



Phytochemical Screening and Evaluation of Antimicrobial Properties for *Glycyrrhiza Glabra* Linn

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Abstract

The current study aimed to explore the anti-bacterial, anti-oxidant, and anti-bacterial effects of the hydro-methanolic root extract of *Glycyrrhiza glabra* (*Leguminoseaanti*-bacterial) using standard screening approaches, disc diffusion, and deoxyribose methods, respectively. Different levels of effect were seen when various study approaches were applied. During phytochemical screening, *Glycyrrhiza glabra* revealed the presence of secondary metabolites including flavonoids, saponins, glycosides, terpenoids, etc. Moreover, it effectively inhibited virtually all of the test species' microorganisms. With a 10 mm inhibitory zone, it showed the highest degree of sensitivity to *Shigella flexineri*. compared to the ascorbic acid positive control standard.

Keywords: *Glycyrrhiza glabra*, anti-oxidant, and anti-bacterial, disc diffusion

Introduction

India benefits from established traditional medical systems that rely on natural cures [1].

Plant-based natural products have been utilized for medicinal, medical, and other purposes since the dawn of humanity [2]. The conventional method finally reveals that plants have provided mankind a variety of unique medications to relieve the pain caused by disease [3,4]. Yet, whether in its unprocessed or prepared forms, every drug used in current medical systems is an unrefined drug [3,6]. Up to the eighteenth century, even allopathic western medicine made extensive use of traditional medicines. the growth of the pharmaceutical industry and the advancement of chemical processes throughout the 20th century. Chemical (synthetic) drugs gradually replaced traditional ones in medical practice. *Glycyrrhiza Glabra* Linn's chemical composition is shown in Figure 1. Synthetic drugs may therefore treat serious diseases and even save lives, but they should not be used often to treat chronic conditions [5, 7, 8].

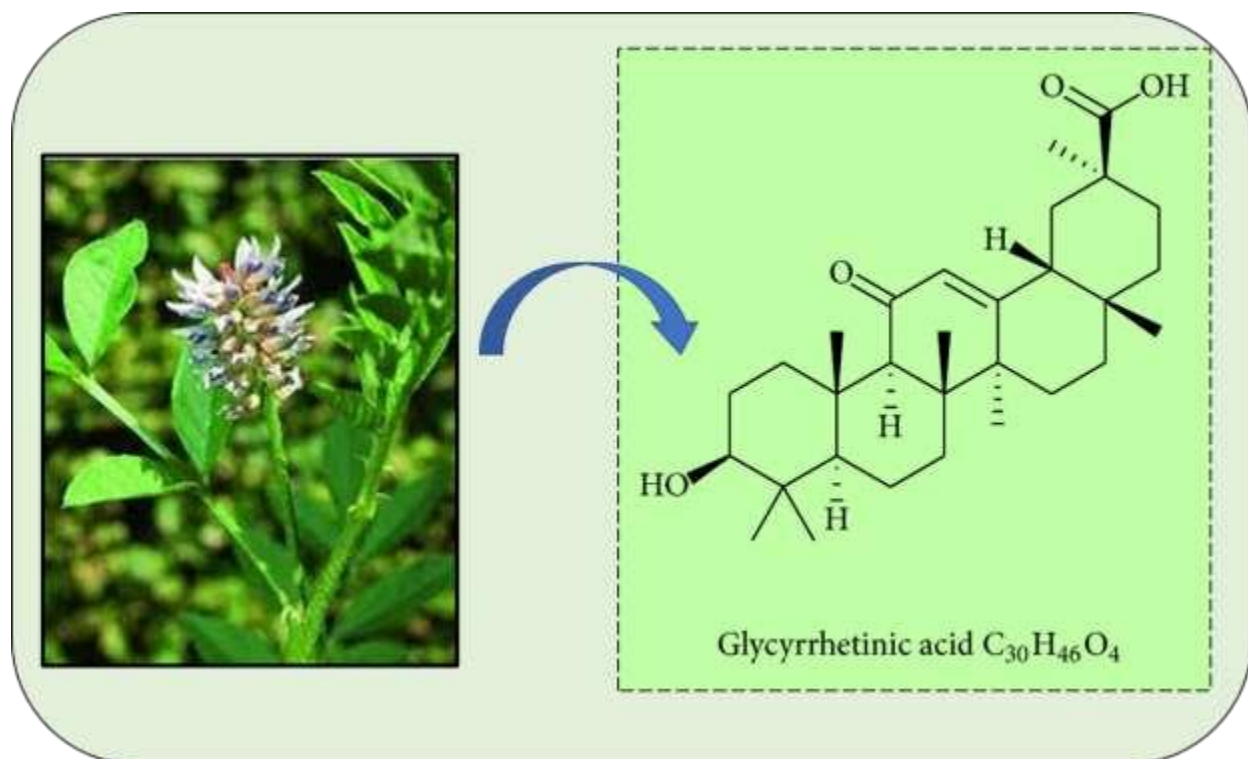


Figure 1: *Glycyrrhiza Glabra* Linn and its chemical structure

Extraction of plants [4,14]

The 1.5 kg of coarsely ground, shade-dried stem of *G. glabra* were extracted with 95% ethanol in a Soxhlet extractor. The ethanolic extract was then compressed using a rotary flash evaporator to 1/10th volume. It was time to purify the combination after fractionating the concentrated

ethanolic extract with n-butanol, ethyl acetate, solvent ether, and petroleum ether (all at 40–60 oC). The chemical components are shown in Table 2.

An aqueous extract was also produced by using chloroform water I.P. as a carrier during the maceration procedure. A rotary flash evaporator was used to vacuum-concentrate the aqueous extract until it was dry. Sesquiterpenes, terpenes, alkaloids, anthraquinones, saponins (foam formation), flavonoids (using magnesium and dil. HCl), and anthraquinones (Dragendorff's test) were among the phytochemical components whose presence and absence were also attempted to be determined using tried-and-true methods[9].

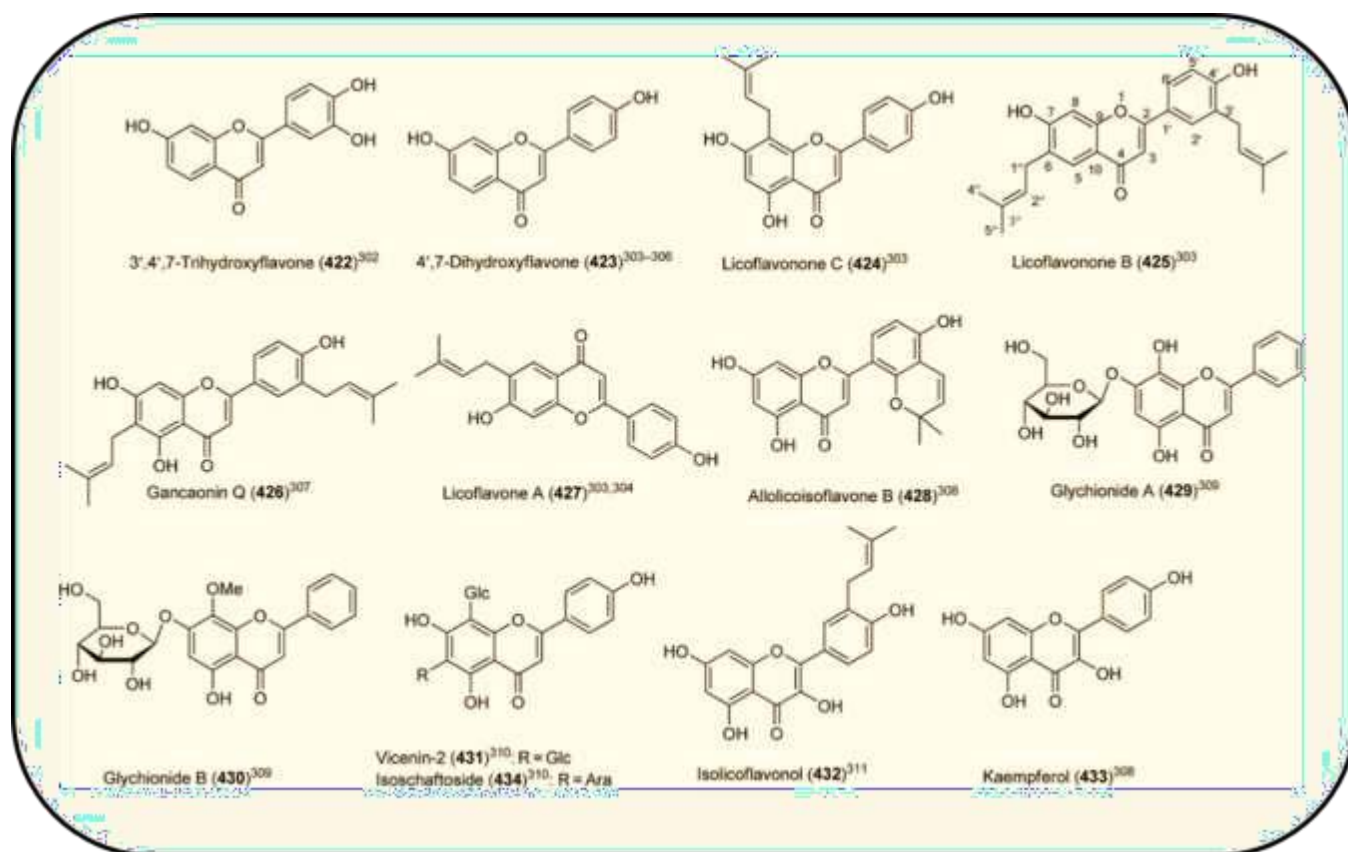


Figure 2. Chemical constituents present in *Glycyrrhiza glabra*

Research strategy

The latest discovery relates to the investigation of substances that encourage hair growth. The present invention is concentrated mainly on a formulation and method for producing and evaluating *Glycyrrhiza Glabra* L root extract in petroleum ether on female rats for hair growth[10].

Materials and Procedures

As hair loss is a dermatologic problem, the search for all-natural products that might encourage hair growth is continuing. Alopecia, or hair loss, is a common patient complaint and a significant

source of both physical and mental stress. Androgens are considered to be one of the most important causes of alopecia, among a number of other factors. Market-available herbal formulations that are used as a hair tonic, to encourage hair growth, to condition hair, to get rid of dandruff, to treat alopecia, and to treat lice infestations are all natural products.

The Indian states of Jammu and Kashmir, Punjab, and the Sub-Himalayan areas are where *G. glabra*, also known as licorice in English, *Yasthi-madhu* in Sanskrit (all Indian languages), *Jethi-madhu* in Hindi, and *Jashtimadhu* in Bengali, is cultivated. It is a hardy undershrub or plant that reaches a height of 1.8 metres. It has deep roots and many branches with exteriors that are red or lemon-colored and interiors that are yellowish or pale. It is known as *Keshya* in ancient Ayurveda texts (hair growth booster). The decoction of the root is an effective shampoo for greying and thinning hair[11].

The effects of mixing five distinct plant extracts—loguath leaf, cork tree, artemisia capillaries, mulberry root skin, and glycyrrhiza glabra root—with a compound plant extract for oil control and acne eradication are described in the literature review on *Glycyrrhiza glabra* Linn 3 CN109646358A. Five effects that are shared by five plant extracts and are acted upon synergistically include: realising the effects of oil-control, antibacterial, anti-oxidant, and light print inhibit acne; preventing the release of lipase; reducing the generation of inflammation; and inhibiting the growth and reproduction of *Propionibacterium acnes*.

An outstanding oil-control and acne-removing cosmetic, the compound plant extract of the innovation may safely and effectively reduce skin oil and fat production while keeping the skin unstimulated, according to a clinical research.

The composition for treating chloasma includes ingredients like persimmon leaves, vaseline, aloe vera gel, centella asiatica extract, chondrus crispus extract, betaine, acryloyl dimethyl ammonium taurate/VP copolymer, methylparaben, carbomer, glycyrrhiza glabra root extract, propylene glycol, vitamin C, androgenic acid, and kojic acid. The production of the mixture for treating chloasma particularly includes the following steps: In S1, the raw materials are quantitatively weighed and combined uniformly using a mixing device; in S2, water is added to the raw materials and combined with the raw materials to produce a colloid composition for treating chloasma. may constantly protect the face, avoid circumstances where direct sunlight stimulates the chloasma, and promote the acceleration of the face's metabolism, which clears out chemical buildup at the chloasma and lightens it[12].

Ginseng, *Bletilla striata*, *Salvia miltiorrhiza*, Clove, Four Types of Licorice, *Rhodiola Rosea*, *Scutellaria baicalensis*, *Centella asiatica*, *Polygala tenuifolia*, and *Rhizoma alismatis* are included in the herbal plant composition disclosed in CN112587455A for lightening and eliminating freckles[11].

Nonetheless, the aforementioned earlier works of art highlight the several applications of *Glycyrrhiza glabra* Linn that aid in treating a number of skin-related issues, such as controlling oil production and removing acne, treating chloasma, whitening, and freckle removal. None of

the older works included here, however, mention the use of Glycyrrhiza glabra Linn as astimulant for hair growth.

Thus, it is essential to construct a composition and a method for producing and analysing petroleum ether extract of Glycyrrhiza Glabra L. in order to encourage hair formation in female rats.

The technical advancements of the contemporary invention go beyond the limitations and flaws of the customary methods and practises that are already in use.

Test for glycosides (Keller-Killani test)

A part of the hydro-methanolic plant extract, a few drops of ferric chloride, and concentrated sulfuric acid were dissolved in glacial acetic acid. The presence of glycosides was then determined by looking for a reddish brown colouring at the junction of two layers, and by looking for a blue green colour in the top layer of this combination.

Test for flavonoids

Three different methods were used to ascertain if flavonoids were present in the plant sample.

Tests to Detect Free Flavonoids

A portion of the powdered plant sample was heated with 10ml of ethyl acetate over a steam bath for three minutes. 1ml of diluted ammonium solution was added to 4ml of the filtrate after the mixture had been filtered. The presence of a yellow colour indicated that the flavonoids test was successful[13].

Test for lead acetate

0.5g of plant extract in double-distilled water was heated, and 1ml of a 10% lead acetate solution was added. Yellow precipitate is a sign of flavonoids' existence.

Reaction with sodium hydroxide

A 0.5 gramme plant sample was warmed in double-distilled water before being mixed with diluted sodium hydroxide. The yellow hue indicates the presence of flavonoids.

Investigate the alkaloids

First, 100 mg of plant extract were dissolved in diluted HCl, filtered, and the filtrate was then tested using Dragendroff and Mayer reagents.

Validation test

After the 5gm of the extract was obviously alkaline on litmus paper after being treated with a 40% solution of calcium hydroxide, it was extracted twice with 10ml of chloroform. Plates with thin layers revealed chloroform extract. In order to identify the drug, the chromatograms were first produced using a solvent solution (n-haxaneethyl acetate 4:1) and then sprayed with freshly

prepared Dragendorff's spray reagent. A pale yellow background contrasts with dark orange or yellow dots. Alkaloids were confirmed using the available information.

(The Liebman test) Test for steroids

0.5g of hydro-methanolic plant extract was dissolved in 2ml of acetic anhydride that had been refrigerated in an ice bath. Then H₂SO₄ was added. Steroid compounds are present when a colour shifts from violet to blue or green.

Testing terpenoids using Salkowski's method is necessary.

0.5g of plant extract were dissolved in 2ml of CHCl₃, and then cautiously added conc. H₂SO₄ to form a layer. The contact became reddish brown, indicating the presence of terpenoids.

Find saponins (Froth test)

0.5gm of the extract was dissolved in 10ml of distilled water for around 30 seconds. The test tube was vigorously shaken for about 30 seconds with the stopper in place. The test tube was allowed to stand upright for 30 minutes while being observed. After 30 minutes, a "honey comb" froth may still be developing on the liquid's surface, indicating the presence of saponins[14].

Test for tanin (Ferric chloride test)

About 0.5g of the dried powdered samples were boiled in 20ml of double-distilled water in a test tube, and then they were filtered. After adding a few drops of 0.1% ferric chloride, the colour was examined for any brownish green or blue-black hues.

Phlobatannin analysis

The production of a red precipitate after boiling a hydromethanolic extract of a plant sample in 1% aqueous hydrochloric acid served as proof that phlobatannins were present.

Testing for anthraquinones

The combination of 200 mg of plant extract and 6 ml of 1% HCl was heated before filtering. After filtering the filtrate and stirring it with 5 ml of benzene, a 10% ammonia solution was added in a volume of 2 ml.

Anthraquinones were detected when the mixture took on pink and violet colours after being shaken.

Phenolic compounds

A little amount of ferric sulphate crystals and around 100 mg of the dried plant extract were dissolved in double-distilled water. A phenolic component is present if a dark violet colour starts to appear.

Anti-bacterial techniques

The hydro-methanolic extract from *Glycyrrhiza glabra* roots was examined for its antibacterial activity using the Kerby-Bauer Disk Diffusion Susceptibility Test.

bacterial variety The gram-positive and gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexneri*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis* were used for antibacterial activity from our lab's stock culture.

Taking the Inhibitory Zone's Size (In mm)

The nutrient agar broth medium was used to conduct the antibacterial activities. Six different test tubes are used to divide the 1.3 grammes of nutritional broth into 100 millilitres of double- distilled water, where six bacterial strains are added to each test tube.

The bacterial cultures are swabbed onto the Petriplates once the nutrient agar medium has hardened, and the plates are then incubated at 37°C for 24 hours. Concentration We synthesized four different crude extract concentrations (100%, 75%, 50%, and 25%).

100% = 1g of crude extract in 1ml of freshly manufactured, double-distilled water, with created serial dilutions of 25%, 50%, and 75% following at 25mg, 50mg, and 75mg in 1ml each.

Antioxidant mechanisms

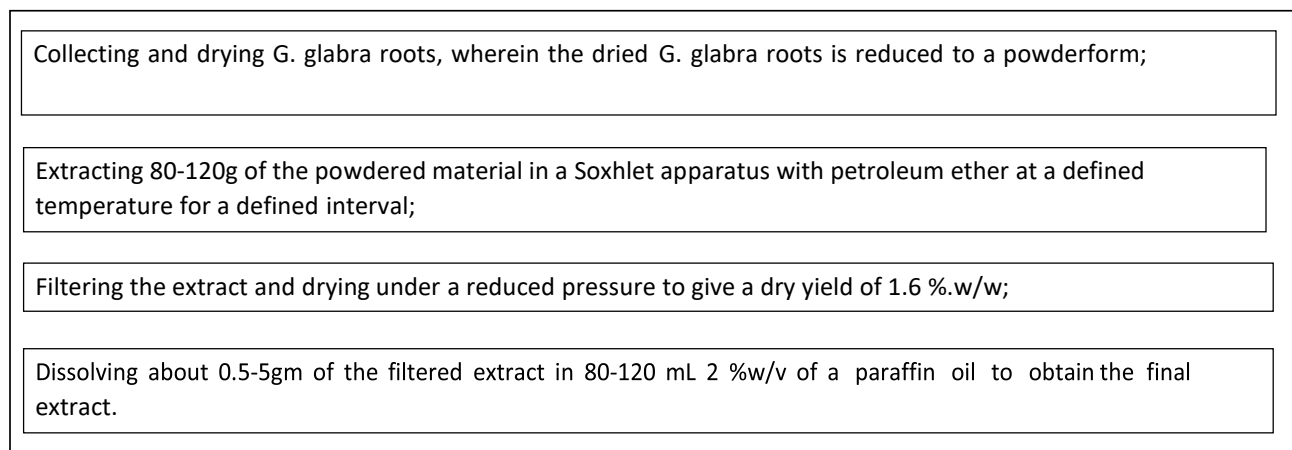
The anti-oxidant qualities of *G. glabra* root extract (10-100 g/ml) were evaluated using the Deoxyribose approach (Fenton reaction) of Halliwell and Aruoma, as shown in Table 2. reaction with thiobarbituric acid As the hydroxyl radical attacked deoxyribose, a chain of events that resulted in the creation of substances began[15].

Table:1 Preliminary phytochemical screening for hydro-methanolic root extract of *Glycyrrhiza glabra Linn*

S/N	Phytoconstituents	Test performed	Result
1.	Carbohydrates	Molisch's test	(-)
2.	Proteins	Copper sulphate test	(-)
3.	Flavonoids	Lead acetate test, NaOH solution test	(+)
4.	Alkaloids	Dragendroff's test	(+)
5.	Steroids	Lieberman's test	(+)
6.	Terpenoids	Salkowski's test	(+)
7.	Saponins	Froth test	(+)
8.	Tannins	Ferric chloride test	(+)
9.	Phlobatannins	HCL test	(-)

10.	Anthraquinones	Benzene test	(-)
11.	Glycosides	Keller-Killani test	(+)
12.	Phenolic Compounds	Ferric sulphate test	(-)

Figure:2 Extraction process

Table 2: The study of anti-oxidant activities of *Glycyrrhiza glabra* root extracts.

S.No	Concentration ($\mu\text{g/ml}$)	<i>Glycyrrhiza glabra</i> root extract
1.	10	9.90 \pm 0.72
2.	20	18.77 \pm 0.28
3.	30	14.86 \pm 0.97
4.	40	20.21 \pm 0.41
5.	50	32.63 \pm 0.83
6.	60	38.48 \pm 0.80
7.	70	46.29 \pm 1.12
8.	80	52.5 \pm 0.78
9.	90	62.33 \pm 1.36
10.	100	73.77 \pm 0.85

The Findings and Discussion

The principles of the invention will now be discussed in relation to the embodiment shown in the figures, with the embodiment being detailed using precise terminology.

However, it should be noted that this does **not** limit the scope of the invention; rather, additional alterations and modifications to the system shown therein as well as additional applications of

the invention's core concepts are currently being considered, as would typically be the case for a person skilled in the relevant field.

As those versed in the art would recognize, the aforementioned broad description and the following comprehensive explanation are just meant to show and explain the invention, not to restrict it.

The phrases "an aspect," "another aspect," or similar expressions refer to a specific feature, structure, or characteristic that is present in at least one embodiment of the present invention throughout this specification. Hence, references to "in an embodiment," "in another embodiment," and other similar expressions in this specification may or may not pertain to the same embodiment.

The phrases "comprises," "comprising," or any variants thereof, are meant to refer to a non-exclusive inclusion. For instance, a process or method that contains a list of stages could also include extra steps that are suggested by the process or method or that are not explicitly specified in the list but are nonetheless included in the list. The phrase "comprises...a" followed by one or more devices, subsystems, elements, structures, or components works similarly to the example before it in that it does not exclude the inclusion of other devices, subsystems, elements, structures, or components.

Unless otherwise specified, any technical and scientific terminology used herein have the same meaning as those that a person of ordinary competence in the applicable field would typically understand. The system, procedures, and examples given here are just meant to be used as guides; they are not meant to be comprehensive.

With reference to the accompanying drawings, the embodiments of the present invention will be described in further depth below.

Glycyrrhiza Glabra L petroleum ether extract is made and tested using 80–120 grammes of powdered G. glabra roots at a weight percentage of 1.6% and 80–120 milliliters of paraffin oil at a volume percentage of 2%.

Dr. DV Amla of the National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh, India, found G. glabra roots, and a voucher specimen (number NBRI-SOP-202) is maintained at NBRI for use in the future[18]. The roots were procured at Ramnagar, Uttarakhand, India's Corbett National Park during the month of June 2011.

Hair Increase The beginning and conclusion of hair development are seen in each of the three classes of animals. As compared to untreated mice and mice administered minoxidil, hair growth start and completion durations in animals treated with extract were significantly slowed down [18–20].

The beginning and finish of hair production happened 50% to 56% slower in mice treated with extract than in untreated mice. In comparison to the control group and animals treated with minoxidil, the hair on the animals treated with extract was 26.2% and 36.6% longer.

Compared to the mice treated with extract, which showed a greater follicle density and more anagenic hair follicles overall, the mice treated with minoxidil had less anagenic hair follicles.

The group that received extract treatment had more anagenic than telogenic hair on their skin. Also, compared to the control and minoxidil-treated groups, it had 69.6 and 13.0% more anagenic hair follicles.

As the Food and Drug Administration (FDA) of the United States has only authorized topical minoxidil and oral finasteride as therapies for alopecia, there is an unmet demand for innovative, effective hair growth enhancers. A thorough randomised placebo-controlled human study on the potassium channel opener minoxidil was undertaken by the Upjohn Company, and 54% of the treated patients showed success, compared to 34% of the placebo (control) group. Among the significant side effects of minoxidil that impact the skin include pruritis, dryness, scaling, localized irritation, and dermatitis. Within a year, 48% of those using finasteride medication had a resumption of hair growth. While finasteride is often well-tolerated, users noted that a tiny proportion of individuals stopped their treatment because of sexual problems brought on by the medication. For female users, finasteride is not advised[21].

The *G. glabra* petroleum ether extract demonstrated hair growth enhancement activities when compared to the control and minoxidil-treated groups. The animals that had been exposed to the extract generated longer, denser, and more anagenic hair and took less time to cover the female rats' bare skin with hair. Although *G. glabra* extract contains some estrogenic properties, minoxidil was thus less effective than the group that received 2% of the extract but more successful than the control group in promoting hair growth. The anagenic phase of hair development is prolonged by oestrogen, which encourages hair growth. The extract probably had an impact on testosterone metabolism. The 12 androgens are to blame for hair loss. They cause women's serum testosterone levels to drop. Given this, it is likely that hormonal regulation is what causes the extract to promote hair growth. After being applied topically, it seems that the extract would not have any negative systemic effects. Hence, the petroleum ether extract of *G. glabra* possesses effects that promote hair development.

According to a recent research on the herb *Eclipta alba*, which supported its role as a hair growth promoter, the antigens FGF-7 and Shh, as well as a deficiency in BMP4, favor the anagenic condition of hair. It was discovered that extract-treated rat skin had a larger percentage of anagenic than telogenic hair follicles when compared to control and minoxidil-treated rat skin. When the follicle develops in the anagen stage, it sheds its skin during the telogen stage.

An alternative hypothesis is that in order to completely understand the mechanism of the stimulation of hair formation by the petroleum ether root extract of *G. glabra*, investigations of skin cells' immunohistochemistry are necessary[22].

Examples of embodiments are given in both the explanation that occurred before the images. One or more of the aforementioned components may be combined into a single functional part with reasonable ease, as those trained in the art will observe. On the other hand, a specific component may be divided into a number of functional components. One embodiment's element

could appear in another. For instance, the sequence of the actions given below is only an example and is not required. The steps in a flow diagram don't necessarily have to be carried out in the exact same sequence or in their whole. The simultaneous performance of separate actions is another possibility. These particular examples in no way restrict the variety of embodiments.

Changes to the structure, size, and material use are only a few examples of the many adjustments that may be made, whether or not they are explicitly included in the specification. At a minimum, the following claims' outlines are presented.

The aforementioned discussion of advantages, additional benefits, and problem-solving techniques was related to specific embodiments. Benefits, advantages, solutions to difficulties, and any other elements that could lead to the occurrence or improvement of any benefit, advantage, or solution are not to be interpreted as necessary, impermissible, or constitutive elements of any one or more of the claims [23–24].

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