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FORMULATION AND EVALUATION OF PHYTO-HYDROGEL CONTAINING *AZADIRACHTA INDICA* AND *TRIDAX PROCUMBENS* FOR THE TREATMENT OF SEBORRHEIC DERMATITIS

[1]Shweta Soni, [2]Niharika Thakur, [3]Rajesh Singh Pawar, [4]Prem Samundre, [5]Sarika Shrivastava

[1]Truba Institute of Pharmacy, RGPV University, Bhopal, MP 4262037, India. Email ID: shewon170921@gmail.com

[2]Truba Institute of Pharmacy, RGPV University, Bhopal, MP 4262037, India. Email ID: niharika87thakur@gmail.com

[3]Truba Institute of Pharmacy, RGPV University, Bhopal, MP 4262037, India. Email ID: drrajesh.pawar@trubainstitute.ac.in

[4] Sagar Institute of Research & Technology- Pharmacy, Sage University Bhopal, M.P, 462022, India. Email ID: premsamundre786@gmail.com

[5] Sagar Institute of Research & Technology- Pharmacy, Sage University Bhopal, M.P, 462022, India. Email ID: sarika.kanaha@gmail.com

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Abstract:

The aim of the present study was to evaluate the hydrogel containing the extract of azadirachta indica and tridax procumbens for the treatment of seborrheic dermatitis and to check the efficacy of both extracts against malassezia furfur as compare to other standards available in the market. The selection of tridax procumbens (hair growth stimulator, antifungal, anti-oxidant, anti-inflammatory) and azadirachta indica (insecticidal, antibacterial, antifungal, anti-inflammatory) was based on the properties of both the plant extracts and these extracts were loaded in a Carbopol to obtained the gel consistency with other ingredients. The combination of the plant extracts (azadirachta indica and tridax procumbens) was selected to enhance the efficacy of the hydrogel. As the scalp needs an adequate amount of moisture & smoothness to get rid of seborrheic dermatitis with dry & itchy skin, hydrogels would be the better choice with their unique properties of high-water content, softness, flexibility and ease of application as compare to other marketed formulation. The prepared phyto - hydrogel have passed all the test including skin irritation test, stability test & many other tests with the hope of good future prospect, investigations and other herbal marketed formulations.

Corresponding author:

Shweta Soni,

Truba Institute of Pharmacy,
RGPV University, Bhopal, MP 4262037,
India. Email ID: shewon170921@gmail.com

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INTRODUCTION:

1. Topical drug delivery system – Topical drug delivery system is a system deals with numerous medications, prescription, prevention and pre-defined prospect of wellness, care, hygiene and beauty of skin for both human and animals. It is also a preventive measure to mucous membrane. It is used in plants to overcome with the problems of insects, fungi, bacteria and to promote production.

Topical dosage forms - Topical dosage forms are available in solid, liquid and semisolid forms which are covered, enclosed or packed by different types of packing or suitable containers.

Formulation Example: Creams, lotion, gel, ointment, spray etc.

Topical route of drug administration - Any formulation which avoids the passage of first pass metabolism and gastrointestinal tract is known as topical route of administration and the specific routes are: oral, nasal, vaginal, anorectal, transdermal, auricular and ocular respectively [26].

- **Advantages of topical drug delivery system –**
  1. Riddance of gastrointestinal absorption and pre-systemic effect.
  2. Availability & intractability of drug formulations in different forms (solid, liquid, semisolid, gas etc.)
  3. Simple and convenient way of usage.
  4. More choices of formulation selection according to person's demand and as compare to other drug delivery systems.
  5. Washable and removable medicament for skin.
  6. Better acceptance with different fragrance, flavor, smooth texture & appearance as compared to bitter and common oral solid dosage forms.
  7. Effectivity in low doses at a specific site.
  8. Vast area of application and utility in daily household demands along with medical products.

- **Disadvantages of topical drug delivery system –**

1. Irritation, itching, allergic reaction will occur in any unsuitability on particular site.
2. Drugs having poor permeability & larger particle size are difficult to penetrate.
3. Requirement of low plasma concentration of drug.
4. Enzymes present in epidermis may denature

1.1 Skin – The skin is a protective, preventive, well organized and largest sense organ or cover which encloses all the body and its parts. It consists of epidermis, dermis and hypodermis layer to provide strength stability and flexibility to protect us from outer environment. There are two types of skin, hairy skin and glabrous skin (ex: palm and sole). whole skin surface is hairy but, on the scalp, there are so many hairs[16].

1.2 Scalp – Scalp is a part of the head where the hair grows with their different type, size or length. It consists of five layers: the skin, connective tissue, epicranial aponeurosis, loose areolar tissue, and pericranium. The skin contains several hair follicles and sebaceous glands (secrete sebum or natural oil that lubricates the scalp). Connective tissue is a dense subcutaneous layer of fat & fibrous tissue. Epicranial Aponeurosis keeps the frontalis muscle & occipitalis muscle connected and also control the action of scalp movement in forward & backward direction. Loose Areolar connective tissue acts as a flexible plane separating the three other layers from the pericranium. This layer is considered as a ''danger zone'' because when infected, it can spread easily in whole tissue & can pass on the underlying pericranium. [10]

1.3 Hair – hair is a thin, smooth and adjustable, protein filament which grows leisurely and separately. The hair papilla cells are mesenchymal cells which contain nerve endings & blood capillaries in dermal area and are the base of a hair shaft. These dermal cells are important in hair origination and growth. Another part of the hair is hair bulb which is above the hair papilla & below hair follicles. In Hair bulb living cells will divide and grow to develop the hair shaft. In the epidermal area, Hair follicles determines the shape, colour and texture of different types of hair. The follicle shape may also vary (circular, oval, over flat) from person to person having curly, straight, wavy, kinky hairs etc. Hair follicles store a colour pigment (melanin) which is of two types eumelanin (gives black colour in more amount and brownish colour in little amount) and pheomelanin (gives red type of colour). Follicles will die or damaged with age and several other conditions and become white or greyish.[4]

1.3.1 Significance of Hairs –

1. Hairs are the most enticing, observable, tricky and crucial part on the head which are necessary to reflect our look, personality, gender and manner.

2. By different hair styles, hair-cut and hair colours, one can show their trends, simplicity, modernity and a glimpse of professional livelihood.

3. Way of arranging the hairs will impact an impression to all viewers.

- **Hair products –** Products used to provide care, nutrition/nourishment, lustre, volume and smoothness to our hairs are known as hair products. These products are used for hair problems (hair fall, split ends) and as well as for scalp problems (Dandruff, ringworms, scalp acne etc.)

- **Herbal products for hair –** Products which are natural & free from chemicals are known as herbal products. Herbal hair products can be prepared by different types of herbs, shrubs, plants etc. [27].

- **Advantages of herbal hair products–**

1. Herbal hair products are easily available products.
2. These natural products contain natural ingredients which are essential for proper care and growth of hairs.
3. These herbal products can be prepared at home also.

- **Disadvantages of herbal hair products–**

1. Ingredients suitability is not same and may vary from person to person.
2. Side effects and adverse effect may possible.

- **Other application of herbal products –** Herbal products are used in the form of tablets, capsules, powders, gels, creams and liquids in Ayurvedic, homeopathic, and allopathic medicines for the treatment of arthritis, malaria, diabetes, cancer, heart disorders, mental illness, physical dysfunction, digestive problems, chronic inflammation, headache, skin reactions/infections and in so many conditions. However, research is an important part in all types of medicines and for several rare and new diseases.
- Hair accessories – These products are used to enhance the beauty of hairs or to design particular hair style. Numerous numbers of hair products are available in market for purchasing. Ex: hair straighteners, hair extension, hair clips, hair bands, hair ribbons / ties, hair stickers, hair buns etc.

Table 1: Different types of marketed topical products for hairs.

S.no.	Marketed topical products for hairs	Brand name
1.	Hair shampoo	L'OREAL PARIS
2.	Hair oil	Hair Best
3.	Hair serum	Livon
4.	Hair tonic	innisfree
5.	Hair cream	YARDLEY LONDON
6.	Hair conditioner	Tresemme
7.	Hair spray	Nova Gold
8.	Hair dye	Garnier
9.	Hair mehndi	Black gold
10.	Hair spa	Zuke
11.	Hair perfume	in the STARS
12.	Hair moisturizer	PANTENE
13.	Hair cleanser	Triclenz
14.	Hair wax	Set Wet
15.	Hair gel	Balmain Paris
16.	Hair powder	NEWSHA

17.	Hair balm	Paul Penders
18.	Hair lotion	qilib
19.	Hair solution	RENOCIA
20.	Hair care drops	Bajaj
21.	Hair vitalizer	USTRAA
22.	Hair thickening fibres	Berkowits
23.	Hair volumizer	BBlunt
24.	Hair concealer	The Cosmetic Republic
25.	Hair tincture	Folifast
26.	Hair soap	Scaberin
27.	Hair mask	Re'equil
28.	Hair highlighter	Streax
29.	Hair illuminator	IT
30.	Hair rejuvenator	Hair Gia

Hair products can be formulated in different combinations, variety of ingredients /mixture, texture, applicability and their natural/synthetic origins. At a glance these products are reviewed, purchased and identified by their key ingredients or usage. But sometimes all ingredients are not exposed by manufacturer and addition of substitute material or other harmful additives is a common problem in a hidden way. Adulteration is also a problematic issue. Other than topical products, there are so many oral solid dosage forms like tablets, capsules, pills and liquid dosage forms like multivitamin syrups are available for hair growth. Different types of protein- powders, gummies, dietary supplements with vitamins, biotin, iron, zinc are also available.

Table 2: Herbal hair topical products with their herbal ingredients [28] [13].

S.no.	**Herbal topical products for hairs**	**Herbal ingredients**	**Brand name**
1.	Hair shampoo	Amla, Shikakai, Reetha	Ayur
2.	Hair oil	Bhringraj, Brahmi, Til, Dina, Mallika, Madaynti, Jatamansi, Gandha, Neem, Kanyasara	Tru hair
3.	Hair serum	Hibiscus, Flax seed oil, Waterlily	Moha
4.	Hair tonic	Henna, Hibiscus, Wedella, Phyllanthus, Aloe, Fenugreek	Tampcol
5.	Hair cream	Amla, Argan oil	INATUR
6.	Hair conditioner	Rosemary,Mint, Sandalwood	Herbal Essences
7.	Hair spray	Flax seeds, Rice water	Ktein
8.	Hair dye	Ginger extract	Mokeru
9.	Hair mehndi	Indigo, Henna, Amla, Bhringraj, Brahmi, Neem,Shikakai	Atulya Khadi
10.	Hair spa	Rosemary, Almond, Coconut, Methi, Wheat gram, Neem, Basil, Lavender	Indus Valley
11.	Hair perfume	Lemongrass/Sandal/ Rose/Jasmine/Lavender	Himgiri
12.	Hair moisturizer	Aloe Vera	Khadi Natural
13.	Hair cleanser	Shikakai	Patanjali
14.	Hair wax	Sunflower	Rawnature
15.	Hair gel	Aloe Vera, Hibiscus	V Herbz

16.	Hair powder	Methi	Guduchi
17.	Hair balm	Rosemary, Lavender, Lemon, Castor oil, walnutoil	Aerth Naturals
18.	Hair lotion	Amla, Bhringraj, Henna, Jatamansi, Brahmi	Nutriley
19.	Hair solution	Korphad, Neem, Amla, Bhringraj, Brahmi	Charvi
20.	Hair/scalp care drops	Cinchona officinalis,Arnica montana, Jaborandi	GERMAN‟S
21.	Hair vitalizer	Argan oil	Full Grow
22.	Hair thickening fibres	Natural celluloid fibres, Henna,Silk amino	Kera Gain
23.	Hair volumizer	Javasum, Indian gooseberry, Castor oil, Methi, Vetiver oil, Wheat gram, Coconut oil, Hibiscus, Shikakai,	Just Herbs
24.	Hair touch up	Natural oils	Shahnaz Husain
25.	Hair tincture	Thuja occidentalis	SBL
26.	Hair soap	Shikakai, Multani mitti	Earthy Sapo
27.	Hair mask	Bhringraj, Amla, Shikakai	mama earth
28.	Hair highlighter	Natural colours, Bees wax	LiLiUM
29.	Hair illuminator	Pine bark, Rosemary Soya protein,	The Earth
30.	Hair rejuvenator	Bhringraj, Amla, Brahmi, Hibiscus, Liquorice	VedantiKA

Table 3: Medications available for scalp problems (Seborrheic dermatitis). [5]

S.no.	Medication type	Formulation
1.	Ketoconazole	Shampoo, Gel, Cream, Foam
2.	Clobetasol propionate	Gel, Foam, Shampoo, Lotion, Spray
3.	Fluocinonide	Cream
4.	Triamcinolone acetonide	Cream
5.	Betamethasone dipropionate	Cream, Spray (Aerosol)
6.	Desonide	Ointment, Lotion, Foam
7.	Clobex	Spray, Shampoo, Lotion
8.	Mometasone furoate	Ointment
9.	Desoximetasone	Spray (Non – Aerosol), Ointment
10.	Hydrocortisone	Cream
11.	Halobetasol propionate	Cream, Foam
12.	Topicort	Ointment, Spray (Non-Aerosol)

13.	Prascion	Cleanser
14.	Ciciopirox	Shampoo, Gel, Solution, Cream
15.	Nizoral	Cream, Shampoo
16.	Salicylic acid	Foam, Shampoo, Gel
17.	Dermatop	Ointment, Gel
18.	Rosaderm	Cleanser
19.	Trianex	Ointment
20.	Cloderm	Cream

1.4 Hair problems – There are many hair problems from which more than billions & trillions of people are suffered daily and commonly like hair fall, split ends, greyish & premature white hairs, thin hairs but these common problems are often leads to severe hair disorders (Alopecia areata, Male/Female pattern baldness, Telogen effluvium, Anagen effluvium, Bamboo hair, Folliculitis) and then adversely affect our mental strength as well as our social outlook. [1]

1.5 Scalp problems – Scalp problems are common in every age group of people and may vary in age, condition, and person to person with the spreadibility on the whole skin or particular part of the skin. These problems are caused by **bacterial infections** (Impetigo, Dry scalp) **viral infections** (Shingles) **autoimmune defects** (Discoid Lupus Erythematosus, Scleroderma) **inflammation** (Contact dermatitis, Seborrheic dermatitis, Psoriasis, Lichen planus, skin allergies) **parasites** (Leishmaniosis, Lice) and **fungal infections** (Ringworm, seborrheic dermatitis). [12],[5]

1.5.1 Seborrheic dermatitis – Seborrheic dermatitis is a superficial fungal as well as inflammatory disease of the skin occurring in areas rich in sebaceous glands. It is a condition that causes scaly patches and red skin, mainly on the scalp [2], [18]. It can also occur on other oily areas of the body, such as the face, upper chest, groin, anogenital area, bellybutton, crease of the nose, eyebrows, eyelids, armpitsetc.

Dandruff (Pityriasis capitis) is a non- inflammatory form of seborrheic dermatitis. In dandruff condition, small pieces of dry white or yellowish skin flake off (desquamation) on the scalp which appears clearly and it may affect the whole hairy skin (eyebrows, ears, beard, moustache, chest, armpits and other parts of the body) [21].

Figure 1: Figure shows scalp with seborrheic dermatitis.

1.5.2 Causes of seborrheic dermatitis – The exact cause of seborrheic dermatitis is still unknown but according to research studies, it may cause due to several environmental factors (pollution, Dry cold air), irregular immune response, Stress, genetical factors, certain medications, nutritional deficiency and a lipophilic yeast (Malassezia) which usually lives on our skin[15].

1.5.3 Management – Various types of medicated shampoos, creams, gels, and lotions containing salicylic acid, coal tar, ketoconazole & zinc with other chemical products and some herbal formulations (containing antifungal, anti-inflammatory, antibacterial, moisturizing

properties) are there for the treatment of seborrheic dermatitis [20, 23], [3] (see Table no. 3)

1.6 Hydrogels – Hydrogels are pliant, glossy and glaring topical preparation which are arranged in three- dimensional network or a network of cross-linked polymer chains that are hydrophilic, sometimes found as a colloidal gel in which water is the dispersion medium. These are of swelling properties but do not dissolve in water [17, 24, 25].

- **Advantages of hydrogels–**

1. Hydrogels have good transport properties.
2. These are biocompatible, biodegradable and are

also injectable.

3. These are known by their degree of flexibility like natural tissue.

4. These can be prepared according to the changes in temperature, pH, metabolite concentrations and their release by sensing properties.

➢ **Disadvantages of hydrogels–**

1. Hydrogels are expensive & are of low mechanical strength.
2. These are somewhat difficult in drug loading.

1.6.1 Applications of hydrogels – [6], [22]

- **In oral drug delivery** ——— Drug delivery in the treatment of mouth diseases, stomatitis, fungal, viral infections and oralcavity cancers.
- **In ocular drug delivery** ——— Drug delivery in the treatment of eye infections, discomfort and pain.
- **In nasal drug delivery –** Drug delivery in the treatment of certain allergies, inflammation and other nose infections.
- **In protein drug delivery –** Delivery of interleukins for regulating cell growth and immune responses.
- **In parenteral drug delivery –** Injectable hydrogels for cartilage repairing.
- **In rectal drug delivery –** Drug delivery in the treatment of hemorrhoids and other diseases associated with the rectum.
- **In tissue engineering –** Hydrogels scaffolds are used in the treatment of damaged tissue, injury and disease due to their ability to support cell proliferation, migration and differentiation, to permit oxygen and nutrient transport, and to mimic native soft tissue.
- **In brain drug delivery –** Local delivery of drug to the brain through polysaccharide hydrogels in the treatment of nerve injury & otherbrain disorders.
- **In transdermal drug delivery –** Systemic Drug delivery to the skin for a long duration and at a constant rate in the treatment of variousskin diseases.
- **Drug delivery in G.I.T –** Hydrogel based devices can be designed to deliver drugs locally to specific sites in the GI tract or delivery of some antibiotic for the treatment of H. pylori infection in peptic ulcer another conditions.
- **In gene delivery –** Gene delivery can be used to modify cells through vector to replace defective cells or genes that are causing problems.
- **In topical drug delivery –** Hydrogels are widely used in topical drug delivery system. These are used in antifungal, anti-inflammatory, wound healing & cosmetic applications. Specifically known by their moisturizing properties and smoothness which avoids scaling and dryness and has better patient compliance.
- **Other applications –** Hydrogels are used in diapers, sanitary products, dental products, contact lenses etc.

Materials and Method

1. **Preformulation study –**Preformulation study was based on physical, chemical and medicinal property of the crude drugs.

i) **Herbarium file –** Leaf part of both Tridax procumbens & Azedarach indica was studied with whole description of micro, macro specific characters & scientific classification and was authenticated by Saifia College (Botanical department).

ii) **Solubility test –** Different solvents were used to check the solubility of the crude & powdered drug (Tridax procumbens &Azadirachta indica). About 5mg crude drug was taken in 5 ml of each solvent in Eppendorf tubes.

Table 4: Solubility of crude drugs in different solvents.

S.no.	Solvents	Solubility (for neem)	Solubility (for ghamra)
1.	Water	Sparingly soluble	Practically insoluble
2.	Methanol	Sparingly soluble	Sparingly soluble
3.	Ethanol	Slightly Soluble	Slightly Soluble
4.	Hexane	Practically insoluble	Slightly Soluble

iii) Organoleptic characters – The crude drug was observed by sense in taste, appearance, odour and type of the powderedform.

Table 5: Organoleptic properties of crude drugs.

S.no.	Crude drug	Colour	Odour	Taste	Powdered form
1.	Neem	Green	Strong aroma	Bitter	Coarse
2.	Ghamra	Green	Characteristic	acrid	Coarse

2 Extraction –For extraction, neem and ghamra leaves were picked in the month of October and were washed, soaked properly and were shade dried for one week. After one week, leaves are coarsely crushed in the grinder and stored in air tight containers for extraction and furtheruse.

a) Soxhlet extraction method –Before performing extraction, crushed neem leaves (50gm) were kept in a thimble with some cotton fitted with a round bottom flask. About 250 ml of methanol was poured in a round bottom flask for 6 hours on heating mantle. Then after few hours the condensed extract was passed out from the siphon and dropwise collected in a flask. Full extraction was observed until the colour of the extract becomes yellowish from green and it was completed in 6,7 cycles in the whole process of extraction. For the removal of methanol, rotatory evaporator was used at 40°C and the remaining extract was kept in a petri dish for one week and scrapped off with spatula and stored in a polybag.[19] Same process was applied for the extraction of ghamra leaves.[11]

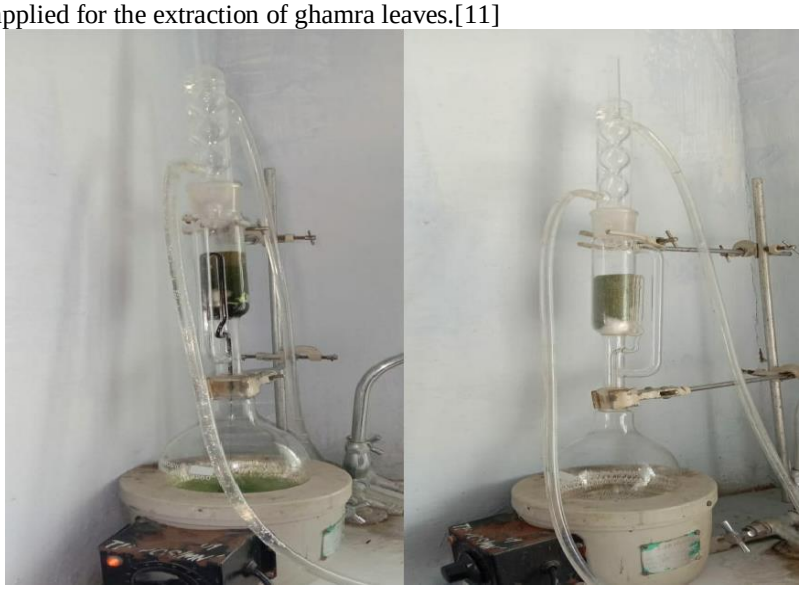


Figure 2: Extraction procedure for ghamra & neem.

2.1 Extraction parameters –

i)TLC – TLC plates were properly washed with methanol and were dried in hot air oven for 10 min. A slurry of 5 % silica gel with distilled water was prepared in a beaker and stirred well carefully. Prepared slurry was poured on activated glass plates and was distributed uniformly. Then all the plates were kept in an oven for 1 hour at 120-degree C.

a) Method –Three proper and uniform plates were taken for TLC chamber. Then sample (neem, ghamra & combination of neem ghamra) was marked above 1cm from the end of the prepared TLC plates with the help of sharp capillary and were placed in a TLC chamber for solvent (methanol) evaporation for 30 minutes. Then for spot visualization, plates were placed in another chamber and few crystals of iodine were added. Pink colour was observed in the whole chamber with fumes. Then measurement of the sample running was noted with the help of measuringscale.

Figure 3: TLC test of neem, ghamra extract & its combination form.

Retention factor (Rf) = Distance moved/ travelled by solute

Distance moved/ travelled by solvent

Table 6: Table showing Rf value of the formulations.

S.no.	Formulation code	Rf value
1.	FN1	4.3/5 = 0.86
2.	FG1	3.7/5 = 0.74
3.	FNG	4.5/5 = 0.9

i) Ash value – The extract (10g) was placed in a small china dish in muffle furnance for 1 hour at 30°C and was carefully kept out with the help of cotton cloth &tong.

Ash value (%) = weight of total ash obtained

weight of crude drug taken

Table 7: Table showing the ash value of the crude drugs.

S.no.	Formulation code	Ash value %
1.	FN1	1.6/10 ×100 = 16%
2.	FG1	1.2/10 × 100 = 12%

iv) Phytochemical test – After obtaining the crude extract from both plants (Azadirachta indica & Tridax procumbens), various tests were performed to check the presence & absence of phytocompounds.

Table 8: Phytochemical test for neem and ghamra extract. [14]

Phytochemical test	Test included	Obs. for neem	Obs. for ghamra
Alkaloid test	Wagner's test	+ ve	+ ve
Amino acid test	Millon's test	_ ve	+ ve
Tannin test	Ferric chloride test	+ ve	+ ve
Flavonoid test	Alkaline reagent test	+ ve	+ ve
Saponin test	Froth test	+ ve	_ ve
Cardiac glycoside test	Baljet's test	_ ve	_ ve

v) HPLC – (High performance liquid chromatography) [14]

a) Reagents and Chemicals – Standard Quercetin was procured from Hi Media Pvt Ltd. Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, New Delhi, India.

b) Instrumentation – A double beam UV-Vis Spectrophotometer model of Labindia 3000+ with 1cm matched quartz cells was used for determination of λ max of standard marker compound "Quercetin" which was utilized for estimation of marker compound in given sample. The HPLC system (Waters India Pvt Ltd) consisted of a pump, a U.V. Visible detector, a Thermo C18 (250 X 4.6 mm, 5µm) column, a Data Acesoftware.

c) Chromatographic condition – The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 ml min–1. A volume of 20 µl was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detector at the wavelength of 256nm.

Table 9: Selection of Separation Variable in HPLC

Variable	Condition
Column	
Dimension	250mm 4.60mm
Particle Size	5µm
Bonded Phase	Octadecylsilane (C18)
Mobile phase	
Acetonitrile	50
Methanol	50
Flow rate	1ml/min
Temperature	Room temperature
Sample size	20µl
Detection wavelength	256nm
Retention time	2.60 ± 0.5 min

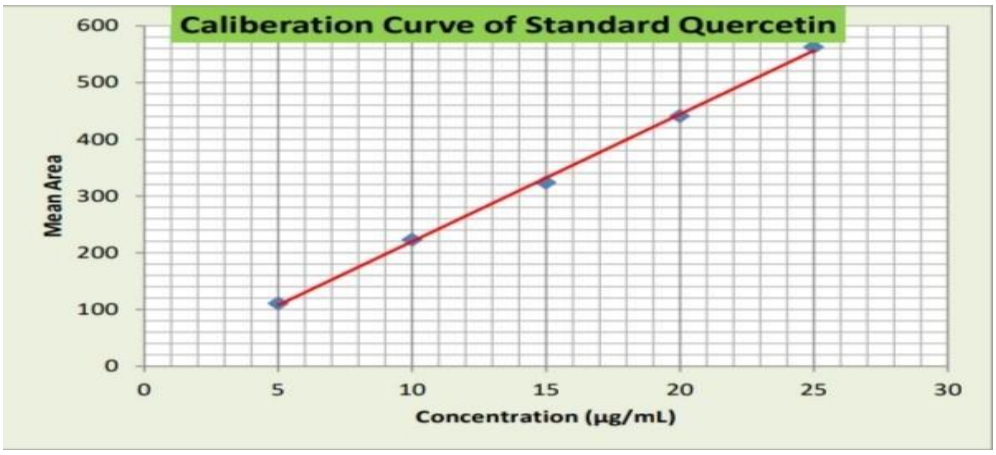
d) Preparation of Standard Stock Solution – 10mg of Quercetin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of1000ppm.

e) Preparation of Working Standard Solution – Standard concentration of marker compound (5, 10, 15, 20, 25µg/ ml) was prepared from stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10 ml volumetric flasks and made upto 10 ml volume with mobile phase.

solution was filtered through Whatman filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was passed through 0.45 µm dissociable nylon syringe filter of using and sonicated for 10min.

f) Identification of Marker Compound (Quercetin) by HPLC – The reverse phase C18 column equilibrated with mobile phase methanol: acetonitrile (50:50 v/v) was used. The filtered and degased mobile phase was maintained at a flow rate 1 ml/min and eluent was monitored at 256 nm. The sample was injected using a 20 µl fixed loop, and the total run time was 10 min. The sample solution was chromatographed and a concentration of Quercetin in extract sample was found out using regression equation.

Graph 1: Calibration curve for standard quercetin.



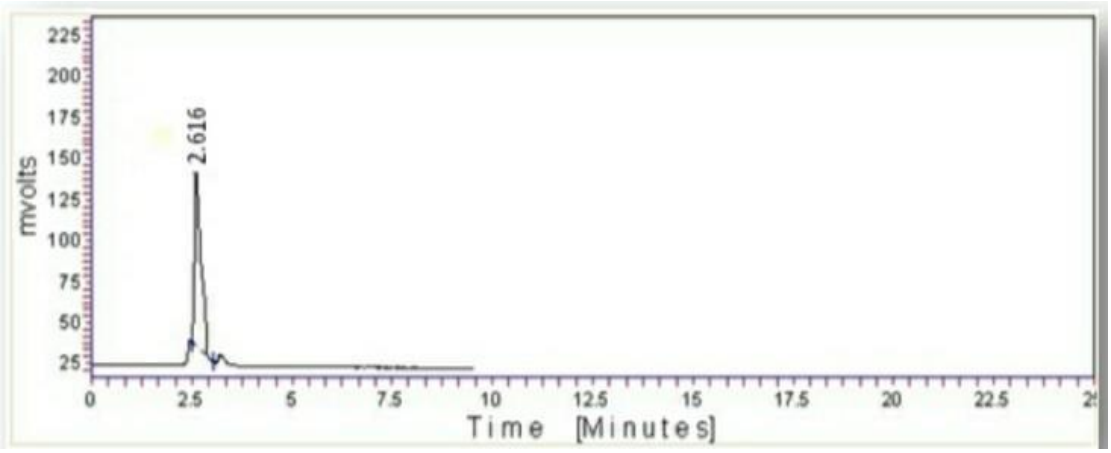


Figure 4: Chromatogram of standard quercetin marker.

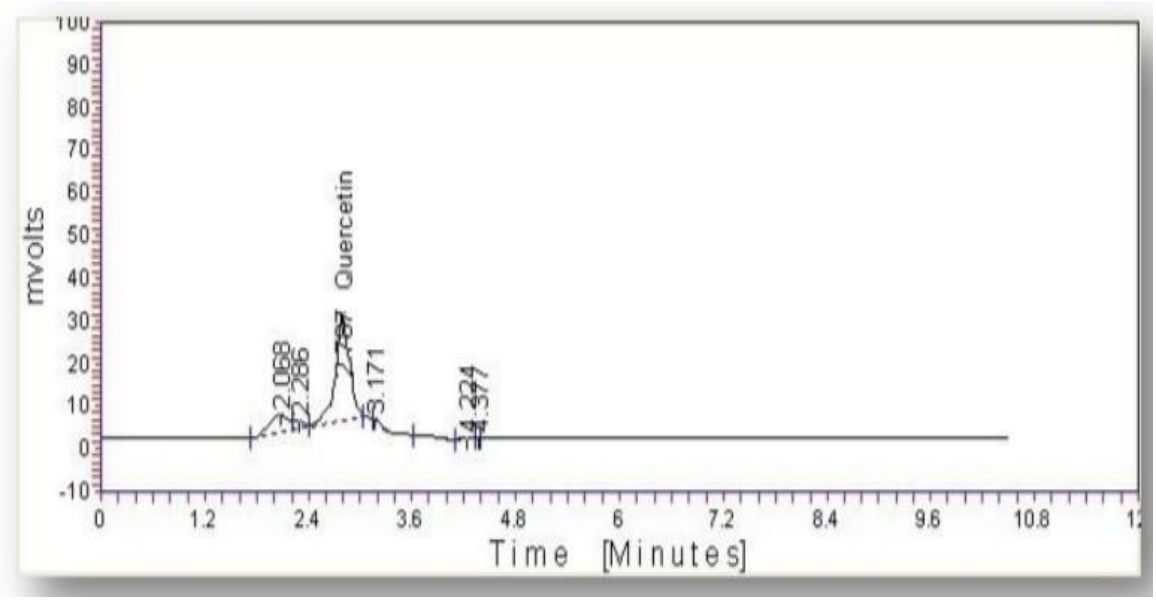


Figure 5: Chromatogram of test sample extract of tridax procumbens for detection of marker compound quercetin.

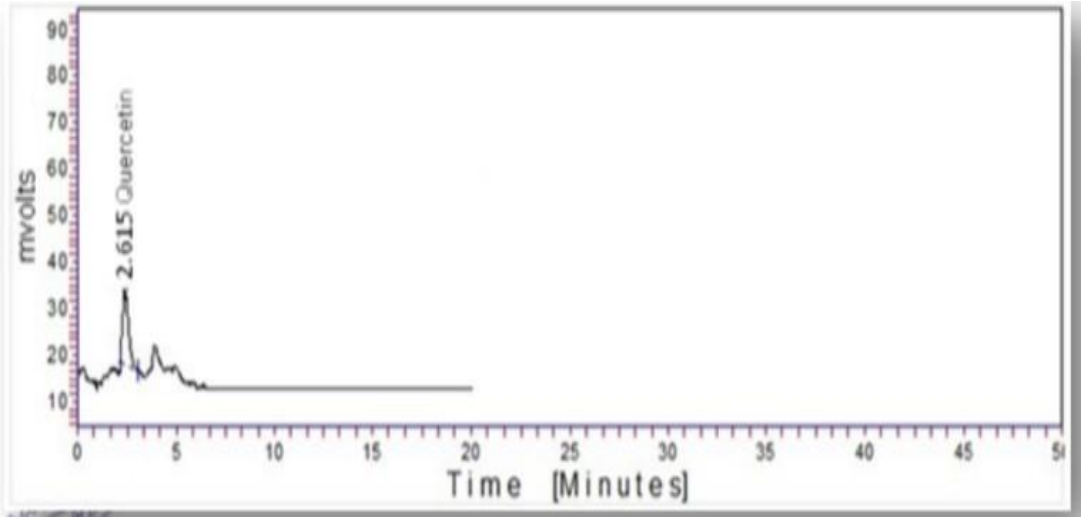
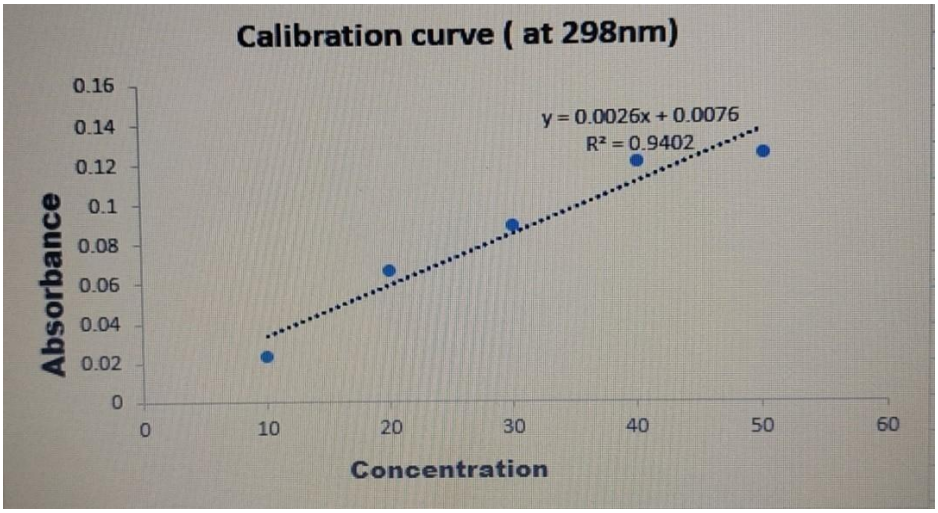


Figure 6: Chromatogram of test sample extract of azadirachta indica for detection of marker compound quercetin

iv) Ultra-violet spectroscopy – [14] UV Shimadzu 1800 double beam spectrophotometer was used in the analysis of sample extract of Azadirachta indica and Tridax procumbens. The extract form of A. indica was weighed and taken about 1 mg and was dissolved in 1 ml methanol to make a 1000 ppm solution and filled up to the mark in a small volumetric flask. Then again 1 ml was withdrawn and was filled up to the mark to make a 100ppm solution. Again, 1ml was taken from 100 ppm and was filled up to the mark to make a 10-ppm solution in another flask. At last, 1ml, 2ml, 3ml, 4ml & 5ml solution was withdrawn with the help of a pipette from 10ppm solution and filled upto the mark to make1µg/ml, 2g/ml, 3g/ml, 4g/ml, 5g/ml solution. In UV -spectrophotometer, methanol was kept in a cuvette in back column as a solvent and prepared sample solution was kept in front column in a cuvette. On spectrum mode, peak and wavelength of sample was observed and noted. And on photometric mode, absorbance was also noted for each solution.



Graph 2: Calibration curve of *tridax procumbens* at different conc.

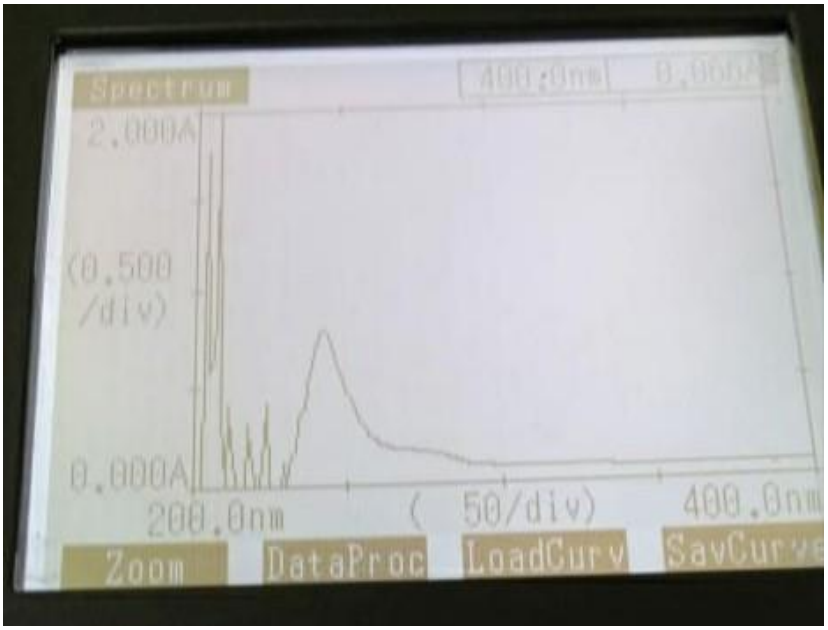


Figure 7: Peak of *tridaxprocumens* at 298 nm on spectrum mode.

3. Formulation parameters –[8]

1. **Placebo (Without drug) –** Carbopol 940 was properly weighed & taken (1%) to prepared a placebo for drug and its combination form. Weighed carbopol was dispersed in water with propylene glycol (5%) and methyl paraben (0.5%) and was kept for 24 hours for swelling. Then for proper stirring and gel formulation, whole mixture was kept in a beaker with a bead on magnetic stirrer for about 15 minutes. Gel was neutralized with triethanolamine to maintain pH 5.0 and kept in a jar for 1 week at room temperature properly.

a. **Viscosity –** Brookfield viscometer was used with spindle 7 at 100 rpm and at room temperature to determine the viscosity of the gel.

Table 10: Showing viscosity of the carbopol gel at different conc.

S.no.	Concentration (%)	Viscosity (cps)
1.	1%	155.61 ±6.12
2.	2%	157.22 ± 3.12
3.	2.5%	158.11 ± 2.21

a. Swelling index – Carbopol mixture was prepared in 5 different concentrations (0.5%, 1%, 1.5%, 2%, 2.5%) and was stirred at magnetic stirrer for 15 minutes. All concentrations of carbopol were equally weighed in 5 different tea bags and were tied with thread. Then each tea bags were dip in beakers filled with distilled water for 3 hours and were weighed separately. The observed weight of all the carbopol mixtures were in increasing order according to the concentration increased.

$$\text{Swelling index} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Table 11: Table showing swelling index of the carbopol gel in different conc.

S.no.	Conc.	Initial weight (gm)	Weight after swelling(gm)	Swelling index (%)
1.	0.5%	5gm	4.450	- 11
2.	1%	5gm	6.020	20.4
3.	1.5%	5gm	6.340	26.8
4.	2%	5gm	6.430	28.6
5.	2.5%	5gm	7.010	40.2

b. Spreadability test –Gel was placed between two slides by added some weight (20g) on the upper slide and was kept for 5 -6 minutes. The length of the slide was 6 cm and was placed horizontally. Spreadability was calculated for each formulation with the help of given formula:
Spreadability = Mass × length / Time

c. pH test – 1gm of Carbopol gel was dissolved in 25 ml of water. Then the electrode of digital pH meter was dipped into the beaker filled with gel formulation. Readings were noted after re-test of the gel formulation.

d. Physical evaluation –Carbopol gel was transparent, viscous and was white in colour. It was soft & smoothly applicable. It was easily washable or removable with water.

4. Drug loaded evaluation parameter –[8]

i) Drug loading –5%drug was diluted with propylene glycol loaded in a prepared placebo (Carbopol gel mixture) with 0.5% methyl paraben and was packed in aluminiumtubes.

ii) Skin irritation test – 7, 8 Healthy volunteers were selected for skin irritation test. About 120 mg of gel was applied on their skin in small area. No irritation, rashes, allergic reaction etc. was observed.

iii) Accelerated stability test – Accelerated stability test was performed for 1 month. Changes in formulation was not observed on accelerated temperature & humidity. Little difference or fluctuation was observed in repeated physical evaluation of the formulation.

iv) Physical evaluation – Drug loaded hydrogel was viscous, smooth and green in colour and was attractive in texture and applicability.

v) Extrudability –The prepared hydrogel was filled in aluminum tubes and was packed. Then the tube was pressed in the middle portion and filled hydrogel was extruded outside with ease.

Table 12: Showing viscosity & extrudability of formulations.

S.no.	Formulation code	Viscosity (cps)	Extrudability
1.	FN1 (Neem)	9179 ± 2	++
2.	FG1 (Ghamra)	9182 ± 1.5	++
3.	FNG (Combination)	9589 ± 2	++

Table 13: Showing swelling index of drug loaded hydrogel.

S.no.	Formulation code	Swelling index (%)
1.	FN1	32.1
2.	FG1	35.0
3.	FNG	42.5

Table 14: Spreadability of the formulations.

S.no.	Formulation code	Spreadability gm cm/sec
1.	FN1 (with neem extract)	20×6/5= 24
2.	FG1 (with ghamra extract)	20×6/4= 24
3.	FNG (combination)	20×6/6= 20

Table 15: Showing pH of various formulations.

S.no.	Formulation code	pH
1.	FN1	5.03
2.	FG1	5.00
3.	FNG	5.05

i) **Drug entrapment efficiency –** 20mg hydrogels were weighed & dispersed in phosphate buffer (250 ml) for 24 hours. Then solution was properly shaken& filtered and drug content was analyzed in Spectrophotometer.

Drug Entrapment Efficiency = Actual drug content × 100

Theoretical drug content

Table 16: Entrapment efficiency of the formulated gel.

S.no.	Formulation code	Entrapment efficiency (%)
1.	FN1	5/8 ×100 = 62.5 %
2.	FG1	5/ 7.5 × 100 = 66.6 %
3.	FNG	5/9 × 100 = 55%

ii) **Drug release study–**

a) **Separation of egg shell membrane –** For drug release study, membrane method was used. Fresh eggs were pricked from the top with the help of a glass rod and the yellow part was drain in a beaker and then discarded. Then empty egg shell was dipped in a concentrated HCl for few minutes and egg membrane was carefully separated and rinsed with distilled water.

b) **Process of drug release –** The prepared membrane was tied on two-sided open test tube with the help of a thread on one side and from another end formulated gel was placed. Glass tubes were fitted on a stand and was dipped in a beaker filled with phosphate buffer solution, kept on a magnetic stirrer. Three assemblies were running at a time to check the release of FN1, FG1 and FNG. Readings were taken in 15, 30, 60, 90 & 120 minutes and sink condition was maintained by withdrawing 1 ml into the volumetric flask and by pouring 1 ml freshly prepared buffer solution in each beaker. Dilutions were made according to the time and marked upto the volume by fresh buffer solution.

Absorbance was taken in UV spectrophotometer and observations were noted.

Table 17: Table showing drug release of the formulations.

Time of release (In min.)	% Drug release for neem (FN1)	% Drug release for ghamra (FG1)	% Drug release for Combination (FNG)
15 min.	40%	40%	40%
30 min.	50%	55%	55%
60 min.	60%	65%	65%
90 min.	65%	70%	70%
120 min.	70%	75%	75%

f) Other Evaluation parameter (Related with biotechnology)–

1. **Collection and isolation of M. furfur –** Flakes of dandruff were collected with the help of clean & sterile comb. Collected sample was inoculated in sabouraud dextrose broth and was incubated for overnight. Then prepared culture was streaked on SDA (sabouraud dextrose agar) containing chloramphenicol and further incubated for three days at room temperature. As M. furfur is a lipophilic yeast, colonies were overlaid with olive oil and separated by their morphology and were identified with catalase test and tween assimilation test. Then cell shape and type of budding was observed in a microscope.[9]

a) Sabouraud dextrose agar preparation – Dextrose (40%), Peptone (10%) and Agar (15%) are poured in 900 ml distilled water with stirring and final volume was adjusted by hydrochloric acid to maintain the pH up to 5.6 and was heated to dissolve the medium. After that, it was autoclaved for 15 minutes at 121°C and was poured into petri dish.[11]

2. **Antifungal assay –** For antifungal assay, well diffusion method was used. Cell suspension was spread over the agar uniformly in a petri – dish. Then holes/wells were prepared with a diameter of 6 to 7mm aseptically with a cork borer. The standard drug (quercetin), placebo and formulated gel (FN1), (FG1), (FNG) were placed in all wells and incubated for 3 days at room temperature. Inhibition zone was observed by diameter and radius respectively. [7],[9]

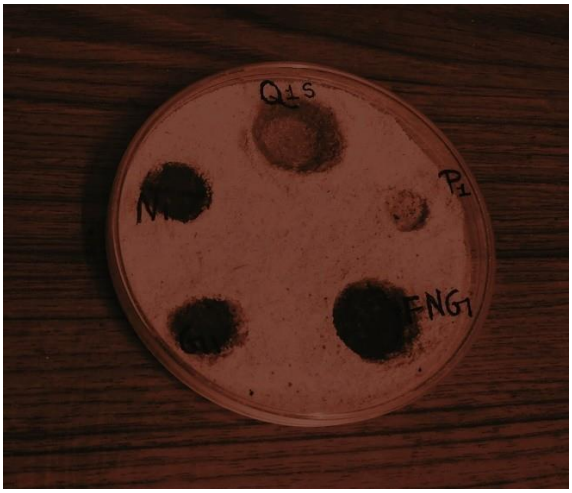


Figure 8: Well diffusion method of antifungal activity against M. furfur. using quercetin (Q1S) as a standard, placebo (P1) for negative effect and formulated hydrogel containing *azadirachta indica* (neem) (N1) &*tridax procumbens* (ghamra) (G1) with combination form (FNG).

RESULTS AND DISCUSSION:

In the experimental work, several tests were performed for the formulation and evaluation of phyto - hydrogel and we had faced lot of consequences and conditions also like weather changes, humidity, drug degradation etc. After facing all the problems many tests were performed twice or thrice times and we arranged our materials and plants extract again. In plants extract, neem extract & ghamra extract were taken for the treatment of seborrheic dermatitis, in which, extract of ghamra leaves was very effective with antifungal &anti- inflammatory properties in almost all the tests. Neem is also effective with the same properties but it works magically with the combination of ghamra and it was something we want in our formulation. In solubility test, neem was sparingly soluble in water but ghamra was insoluble in water but both neem & ghamra were soluble in methanol. So, the ratio of methanol & water was taken for better efficacy and the solubility results are good in methanol - water ratio. The extraction process was performed in 80:20 ratio of methanol & water and green colour was observed which was changed into yellow colour after 6,7 cycles. In TLC, pink colour fume was observed and the distance of sample running was noted and the Rf value of neem & ghamra combination was good. The sample extract was kept in a muffle furnance and it was converted into black ash. The ash value was noted for each sample. In phytochemical test different colors were observed with the presence or absence of phytoconstituents. The presence of quercetin was confirmed in the HPLC test which is responsible for antifungal & anti-inflammatory action. The wavelength and absorbance of the sample extract was noted by UV spectrophotometer. The formulation parameters without drug were performed & the efficacy and readings of the placebo were noted with swelling index, pH test, viscosity etc. The drug loaded evaluation parameters shown better results in skin irritation test, extrudability test, accelerated stability test. The drug entrapment efficiency of the formulated gel was good with ghamra extract. The drug release study was taken at different time intervals and the combination of *azadirachta indica* (neem) and *tridax procumbens* (ghamra) was better and the maximum drug release was found in the combination form. In antifungal assay the quercetin was taken as a standard, placebo for negative effect and the extract of neem (FN1), ghamra (FG1) and its combination (FNG) form was filled in the wells separately. We found that the combination form of the extract was effective as similar to the standard or the inhibition zone against Malassezia furfur was more than single extract of neem and ghamra. Thus, in many tests ghamra was more effective than neem and the combination of neem & ghamra was much more effective for the treatment of seborrheic dermatitis.

SUMMARY & CONCLUSION:

Seborrheic dermatitis is a common worldwide problem with a prevalence rate of 3 – 5 % and the mildest form (dandruff) of this dermatitis was estimated about 15 – 20 % of the population. Its occurrence appears on scalp or on multiple body sites even after treatment has a negative impact on individual's belief but the availability of natural sources as well as other pharmaceutical products (that decrease colonization of lipophilic yeast and inflammation) is a positive thought to handle with the recurrence of this problem by some necessary care, prevention and hygiene habits. Due to scalp problems hair fall rate is also increased continuously. Hence, treatment choice should be beneficial for both scalp & hairs. Due to scalp problems, hair fall rate is also increased continuously. The marketed products (shampoos, conditioner, highlighters, colourants etc.) contain so many types of chemicals and ingredients which are harmful for our skin and will lead to skin irritation, infections and hair fall problems. The selection of *tridax procumbens* was not only based on its antifungal & anti-inflammatory properties even it is based on its hair growth promoter activity also and the selection of *azadirachta indica* was based on its anti-parasitic activity, besides antifungal, anti-inflammatory or healing properties. So, the scalp will be free from all the parasites, and hairs can grow naturally. The efficacy of the formulated hydrogel will also enhance by the combination form of both plant extracts (*azadirachta indica*&*tridax procumbens*). The hydrogel selection was based on its high water - content, smoothness, flexibility and ease of application to get rid of dry and itchy scalp. The current experiment of our neem (*azadirachta indica*) and ghamra (*tridax procumbens*) combination as a phyto hydrogel for scalp problems was effective and it may come in the market with further investigations, studies and experiments. In future, hydrogels will be profitable in rare diseases & disorders with new advancement and technology.

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