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Formulation Development and Evaluation of Polyherbal Gel Containing Extract of *Eclipta Alba (L.) (Asteraceae)* For The Management of Alopecia

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KEYWORDS
phytoconstituents, alopecia, deforestation, Plant-based commodities, Conventional pharmaceuticals ect.

ABSTRACT:
It is well known that Eclipta alba (L.) plant thrives best in climates that can be classified as either tropical or subtropical. The Hassak plant, belonging to the Asteraceae family, holds considerable importance in the field of medicine. The application of this specific therapeutic approach is frequently noticed in the care of various skin, liver, and gastrointestinal disorders in nations such as India, Nepal, Bangladesh, and other comparable countries. The main aim of this paper was to gather and examine the current body of literature regarding the biological functions, phytoconstituents, and traditional uses of E. alba. The compilation of scientific literature involved the utilisation of various resources such as books, proceedings, and electronic bibliographic databases like Scopus, MEDLINE/PubMed, Google Scholar, and SciFinder. The investigation identified several active phytochemicals, including phenolic acid, flavonoids, triterpenoid saponins, steroid saponins, and substituted thiophenes. Different extracts and chemicals isolated from E. alba have shown a wide range of biological properties, such as the ability to fight bacteria and cancer, protect the liver and brain, and promote hair growth. The study provides strong evidence to justify the utilization of a botanical blend in the context of hair care therapy. Hence, the botanical specimen could be regarded as an organic reservoir and potentially harnessed to develop an alternative therapeutic approach for alopecia.

1. Introduction

Medicinal plants contain naturally occurring active compounds that possess the potential to effectively heal various ailments or alleviate symptoms [1]. Traditional medicines and medicinal plants are widely acknowledged to play a significant part in the facilitation and maintenance of health in a large

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number of low-income nations. According to the presence of phytochemicals, plants might possess medicinal capabilities due to their anti-oxidant, antibacterial, and antipyretic characteristics. [2,3]. Throughout history, herbal treatments have been widely employed across different cultures as a means of addressing various ailments, owing to their inherent non-toxic properties. Despite several instances in the literature demonstrating the adverse effects of plant usage, both the general public and professional organizations within the industry have failed to acknowledge the possible toxicity associated with herbal remedies [4]. There is a growing trend in the pharmaceutical industry towards the utilization of medicinal plants as primary sources for the production of various medications[5].

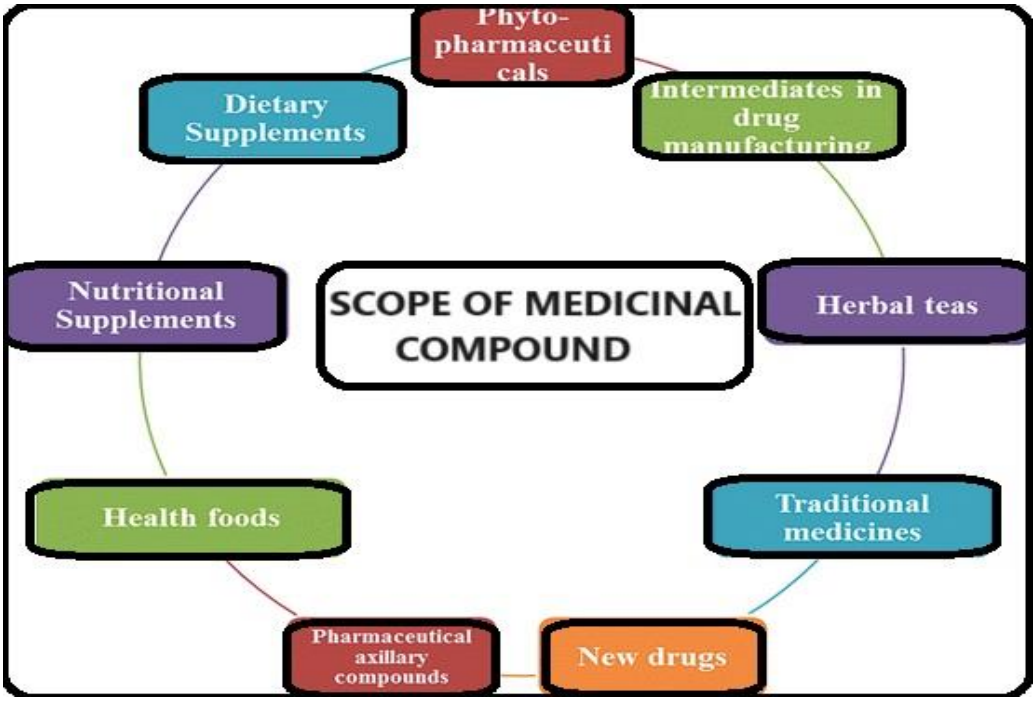

Fig.1 Diagram illustrating the scope of medicinal plants

2. The Significance of Plant-Based Contemporary Medications[6]

Plant-based commodities are derived from a variety of sources, including both cultivated and wild plants. Due to a limited understanding and technological capacity, our predecessors exclusively relied upon plants for medicinal purposes, as they were unable to identify the specific constituents inside plants that provide therapeutic properties[7,8]. Globally, the growth of the human population, the expansion of metropolitan areas, the advancement of industrial activities, and the cultivation of crops have collectively contributed to the phenomenon of deforestation and excessive exploitation of natural resources. Consequently, these activities have had detrimental effects on the environment, such as the contamination of air and water as well as the extinction of numerous species, including those with medicinal properties [9]. The user's text is already academic and does not require any rewriting. In 1638, the early immigrants to Peru employed Cinchona bark as a remedy for the treatment of malaria. The extraction of quinine, an alkaloid employed in the treatment of malaria, was performed from the bark [10]. The presence of medicinal potential is inherent in all plants, as they possess the ability to produce biologically active substances as a means of self-defense against illnesses, pests, and environmental adversities. Grain legumes are a source of proteins and antioxidants, while grains provide carbohydrates and fiber. Oilseeds include significant amounts of dietary fatty acids, while vegetable crops are rich in vitamins and minerals [11]. These significant crops underwent domestication from their wild counterparts, and were subsequently improved through genetic and breeding studies, ultimately leading to their cultivation.

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An approach to drug discovery based on plants[12]

The exploration of biologically active compounds in higher plants can be examined from several perspectives. To identify potential compounds with pharmacological properties, it is necessary to investigate novel chemical compositions and seek collaboration with a biologist who possesses the expertise to conduct comprehensive pharmacological evaluations on each product[13]. An additional approach involves the collection of readily available plant specimens, followed by the extraction of their constituents, and subsequent evaluation of each extract for potential pharmacological effects across one or more categories.The utilization of a comprehensive screening and random collection approach represents a viable technique that has promise for the future development of valuable drugs[14]. However, the success of this strategy is contingent upon the presence of adequate research findings and the availability of appropriate, reliable bioassay equipment.

Compared with conventional treatment, herbal treatment is used [15]

Conventional pharmaceuticals possess a limitation whereby a significant number of them may provide adverse effects and primarily target the manifestation of symptoms rather than the fundamental etiology of the issue[16]. Moreover, numerous commonly prescribed drugs are formulated with individual compounds, against which bacteria gradually acquire resistance. In contrast, herbal medicines consist of intricate compounds that pose a formidable challenge for bacteria in terms of metabolism and colonization. One of the notable benefits associated with the utilization of natural medicines lies in their inherent safety and absence of unwanted effects. Furthermore, they have demonstrated efficacy in effectively managing both the symptoms and root causes of the ailment[17,18]. The user's text is not sufficient to rewrite it in an academic manner. Please provide more In addition to the aforementioned advantages, herbal medicines have the potential to enhance immune function, bolster the body's defense mechanisms, and augment its innate ability to resist the infiltration of external pathogens. Several botanical species, such as goldenseal and garlic, possess inherent characteristics that exhibit antibacterial and antiviral effects [19]. Many other medical issues, including those affecting the nervous, digestive, respiratory, and sex systems, have been shown to respond positively to herbal treatments. Natural herbal remedies have been found to be commonly efficacious in mitigating the adverse consequences associated with intensive treatments such as chemotherapy[20]. In summary, the utilization of natural medications offers numerous advantages.

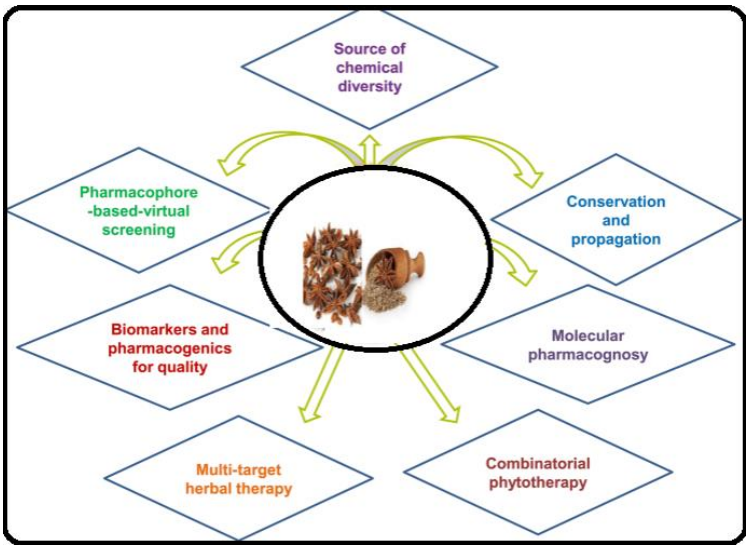

Fig.2 A flowchart Compared with conventional treatment, herbal treatment is used

Hair loss and its importance [21,22]

In addition to sweat glands and sebaceous glands, hair is considered a vital anatomical structure originating from the ectodermal layer of the skin. Hair is categorised as an accessory structure within the integumentary system, fulfilling the role of a

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protective appendage for the human body (23). The role of an individual's hair in enhancing their visual appeal is significant. During the telogen phase, the hair follicles transition from the anagen phase, characterised by vigorous hair growth, to the dormant condition. The duration of this stage spans a period of two to three months. The physical manifestation of hair is a distinctive attribute of the human body, which distinguishes humans from other land-dwelling mammals due to their unique ability to exert direct influence over its appearance. [24]

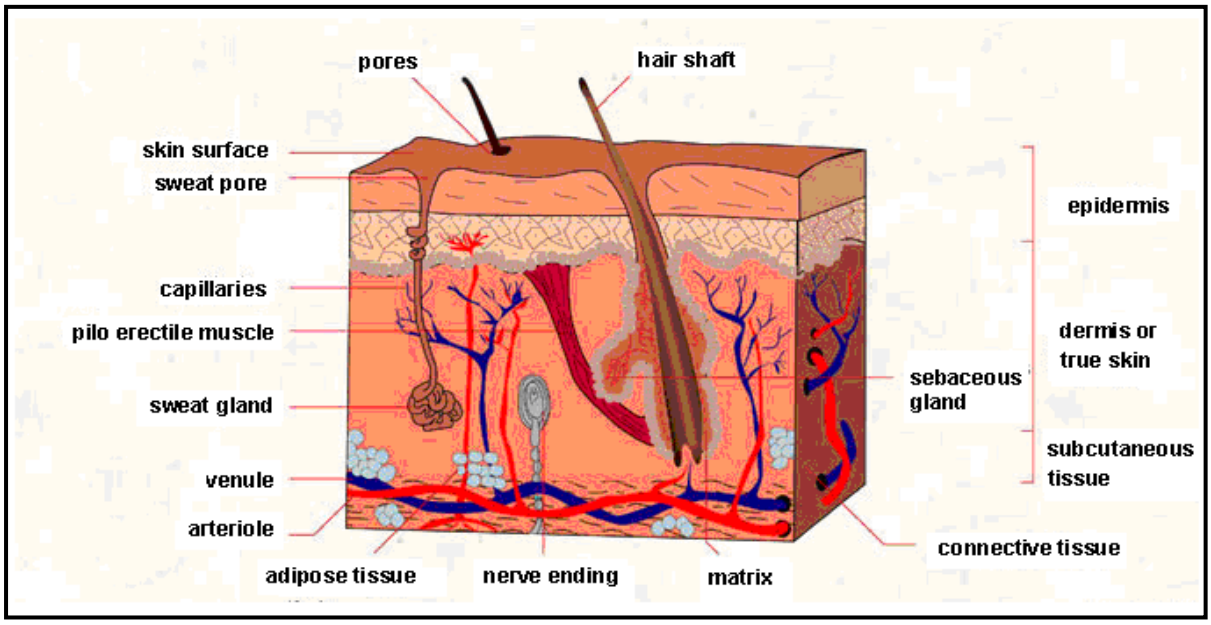

Fig.3 A structure of skin

3. Research on the phytochemistry of the plant E. alba [25]

E. alba is known to possess a diverse range of bioactive constituents, such as proteins, amino acids, volatile and essential oils, tannins, steroids, carbohydrates, glycosides, alkaloids, and flavone glycosides. According to a preliminary phytochemical investigation. Numerous active compounds are included in it, including glycosides, alkaloids, flavanoids, polyacetylenes, and triterpenoids[26]. The hydrolysis of the plant's polypeptides results in the production of the amino acids cystine, glutamic acid, phenylalanine, tyrosine, and methionine are being discussed. [27].

Table 1. An analysis of the chemical composition and phytoconstituents of the E. alba[28]

S.No.	Nature of Phytoconstituents	Phytoconstituent
01	Coumestan	Demethylwedelactone-7-glucoside, demethylwedelactone, and Wedelolactone
02	Glycosides and terpenoids	EclalbasaponinsI-V I,oleanolic acid,ursolic acid
03	Sterol	Stigmasterol, Stigmasterol-3-o-glucoside
04	Flavonoids	Vitamin A precursors lutein-7-glucoside, luteolin, and apigenin
05	Fatty Alcohols	Hentriacontanol, heptacosanol

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S. No.	Parts of the plant	Biological activity of Plant
1	Whole plant parts	Detoxifying, deobstruent, a catarrhal tan, symptoms of hyperacidity, stomach pain, and dysentery, as well as attributes such as anti-catarrhal, spasmogenic, and hypotensive qualities.
2	Extract obtain from leaves	Skin problems, liver problems, asthma, swelling and bloating, enlarged glands, dizziness, vertigo, blurred vision, and allergic urticaria are only few of the symptoms.
3	Solid paste of leaves	To treat rash
4	Fine granule	Chest infections, coughs, rheumatic diseases, and skin disorder
5	Liquid from Decoction	Symptom include menstrual cramps, spotting, infertility, and hair greying.
6	Herb Paste	A review of pain, recovery, and headaches

Table 2. A wide variety of biological processes taking place in different E. alba

Extracts prepared from the plant E. alba [29]

A total of 250–250 g of dry leaf powder from Eclipta alba (L.) was treated with various soxhlets. It was defatted with petroleum ether and then extensively extracted over the course of 36 hours using a soxhlet system. Between 40 and 50 degrees Celsius were maintained as the temperature. The extraction process involved the distillation of the liquids under reduced pressure, followed by vacuum drying using a flash evaporator. The final result was a semisolid mass.

4. An analysis of the plant's quality, with a focus on E. alba[30]

False daisy, also called Eclipta alba, is a popular botanical medication used in conventional medicine for its many medicinal benefits. E. alba is also known as Eclipta alba. [31] In spite of the fact that I am unable to carry out a physical test in my capacity as AI, I am able to offer you with information regarding the qualitative chemical tests that are generally carried out on E. alba.

Table 3. Listing of qualitative chemical analyses of E. alba [32]

Name of Chemical Tests	Plant extract
Alkaloidal content	
Checking with Dragendorff's Reagent	+
Check with Mayer's Reagent	_
Assay using Hager's Reagent	+
Test with Wagner's Reagent	_
Glycosides moiety	
Brontanger's experiment	+
The Legal Standards Examination	+
Phenols/Tannins components	
Check with Ferric Chloride	+
A Test With Gelatin	_

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Flavonoidal moiety	
Mag-ribbon analysis	+
Saponins moiety	
Examining the Foarth	+
In vitro hemalysis	+
The Lead Acetate Test	+
Fixed oil/Fats content	
Saponification Test on the Spot	–
Saponification test	+
Analysis using Copper Sulphate	–

5. The Development of a Transdermal Delivery System

Plant E. Alba Extract in the Production of Hydrogel and Hydro alcohalic Gel.

In a volume of fifty milliliters of distilled water, Carbopol 934 and Sodium CMC were combined in a number of different proportions, including 3:0, 3:1, 2:1, 1:1, 0:3, 1:3, and 1:2. To facilitate the dissolution of the specified amounts of methyl and propyl paraben in a five millilitre volume of distilled water, a water bath was employed. Subsequently, the first solution, consisting of 5% propylene glycol, was introduced into the combination. Before adding the dissolved plant extracts to the polymer mixture, A minute quantity of ethanol was utilised initially to dissolve various mixtures of plant extracts. After that, each of the components were fully mixed together with the Carbopol 934 gel while the mixture was being continually swirled. Triethanolamine was applied one drop at a time in order to reach the skin pH and gel consistency that are outlined in Table No. 4. The polymer-based gel that included plant extracts was cloudy and lumpy in some of the batches that were tested. Therefore, these batches were ignored, and instead, FE3, FE4, and FE5 were considered for the purposes of further study.

Table.4 extracts of the E. alba plant are used in polyherbal gel compositions [33].

Constitution	FE1	FE2	FE3	FE4	FE5	FE6	FE7	FA
Carbopol 934 (gm)	4	4	3	2	2	-	2	2
Tee tree Oil	0.7ml	0.7ml	0.7ml	0.7ml	0.7ml	0.7ml	0.7ml	0.7ml
Sodium carboxymethyl cellulose (gm)	-	1	1	1	3	2	2	3
Leave extract of Eclipta alba (L.) (% w/w)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Propylene glycol 400 (5%)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Aloe vera	4.8 ml	4.8ml	4.8ml	4.8ml	4.8ml	4.8ml	4.8ml	4.8ml
Methyl 4-hydroxybenzoate (0.5%) (ml)	0.3ml	0.3ml	0.3ml	0.3ml	0.3ml	0.3ml	0.3ml	0.3ml
Propyl 4-hydroxybenzoate (0.2%) (ml)	4.9 ml	4.9ml	4.9 ml	4.9 ml	4.9 ml	4.9 ml	4.9 ml	4.9 ml

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Triethanolamine (ml)	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quanti ty suffici ent
Ethyl alcohol	-	-	-	-	-	-	-	30 ml
ml of purified water	upto 100ml	upto 100ml	upto 100ml	upto 100ml	upto 100ml	upto 100ml	upto 100ml	upto 100ml

* Each formulation make up to 100 ml with purified water.

An improved approach to the process of preparation The term "FA" denotes a hydroalcoholic gel derived from plant material that was specifically chosen for an in-vivo trial on hair growth. Quantitative analyses of hair growth are performed with the use of hair length, hair weight, and research from the field of histology[34].

Fig.4 Preparation of polyherbal Gel from the plant E.alba

6. Evaluation of Gel Formulation

The result showed that the developed herbal gel was brownish in color, translucent in appearance and showed good homogeneity with absence of lumps. Formulation FE4 had good values of spreadability, viscosity, pH, drug content and during the accelerated stability studies the appearance was clear and no significant variation in spreadability, pH and drug content was observed.

In-vitro drug release study

Percentage drug release of Hydroalcohalic gel FE_4 and FA formulation containing combination of plants was observed to be 25.14% and 29.27% (at 30 min.) and 57.51 % & 64.50% (at 180 min.) respectively at 236 nm. It was observed that addition of ethanol in formulation increase the release by increasing permeation properties of gel. The hydrolcohalic gel containing both extract formulation FA showed maximum drug release as compared to other formulation.

Standard curve of Plant extract

Standard calibraction curve of plant extracts was determined by plotting absorbace vs concentraction at 236 nm. Table no.5 and Fig-5 shows the standard curve for herbal extract. The method obeyed Beer's law limit in the concentration range of 2-12 mcg/ml at 230 nm with a regression value of 0.998

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Table 5: Calibration curve of Plants Extracts at 230 nm

S.No	Concentration (µg/ml)	Absorbance
1.	Blank	0.000
2.	0.2	0.082
3.	0.4	0.163
4.	0.6	0.242
5.	0.8	0.314
6.	1.0	0.383

Fig 5: Calibration curve of Plant Extracts at 236 nm

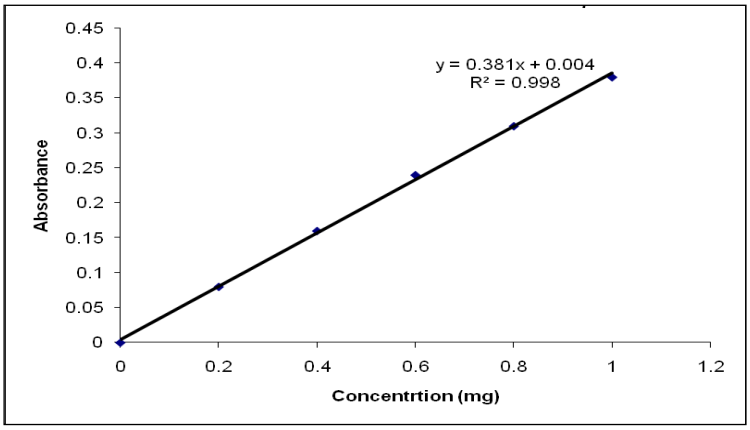

Table 6: % Drug Release of Formulation at 230 nm

Time Interval (Min)	% Drug Release of Formulation at 236 nm 234 nm	
	FE_4	FH
15	18.83	23.51
30	25.14	29.27
45	31.20	37.10
60	37.14	42.10
90	43.11	50.11
120	49.70	58.39
180	57.51	64.50

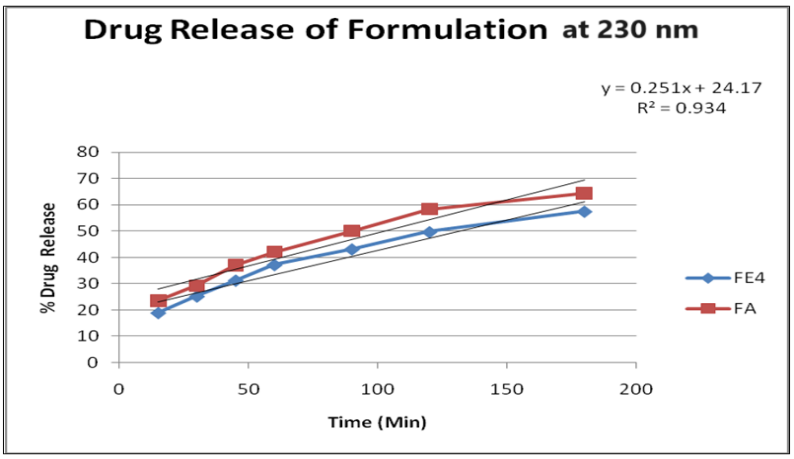

Fig 6: % Drug Release of Formulation at 230 nm

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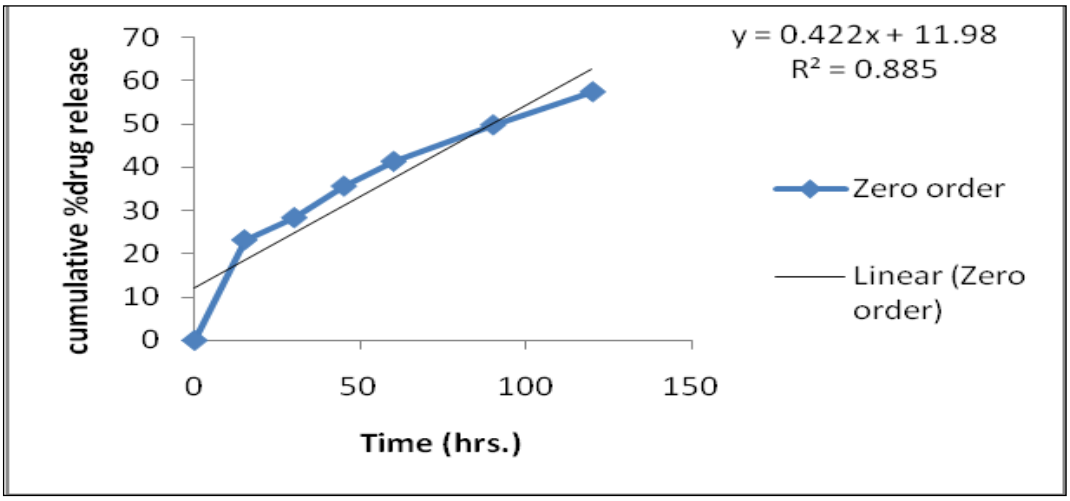

Fig.7 Zero order kinetics of formulatio FA

7. Screening of the E. alba plant for potential pharmacological properties [35]

We investigated whether or not an extract from the E. alba plant could stimulate the growth of hair. In the course of the research on the growth of hair. It was noted that both the quality and quantity of hair was growing. In the context of a qualitative inquiry into hair growth, the initiation and termination periods of hair growth were measured and visually assessed as integral components of the research methodology [36]. Quantitative analyses of hair growth are performed with the use of hair length, hair weight, and research from the field of histology. Institutional Animal Ethics Committee (IAEC) permission No. CPCSEA/IAEC/JLS/18/07/22/018 for animal research at Jeeva Life Sciences,Telengana assured that the research will be conducted in compliance with the guidelines set forth by the CPCSEA.

Animals

In the experiment, healthy Wistar rat weighing between 200 and 250g were employed. Rats were confined in small cages and their environments were strictly regulated (25–20 degrees Celsius, 12 hours of light and dark, and free access to food and water at all times). Throughout the duration of the experiment, the rats were given a diet that consisted of standard lab chow as their food source.

Potential of the E. alba plant to stimulate hair growth has been studied in vivo [37]

Which research project will investigate whether or not polyherbal extract can stimulate the growth of hair in rats that are Wistar albino? There are a total of thirty Wistar rats utilized in the study. There are a total of 30 rats, which are distributed evenly among five groups of six rats each. In the first group, which served as the control group for the experiment, no drug therapy was ever administered. The shaved region was subjected to a 2% minoxidil treatment and employed as a control in the second cohort of participants, serving as the reference group. Topical applications of extract mixtures (PA1–PA5) were made to the third through fifth groups (the test subjects). The dorsal region of each rat was shaved with electrical shavers to create an area that was 3 cm^2 in size, and then a commercial hair remover was applied to remove any remaining hair. The individuals in the remaining groups were subjected to two instances of minoxidil gel application on the areas experiencing hair loss, in addition to one application of each of the polyherbal extracts. Conversely, the control group did not receive any form of treatment. This therapy was maintained for a total of thirty days, during which time qualitative and quantitative elements of the growth of new hair were measured.

Table No. 7 Hair growth duration and rate in response to extracts [38,39]

Batch	Dose regimen	The duration required to commence the process of growth. (in days)	The duration required for full growth. (in days)
Batch No. I	Dose of Control	12±0.80	40.20±1.12

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Batch No. II	2% Minoxidil	7.08±0.91	33.8±0.63
Batch No.III	Combination extract (PA1)	11.5±0.66	40.8±0.36
Batch No. IV	Combination extract (PA2)	9.56±62	23.6±0.71
Batch No. V	Combination extract (PA3)	8.2±66	29.1±0.71
Batch No. VI	Combination extract (PA4)	8.3±0.51	38.9±0.21
Batch No VII	Combination extract (PA5)	8.7±0.66	38.2±0.36

Values are mean ± SEM

8. Histological studies

Development of Hair Follicles

The hair follicle count, skin thickness and color appearance were observed. Formulation FH containing plant extracts showed significantly considerable results and exhibited significant increase in hair regrowth. Increase in the thickness and presence of hair follicles in the subcutis layer were taken as an evidence for transition of follicles from telogen to anagen phase of hair growth. The photomicrographs obtained indicated that formulation FA treated animals had showed maximum percentage of anagenic hair follicles (69.2 %) and hair follicles density while the PE2 extract combination (66.5 %) and minoxidil (70.4 %). (Table.8)

Table No. 8 Effect of Extracts different combination on per cent of hair follicles

Batch of 20 days	Formulation	Anagen	Telogen	T/A ratio
Batch No. I	Control	51.3±0.69	43.4±0.89	0.84
Batch No. II	2% Minoxidil	70.4±0.65	26.2± 0.17	0.37
Batch No. III	Extract combination (PE2)	66.5±58	29.2±0.62	0.45
Batch No. IV	Hydroalcohalic Gel formulation	69.2±0.42	27.5±0.42	0.40

Values are mean ± SEM

9. Result and Discussion

The goals in the current era investigation for the treatment of alopecia using herbal plant are the preparation, characterisation, and assessment of topical studies. Several other formulations, such as hydrogel and hydroalcohalic gel, were developed with the assistance of Eclipta alba (L.), and these gels were also enhanced. After conducting a physical-chemical examination on the powder derived from the aerial portion, the results obtained can now be utilized for the purpose of reliable crude drug identification. Physiochemical analysis studies can serve as a diagnostic tool to accurately identify the species of plants under investigation. Therefore, these standardized characteristics are helpful in recognizing the adulterants that may be present in any plant, which will lead to the picked plant's increased potency and purity. As a result, all of these discoveries are going to be beneficial in correctly identifying, establishing the identity of, and establishing the purity of the chosen endangered plant. The hydrogel and hydroalcohalic gel formulation of hydroalcoholic extracts combination PE2 were designed by using varied concentration of carbopol and sodium CMC polymer. During the trial, the excipients concentrations of carbapol and Sodium CMC were gradually increased and then decreased as several problems like homogeneity, spreadibility and viscosity were encountered. Some batches were discarded due to uniformity and remaining batches (FE3, FE4 and FE5) were characterized for various parameter. The result showed that the developed herbal gel was greenish in colour, translucent in appearance and showed good homogeneity with absence of lumps. Formulation FE5 had good values of spreadability, viscosity, pH, drug content and during the accelerated stability studies .the appearance was clear and no significant variation in spreadability, pH and drug content were observed. Hence hydroalcoholic gel were formulated from hydrogel FE5 formulation and its physiochemical study was found to be good. The research provides solid evidence in support of the utilization of a plant combination in the treatment of hair loss. As a result, the plant could be regarded as a natural source and could be utilized in the process of developing an alternative therapy for alopecia. Evaluation of the

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New and Improved Formulation from a Pharmacological Perspective According to the findings of the FA experiment, the duration of time required for rats treated with the formulation to begin and finish growing hair was drastically reduced. On day 6, there was evidence of the beginning of hair growth. In a manner analogous to this, it took twenty-four days for all of the hair to come back on the area that had been shaved. At 30 days, the hair length measured 2.64 millimeters when treated with the prepared Hydroalcoholicgel FA combo extract. On the market internationally, there is a growing appetite for herbal products. During the course of research, an attempt was made to demonstrate the existence of herbal gel comprising Eclipta alba (L.) extract.

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