26. Bhowmik D. Recent advances in novel topical drug delivery system. The Pharma Innovation. 2012 Nov 1;1(9).

27. Rathi B, Rathi R. Pharmaceutical standardization of Bakuchi vati: A modified dosage form of Dhatryadi Yoga, nternational Journal of Research in Ayurveda and Pharmacy, 2017;8(1):57-61