



# DEVELOPMENT OF ANTIACNE HERBAL GEL

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## Abstract:

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous units, its prevalence is highest in adolescence, where the individual encounters several psycho-social changes. Acne vulgaris affects over 80% of teenagers persists beyond the age of 25 years in 3% of men & 12% of women.

Until now Synthetic medicines or antibiotics were used to treat acne. Herbal medications are considered safer than allopathic medicines. as these are Associated with side effects such as contact allergy, local irritation, scaling, itching, redness, Skin pilling etc. There are very few herbal antiacne. creams & gels available in the market & those are very costly. Keeping this mind our aim was to develop herbal anti-acne gel, to evaluate the physical characteristics of prepared formulations, to study effect on human volunteers.

In this research work following activities was done. Preparation of material for the study. (i.e. selection of crude drugs, polymers, authentication, drying & storage of plants). Extraction of crude drugs by simple maceration process. Pharmacognostical study.

Development of gel.

Following plants were selected for development of antiacne formulation Aloe Vera is the word 'Aloe' comes from the Arabic word alloeh, which means 'a bitter, shiny substance.' This is an apt description for the dagger-shaped leaf, which has a bitter taste and spiky edges. Curcuma Longa is the Genus Name is derived from the Arab word 'kurkum'. Most likely it found its way to the Occident with the caravans of Arab traders. Its Sanskrit name is 'haridra', which means 'yellow wood'. Coriandrum Sativum is the name coriander comes from the Greek word koris, meaning "bug." It is likely named for the offensive bug-like smell coriander has when it is unripe Azadirachta Indica In India, neem is known as "the village pharmacy" because of its healing versatility, and it has been used in Ayurvedic medicine for more than 4,000 years due to its medicinal properties. Neem is also called 'arista' in Sanskrit- a word that means 'perfect, complete and imperishable'.

Ocimum sanctum- Known as "tulsi" or "Holy basil" is small Ocimum sanctum also commonly branched, & aromatic herb. It has height of about 70 cm. All the parts of have been widely used since thousands of years for various diseases & disorders with wide application in Ayurveda system of medicine. It has many properties like antioxidant, hepatoprotective, anti-inflammatory, antibacterial, antifungal with a wide margin of safety. Rubia cordifolia Rubia Cordifolia is a climbing herb, with red rhizomatous base & roots. It is an essential raw drug for the traditional herbal formulations. It is member of Rubiaceae family, the plant is commonly known as "Indian madder" and Sold under the trade name "manjistha". Rubia cordifolia extracts from the various parts of the plant are shown to have Several pharmacological benefits including ache & wound treatment.

Key word: Acne vulgaris, eczema, fungicide, Cysts, spiky edges

## Introduction

### 1.1 Herbal Medicine: Global view

World Health Organization (WHO) realized at Alma Ata in 1978 the role of traditional, alternative and complementary systems of medicine in the healthcare sectors of both developing and the developed nations with the slogan of "Health for All". Later, this issue has been globally addressed by the Traditional Medicine Program of WHO on several perspectives ranging from cultivation of herbs, manufacturing, dispensing, to preparation of guidelines for common masses in TM.

As per definition, "Traditional medicine (TM) is the knowledge, skills and practices of holistic healthcare, recognized and accepted for its role in the maintenance of health and the treatment of diseases. It is based on indigenous theories, beliefs and experiences that are passed on from generation to generation".

Category 1: Indigenous herbal medicines

Category 2: Herbal medicines in systems

Category 3: Modified herbal medicines

Category 4: Imported products with a herbal medicine base

### Challenges

Countries face major challenges in the development and implementation of the regulation of traditional, complementary/alternative and herbal medicines. These challenges are related to regulatory status, assessment of safety and efficacy, quality control, safety monitoring and lack of knowledge about TM/CAM within national drug regulatory authorities.

### Acne Vulgaris

The word **acne** comes from the word *acme* meaning "the highest point", which comes from the Greek *akme* meaning "point" or "spot" - it was originally misspelled, with an 'n' rather than an 'm' in 1835. Acne, medically known as *Acne Vulgaris*, is a skin disease that involves the oil glands at the base of hair follicles. It commonly occurs during puberty when the sebaceous (oil) glands come to life - the glands are stimulated by male hormones produced by the adrenal glands of both males and females. Pimples form when dead skin cells mix with excess oil (sebum). This mixture plugs the pore, causing swelling. Bacteria can grow in the mix and lead to infection and pus.

The main factors in pathogenesis of acne are:

1. Increased sebum production which is androgen dependent
2. Hyper keratinization of follicular openings producing blockage
3. Growth of the bacteria *Corynebacterium acnes* and *Staphylococci*.
4. Acne is responsible for splitting fats in sebum to form irritant fatty acids.
5. Increased sensitivity of the skin to androgens

### Types of pimples

Whiteheads - Remain under the skin and are very small.

Blackheads - Clearly visible, they are black and appear on the surface of the skin.

Papules - Visible on the surface of the skin. They are small bumps, usually pink.

Pustules - Clearly visible on the surface of the skin. They are red at their base and have pus at the top.

Nobules - Clearly visible on the surface of the skin. They are large, solid pimples. They are painful and are embedded deep in the skin.

Cysts - Clearly visible on the surface of the skin. They are painful, and are filled with pus. Cysts can easily cause scars.

### Treating mild acne

The majority of people who get acne will develop mild acne. Most acne products may contain the following active ingredients:

**Resorcinol** - Helps break down blackheads and whiteheads. It is a crystalline phenol and comes from various resins. Resorcinol is also used for treating dandruff, eczema and psoriasis.

**Benzoyl Peroxide** - Kills bacteria and slows down your glands' production of oil. Benzoyl peroxide is white crystalline peroxide used in bleaching (flour or oils or fats) and as a catalyst for free radical reactions. It works as a peeling agent, accelerating skin turnover and clearing pores, which in turn reduces the bacterial count in the affected area.

**Salicylic Acid** - helps break down blackheads and whiteheads, also reduces shedding of cells which line the follicles of the oil glands, effective in treating inflammation and swelling. Salicylic acid is a white crystalline substance which is also used as a fungicide, or in making aspirin or dyes or perfumes.

### Material and method:

Following plants were selected for development of antiacne formulation.

- Aloe vera
- Curcuma longa
- Coriandrum sativum
- Azadirachta indica
- Ocimum sanctum
- Rubia cordifolia

### ALOE VERA

The word 'Aloe' comes from the Arabic word *alloe*, which means 'a bitter, shiny substance.' This is an apt description for the dagger-shaped leaf, which has a bitter taste and spiky edges.



**Aloe vera**

### CURCUMA LONGA

The Genus Name is derived from the Arab word 'kurkum'. Most likely it found its way to the Occident with the caravans of Arab traders. Its Sanskrit name is 'haridra', which means 'yellow wood'.



**Curcuma longa**

## **CORIANDRUM SATIVUM**

The name coriander comes from the Greek word *koris*, meaning "bug." It is likely named for the offensive bug-like smell coriander has when it is unripe.



**Coriander**

## **AZADIRACHTA INDICA**

In India, neem is known as “the village pharmacy” because of its healing versatility, and it has been used in Ayurvedic medicine for more than 4,000 years due to its medicinal properties. Neem is also called ‘*arista*’ in Sanskrit- a word that means ‘perfect, complete and imperishable’.



**Neem**



**Ocimum Sanctum**



**Rubia Cordifolia**

## **PREPARATION OF MATERIAL FOR THE STUDY**

The fresh drugs were collected from the Pune region in the month of June 2012. They were correctly identified by pharmacognosy department MAEER's Maharashtra Institute of Pharmacy, Pune as Aloe vera, Ocimum sanctum, Azadirachta indica, Rubia cordifolia, Coriandrum sativum, Curcuma longa.

## **EXTRACTION OF CRUDE DRUGS**

Hydro alcoholic extract of Ocimum sanctum, Rubia cordifolia and Curcuma longa and aqueous extraction of Azadirachta indica, Coriandrum sativum were carried out by simple maceration process. The weighed quantity of the crude drug (1000g) was mixed with the distilled water / ethanol and set for maceration at room temperature for 72 hr. It was strained, filtered and concentrated to dryness. Aloe vera concentrate gel powder was used.

## **PHARMACOGNOSTICAL STUDY**

### **Morphological study**

Samples were studied exomorphically by using simple microscope (dissecting microscope with 5x and 10x eye piece)

### **Microscopical study**

Transverse sections of each vegetable product were studied by using different staining reagents like phloroglucinol and HCl (1:1), sudan red-III, ruthenium red, alcoholic picric acid, iodine solution etc. to get the knowledge of the cells and tissue present, their arrangement and cell wall contents.

### Proximate analytical work

1. Loss on drying
2. Ash values
3. Total ash
4. Acid insoluble ash
5. Water soluble ash
6. Extractive values
7. Alcohol soluble extractives
8. Water soluble extractive

## CHEMICAL STUDY

### Preliminary Phytochemical Screening

Extracts were subjected for phytochemical screening. Test for Alkaloids, tannins, flavonoids, saponins, sterols, amino acid, reducing sugar, anthraquinone glycoside, cardiac glycosides.

### Development of Gel

Along with extracts and aloe gel following ingredients was used for development of gel.

- Methyl paraben
- Propyl paraben
- Carbapol 934 and 940
- Propylene glycol 200
- Polyethylene glycol
- Sodium hydroxide 10%

### Procedure

*Aloe vera* concentrate gel powder and Carbapol 934 were dissolved in sufficient quantity of water and kept overnight. To this sodium hydroxide was added to form a gel. *Aloe vera* gel and Carbapol 934 gel were mixed together with vigorous stirring and kept in a beaker. The beaker was kept on a water bath and the temperature was allowed to reach above 50°C. To this mixture the weighed quantities of extracts of *Coriandrum sativum*, *Ocimum sanctum*, *Azadirachta indica*, *Rubia cordifolia* and *Curcuma longa* were added. At the same time in another beaker weighed quantities of methyl paraben and propyl paraben were added in water and heated to dissolve. In another beaker weighed quantities of propylene glycol and polyethylene glycol were taken. Thus the mixtures obtained were finally mixed to obtain a gel. Then remaining quantity of purified water was added and PH was adjusted with 10% sodium hydroxide solution.

### Evaluation of gel

1. Measurement of pH
2. Viscosity
3. Spreadability
4. Extrudability study
5. Stability

## EXPERIMENTAL RESULTS

### PHARMACOGNOSTICAL STUDY

#### Morphological study

##### A) Aloe vera

###### General appearance:

Fibrous and woody stem. It has long thick and brittle leaves with thorns both edges grow in a spiral rosette around a stem. They have short, thin prickles along the sides of the leaves.

**Colour:** Light green to yellowish

**Odour:** Characteristic

**Taste:** Faint

**Shape:** Slightly curve

**Size:** Length: 30 to 70 cm and width: 5 to 9 cm

##### B) Curcuma longa

**General appearance:** Rhizomes are rough and similar to ginger

**Colour:** Yellowish

**Odour:** Characteristic

**Taste:** Characteristic

**Shape:** Irregular and straight

**Size:** Length: 3 to 9 cm and width: 2 to 5 cm

##### C) Coriander sativum

**Colour:** Light green to light yellowish

**Odour:** Aromatic

**Taste:** Sweet and characteristic

**Shape:** Oblong to ovate

**Size:** Length: 1 to 2 cm

##### D) Azadirachta indica

**Colour:** Green to light brown

**Odour:** Characteristic and unpleasant

**Taste:** Bitter

**Shape:** Oblique lanceolate

**Size:** Length: 4 to 5 cm and width: 1 to 1.3 cm

##### E) Ocimum sanctum

**Colour:** Light green

**Odour:** Aromatic

**Taste:** Sweet and characteristic

**Shape:** Eliptic oblong

**Size:** Length: 1 to 3 cm and width: 0.5 to 1 cm

##### F) Rubia cordifolia

**Colour:** Green

**Odour:** Characteristic

**Taste:** Faint  
**Shape:** Heart shape  
**Size:** Length: 5 to 10 cm and width: 3 to 6 cm

## Microscopical study

### A) *Aloe vera*

Outer layer is rind which is epidermis. Sap is present in the pericyclic tubules. Then mucilage layer is present and below which layer of parenchymatous cells are present.

### B) *Curcuma longa*

Transverse section of rhizome shows epidermis with thick-walled, cubical cells of various dimensions, cortex characterised by the presence of mostly thin-walled rounded parenchyma cells scattered collateral vascular bundles, a few layers of cork developed under epidermis and scattered oleo-resin cells with brownish contents; cork generally composed of 4-6 layers of thin-walled, brick-shaped parenchyma, cells of ground tissue contain starch grains of 4-15  $\mu$  in diameter, oil cell with suberized walls containing either orange-yellow globules of volatile oil or amorphous resinous matter, vessels mainly spirally thickened, a few reticulate and annular.

### C) *Coriander sativum*

Single layer upper epidermis and lower epidermis is present. Leaf is dorsiventral. Only below upper epidermis palisade cells are present which are single layered. Vascular bundle is located centrally in midrib. Volatile oil secreting cells are present in the lamina

### D) *Azadirachta indica*

Upper and lower epidermis is singled layer and covered with cuticle. Midrib showed presence of 4 to 5 layer of collenchymatous cells. Vascular bundle is 3 in number. One near lower epidermis and two vascular bundles are present near upper epidermis. Leaf is dorsiventral in nature. Paliside cells are single layered

### E) *Ocimum sanctum*

Consisting of single layered epidermis composed of thin walled, oval cells having a number of covering and glandular trachomes vascular bundles situated centrally. Single layers of pallaside cells which indicate that leaves are of dorsiventral nature. Both anomocytic and diacytic types of stomata present on both surface, slightly raised above the level of epidermis

### F) *Rubia cordifolia*

Single layered epidermis, covered with cuticle and possesses covering trichomes. Palisade cells are single layered and compactly packed and the spongy cells are multilayered and loosely arranged. In the lower portion of the midrib 2-4 layers of collenchymatous cells are present. Vascular bundles are conjoint, collateral and closed



Sr.No	Parameter	Results (%w/w)					
		<i>Aloe vera</i>	<i>Curcuma longa</i>	<i>Coriander sativum</i>	<i>Azadirachta indica</i>	<i>Ocimum sanctum</i>	<i>Rubia cordifolia</i>
1.	Loss on drying	80-90	5-6	0.9-3	5-6	16-17	13-15
2.	Total Ash value	1-2	4.5-5.6	14-15	7-8	15-16	4-6
3.	Acid Insoluble ash value	0.2-0.6	1.3-1.6	3-5	0.9-1.3	17-18	0.4-0.7
4.	Water soluble ash value	0.8-1.0	0.5-0.7	7-9	2-3	46-48	0.8-1
5.	Water soluble extractive value	30-50	1-2	1.5-1.8	12-14	7-8	18-23
6.	Alcohol soluble extractive values	30-55	10-25	1.9-2.5	6-7	4-5	92-93
7.	Crude fibers	20-35	2-4	4-7	2-4	2-3	3-5

### Proximate analytical work

Research Through Innovation

**Chemical Study****Preliminary Phytochemical Screening: Extracts were subjected for phytochemical screening.****Phytochemical screening of extracts and mashi of unripe coconut husk**

<b>Chemical Test</b>	<b><i>Aloe vera</i></b>	<b><i>Curcuma longa</i></b>	<b><i>Coriander sativum</i></b>	<b><i>Azadirachta indica</i></b>	<b><i>Ocimum sanctum</i></b>	<b><i>Rubia cordifolia</i></b>
<b>Alkaloids</b>	-ve	+ve	-ve	+ve	+ve	+ve
<b>Tannins</b>	-ve	+ve	+ve	+ve	+ve	+ve
<b>Flavonoids</b>	-ve	+ve	+ve	+ve	+ve	+ve
<b>Saponin</b>	-ve	-ve	-ve	-ve	+ve	-ve
<b>Sterols</b>	+ve	+ve	+ve	-ve	-ve	-ve
<b>Amino acids</b>	-ve	-ve	-ve	-ve	-ve	-ve

<i>Chemical Test</i>	<i>Aloe vera</i>	<i>Curcuma longa</i>	<i>Coriander sativum</i>	<i>Azadirachta indica</i>	<i>Ocimum sanctum</i>	<i>Rubia cordifolia</i>
<b>Alkaloids</b>	-ve	+ve	-ve	+ve	+ve	+ve
<b>Tannins</b>	-ve	+ve	+ve	+ve	+ve	+ve
<b>Flavonoids</b>	-ve	+ve	+ve	+ve	+ve	+ve
<b>Saponin</b>	-ve	-ve	-ve	-ve	+ve	-ve
<b>Sterols</b>	+ve	+ve	+ve	- ve	- ve	- ve
<b>Amino acids</b>	- ve	- ve	- ve	- ve	- ve	- ve

- ve: indicates Absent and + ve : indicates Present

#### DEVELOPMENT OF GEL:

Five different formulations were prepared with varying concentration of carbopol 934 and 940. pH was in the range of 6.8 to 7.1. Colour of all formulations was yellowish green and all formulations were found stable. Extrudability of formulation 1, 2 and 4 were excellent. Formulation 4 was found optimum in terms of gel consistency, spreadability and extrudability. Hence this formulation was given to the volunteers (willingly to use this gel).

Analysis of the data obtained from the volunteers who had used the gel showed that more than 85% of the volunteers were satisfied with the consistency, spreadability, colour, odour of the gel.

#### CONCLUSION

Acne vulgaris is an extremely common skin disorder that affects areas containing the largest oil glands, including the face, back, and trunk. Although not a serious threat to general health, acne is one of the most socially distressing skin conditions, especially for adolescents, who must deal with a disfiguring disease that erupts just when sexual maturity makes them most sensitive about their appearance. Moreover, severe acne can lead to permanent scarring of the skin that carries the social distress throughout adulthood. For many years, antibiotics have been used to treat acne vulgaris. However, antibiotic resistance has been increasing in prevalence within the

dermatologic setting. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases.

- The present study has thrown light on Pharmacognostical aspects of *Ocimum sanctum*, *Azadirachta indica*, *Rubia cordifolia*, *Aloe vera*, *Curcuma longa* and *Coriandrum sativum*.
- Coriander sativum is very good antibacterial which has been used to developed the gel formulation.
- Very few herbal formulations available in marked and they are very costly. We can develop effect formulation with the use of drugs which are easily and abundantly available in the nature.
- Formulation 4 showed good spreadability and consistency.
- Volunteers like the formulation and more that 80% of volunteers were satisfied with the formulation.

## REFERENCES

1. Chaudhary A, Singh N. Contribution of world health organization in the global acceptance of Ayurveda. *J Ayurveda Integr Med.* 2011 Oct-Dec; 2(4): 179–186.
2. Jagtenberg T, Evans S. Global Herbal Medicine: A Critique. *The journal of alternative and complementary medicine.* Volume 9, Number 2, 2003, pp. 321–329
3. Report of a WHO global survey. National policy on traditional medicine and regulation of herbal medicines. World Health Organization, Geneva, May 2005.
4. Guidelines for the regulation of herbal medicines in the South-East Asia Region, World Health Organization Regional Office for South-East Asia; New Delhi, 2003, p. 02.
5. Sharma A, C. Shanker, Tyagi L. Mahendra Singh, Ch. V. Rao. Herbal Medicine for Market Potential in India: An Overview. *Academic Journal of Plant Sciences* 1(2), 2008, pp 26-36
6. <http://www.medicalnewstoday.com/articles/107146.php>.
7. <http://www.webmd.com/skin-problems-and-treatments/how-pimples-form>
8. Satoskar R., Bhandarkar D., Ainapurkar S. *Pharmacology and pharmacotherapeutics.* 14<sup>th</sup> Ed. Popular prakashan Bombay, pp 757.
9. <http://catalogs.indiamart.com/products/anti-acne-cream.html>
10. Hanieh Azimi, Mehrnaz Fallah-Tafti, Ali Asghar Khakshur, Mohammad Abdollahi. A review of phytotherapy of acne vulgaris: Perspective of new pharmacological treatments. *Fitoterapia* 83 ,2012, pp 1306–1317.
11. Jain A, Basal E. Inhibition of Propionibacterium acnes-induced mediators of inflammation by Indian herbs. *Phytomedicine* 2003,10 pp:34–38.
12. Roopashree TS, Raman D, Shobha Rani RH, Narendra C. Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. *Int J Appl Res Nat Prod* 2008,1, pp 20–28.
13. Takahashi T, Kokubo R, Sakaino M. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from Eucalyptus maculata. *Lett Appl Microbiol* 2004,39 pp 60–64.
14. Nam C, Kim S, Sim Y, Chang I. Anti-acne effects of Oriental herb extracts: a novel screening method to select anti-acne agents. *Skin Pharmacol Appl Skin Physiol* 2003, 16 pp 84–90.
15. Viyoch J, Pisutthanan N, Faikreua A, Nupangta K, Wangtorpol K, Ngokkuen J. Evaluation of in vitro antimicrobial activity of Thai basil oils and their micro-emulsion formulas against Propionibacterium acnes. *Int J Cosmet Sci* 2006, 28 pp 125–133.
16. Mishra A, Kumar S, Bhargava A, Sharma B, Pandey AK. Studies on in vitro antioxidant and antistaphylococcal activities of some important medicinal plants. *Cell Mol Biol (Noisy-le-Grand)* 2011, 57 pp16–25.
17. Tsung-Hsein et al. In vitro antimicrobial and anti-inflammatory effects of herbs against Propionibacterium acnes. *Food Chemistry*, 2010,119 pp 964–968.
18. Jain A, Bansal E, Inhibition of *Propionibacterium acnes*-induced mediators of inflammation by Indian herbs. *Phytomedicine*, 2003,10, pp 34-38.

19. Pandey Chetana et al. Screening of selected Herbal plants for Anti Acne Properties. International Journal of Drug Development & Research, 2012 4(2) pp 216-222.
20. Yolanta saks, rivaka Barkani Golan. Aloe Vera gel activity against plant pathogenic fungi. Postharvest Biology and technology, 1995, 6, pp 159-165.
21. Jayapal S et al. Mosquito larvicidal activity of Aloe vera (Family: Liliaceae) leaf extract and Bacillus sphaericus, against Chikungunya vector, Aedes aegypti. Saudi Journal of Biological Sciences, 2012, 19, pp 503–509.
22. Eriko Misawa et al. Administration of phytosterols isolated from Aloe vera gel reduce visceral fat mass and improve hyperglycemia in Zucker diabetic fatty (ZDF) rats, Obesity Research & Clinical Practice, 2008, 2, pp 239—245.
23. Inder Sehgal et al. An in vitro and in vivo toxicologic evaluation of a stabilized aloe vera gel supplement drink in mice. Food and Chemical Toxicology 2013, 55, pp 363–370.
24. Qiuhui Hu et al. Free radical-scavenging activity of Aloe vera (Aloe barbadensis Miller) extracts by supercritical carbon dioxide extraction. Food Chemistry, 2005, 91, pp 85–90.
25. Saka W.A et al. Changes in Serum Electrolytes, Urea, and Creatinine in Aloe Vera-treated Rats. Journal of Young Pharmacists 4(2), pp 78-79.
26. Akram et al. Curcuma longa and curcumin: a review article. Rom. J. Biol. – Plant biol., 2010, 55, 2, pp. 65–70.
27. <http://www.sharecare.com/question/what-is-coriander>.
28. Begnami A.F et al. Antimicrobial potential of Coriandrum sativum L. against different Candida species in vitro. Food Chemistry, 2010, 118, pp 74–77
29. Filomena S et al. Antifungal activity of Coriandrum sativum essential oil, its mode of action against Candida species and potential synergism with amphotericin B. Phytomedicine, 2011, 19, pp 42-47.
30. Patel D et al. Cardio protective effect of Coriandrum sativum L. on isoproterenol induced myocardial necrosis in rats. Food and Chemical Toxicology, 2012, 50 pp. 3120–3125.
31. Matasyoh J C. et al. Chemical composition and antimicrobial activity of the essential oil of Coriandrum sativum Food Chemistry, 2009, 113, pp 526-529.
32. Eguale T. et al In vitro and in vivo anthelmintic activity of crude extracts of Coriandrum sativum against Haemonchus contortus. Journal of Ethnopharmacology, 2007, 110, pp. 428–433.
33. <http://www.pharmainfo.net/reviews/neem-tree-solving-global-problems>.
34. Pattanayak P. et al. Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev. 2010, 4(7), pp 95–105.
35. Divakar K. et al. Protective effect of the hydro-alcoholic extract of Rubia cordifolia roots against ethylene glycol induced urolithiasis in rats. Food and Chemical Toxicology, 2010, 48 pp 1013–1018.
36. Guntupalli M. et al. Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. Journal of Ethnopharmacology, 2006, 103, pp 484–490.
37. Amrita et al. A review on anti-acne potential of medicinal plant extracts against ropionibacterium acnes. Int J Pharm Bio Sci, 2012, 3(3), pp 987 – 997.
38. Anonymous, Pharmacopoeia of India. Government of India- Ministry of Health and family welfare, 1996, Vol-II, A34-35, A-45, A-55, A72-77, 89, 100-127.
39. Anonymous, Quality controls methods for medicinal plant materials. World Health Organization, Geneva, pp 8-34.
40. Harborne J. B., Phytochemical methods a guide to modern techniques of plant analysis. 2<sup>nd</sup> Ed. Chapman and Hall publication, London, 1973, pp 55-72
41. Khandelwal K.R., Practical Pharmacognosy. 8<sup>th</sup> Ed., Nirali prakashan, Pune, 2000, pp 149-159.
42. <http://www.pharmainfo.net/reviews/topical-gel-review>