# HERBAL PREPARATION FOR STIMULATION OF HAIR GROWTH, CONTROL OF HAIR FALL, DANDRUFF AND INFECTIONS THEREOF USING *AGERATUM* SPP.

## TECHNICAL FIELD

**[0001]** Embodiments are generally related to the field of pharmaceutics, cosmetics and allied industries. Embodiments are also related to herbal extracts for stimulation of hair growth, control of hair fall, dandruff and infections thereof. Embodiments are particularly related to an herbal extraction process for preparing hair growth simulator using herbaceous plants, *Ageratum* spp. (*Ageratum conyzoides* (Synonym: *Ageratum coeruleum*) and *Ageratum houstonianum*)

## BACKGROUND OF THE INVENTION

**[0002]** Hair loss/hair fall has become a ubiquitous affliction of human beings (both male and female gender) with the growing impact of global pollution and lifestyle changes. Most common reasons of radical or premature hair loss/hair fall are genetic predisposition, endocrine disorders, medication, radiation, chemotherapy, and exposure to chemicals, nutritional factors, generalized or local skin diseases, stress, child birth, Alopecia areata and mechanical damage such as Trichotillomania, hair styling treatment, hair braids and weaves.

**[0003]** In general, the hair in humans is generated by the hair follicles implanted in the scalp. A healthy head of hair is said to contain between 100000 and 150000 hairs, and each hair within this head of hair possesses its own cycle. The life cycle of the hair can be described in three successive physiological phases such as, Anagen, Catagen and Telogen. Anagen is a phase of hair growth which may last from a few weeks to 10 years, Catagen phase is a transient phase of involution of the follicle and ceasing of hair growth with degeneration of the root which may last for a few weeks and Telogen is a phase of shedding of the hair with the root moving up towards the surface by lasting 1 to 5 months. At the end of Telogen phase, the hair therefore disappears from the scalp and this disappearance may extend from a few days to a few months before the follicle

is reactivated to give a new hair in the Anagen phase.

**[0004]** Unfortunately, after a certain number of cycles, the follicle permanently ceases its production and the hair may be regarded as dead which may lead to baldness. As baldness progresses, there is a shift in the percentages of hair follicles in each phase with the majority shifting from Anagen to Telogen. The size of hair follicles is also known to decrease while the total number remains relatively constant. A variety of devices and procedures are adapted to simulate the hair follicles and re-generate the hair growth in such cases. Most of the prior art approaches either employ a simulator device or a drug for the hair growth. One such common technique is hair transplantation. Briefly, plugs of skin containing hair are transplanted from areas of the scalp where hair was growing to bald or balding areas of the scalp. The transplantation technique can be a time-consuming and relatively costly affair. Other approaches include ultra-violet radiation and exercise therapy. While cosmetic aids are another significant approach for the stimulation of hair-growth, there is still a need in the art for compounds which may have greater stimulatory effect on hair growth.

**[0005]** Based on the foregoing a need therefore exists for an improved herbal preparation for stimulation of hair growth, control of hair fall, dandruff and infections thereof using plants, *Ageratum* spp., as described in greater detail herein.

## BRIEF SUMMARY

**[0006]** The following summary is provided to facilitate an understanding of some of the innovative features unique to the disclosed embodiment and is not intended to be a full description. A full appreciation of the various aspects of the embodiments disclosed herein can be gained by taking the entire specification, claims, drawings, and abstract as a whole.

**[0007]** It is, therefore, one aspect of the disclosed embodiments to provide an improved herbal extraction for stimulating hair growth.

**[0008]** It is another aspect of the disclosed embodiments to provide an improved method for extracting compounds of plants, *Ageratum* spp.

**[0009]** It is further aspect of the disclosed embodiments to provide an improved herbal preparation for stimulation of hair growth, control of hair fall, dandruff and infections thereof using plants, *Ageratum* spp.

**[0010]** The aforementioned aspects and other objectives and advantages can now be achieved as described herein. An herbal preparation for stimulation of hair growth and control of hair fall, dandruff and infections thereof using herbaceous plants, *Ageratum* spp. (including *Ageratum conyzoides, Ageratum coeruleum* and *Ageratum houstonianum*) is disclosed herein. At least one compound of plants, *Ageratum* spp. such as, N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, 3',4',5,6,7,8-hexamethoxyflavone, Phytol, Precocene, Caryophyllene, Squalene, α-linolenic acid, 9,12-octadecadienoic acid (Z,Z)-, Hexadeconoic acid and Hydrocoumarin can be extracted utilizing a Common Plant Extraction Approach (CPEA). The extraction of compounds can be performed utilizing an ethanol solvent and a methanol solvent (i.e., alcoholic solvents) separately and the concentrated materials obtained at both the solvents are pooled together (or separately) in order to obtain an herbal extract for simulating the follicles of the hair. The compounds of plants, *Ageratum* spp. in

combination with an acceptable carrier or diluent can be further prepared into an herbal extract formulations such as, a liquid, oil or gel formulation for topical application. Such an herbal preparation using plants, *Ageratum* spp. can be utilized as an efficient hair care solution in a wide range of hair growth related problems of humans in a cost effective manner.

**[0011]** The Common Plant Extraction Approach (CPEA) described herein can be a principle extraction technique that is well known in the art for extracting the compounds of a plant. Initially, the leaves and soft stems of *Ageratum* spp. (preferably 60 to 90 days old plants) can be sliced into pieces (approx. 1 cm size) and shade dried at room temperature. The sliced pieces can be also dried using a fluid flash dryer. Further, a mechanized pulverizing machine can be employed to grind the dried plant slices into coarse powder in order to thereby extract the compounds using the ethanol and methanol solvents separately. The solvent extraction process described herein can be performed at a 1:10 (W/V) ratio using a soxhlet apparatus in order to thereby separate the green colored solvent extracts using a rotoevaporator.

**[0012]** The compounds obtained at both the ethanol solvent extraction process and the methanol solvent extraction process are further pooled together (or used separately) in order to form the herbal extract for simulating the hair growth. The compounds extracted at the methanol solvent extraction process can be such as, for example, but not limited to, N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, 1-deoxy-d-mannitol, 15-hydroxypentadecanoic acid, 2-chromenone, 3',4',5,6,7,8-hexamethoxyflavone, 3,5-di-tert-butylbenzoic acid, 3,7,11,15-tetramethylhexadec-2-en-1-ol, 4-tert-butyl-2,6-dimethylacetophenone, 6-vinyl-7-methoxy-2,2-dimethylchromene, Anhydro-d-mannosan, Beta-Funebrene, Caryophyllene, Coumarin, Hexa-o-methylmyricitin, Hydrocoumarin, Linoleoyl chloride, Methyl cis-11,14,17-icosatrienoate, Methyl linoleate, Neophytadiene, p-Octylacetophenone, Phenol, 2,4,6-tris(1,1-dimethylethyl), Phytol, Precocene I and Squalene.

**[0013]** The compounds extracted at the ethanol solvent extraction process can be

such as, for example, but not limited to, N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, (Z)-,Beta-farnesene, 1-(3,5-ditert-butyl-4-hydroxyphenyl)ethanone, 2-(1,3-benzodioxol-5-yl)-5-hydroxy-3,6,7,8-tetramethoxy-4h-chromen-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-4H-1-benzopyran-4-one, 2,4,6-tri-tert-butylphenol, 2-chromanone, 2H-chromen-2-one, 3',4',5,6,7,8-hexamethoxyflavone, 3,5-di-tert-butylbenzoic acid, 3,7,11,15-tetramethylhexadec-2-en-1-ol, 3-thujanol, 4-tert-butyl-2,6-dimethylacetophenone, 6-demethoxyageratochromene, 6-vinyl-7-methoxy-2,2-dimethylchromene, 9,12-octadecadienoic acid (Z,Z)-, All-trans-squalene, Alpha-benzopyrone, Alpha-caryophyllene, Alpha-linolenic acid, Alpha-tocopherol-β-D-mannoside, Beta-sesquiphellandrene, Butylphosphonic acid, hexyl 4-(2-phenylprop-2-yl)phenyl ester, Caryophyllene, Coumarin, Delta-cadinene, Dotriacontane, Gamma-mourolen, Gamma-sitosterol, Germacrene D, Gitoxigenin, Hexacosane, Hexadeconoic acid, Hydrocoumarin, Methyl cis-11,14,17-icosatrienoate, Methyl linoleate, Methyl palmitate, Neophytadiene, N-hexatriacontane, n-tertacontane, N-tetracosane, N-tetratriacontane, N-triacontane, Phytol, Precocene I, Precocene II, Squalene, Stigmasterol, Stimasta-4,2,2-dien-3,β-ol, and Tetracontane.

**[0014]** The solvents utilized for extracting compounds herein should not be construed in any limited sense. Those skilled in the art can understand that similar kind of solvents such as hexane, ethyl acetate, acetone, chloroform, dichloromethane, etc. can also tried for preparing herbal extraction and developing the hair growth stimulant formulation. The herbal extract is further formulated with an acceptable carrier or diluent in order to formulate as liquid, shampoo, oil or gel for topical application. The herbal extract can be dissolved in the sterile Distilled water (at 1-5 g/l) and the aqueous suspension can be filtered (through 1 to 5 µ filter, pH adjusted to neutral) in order to form a liquid formulation of the herbal extract. Similarly, the herbal extract is dissolved in vegetable oil or PG/PL and the suspension is filtered (through 1 to 5 µ filter) in order to form an oil formulation. The herbal extract can be dissolved in a small quantity of ethanol and filtered (through 1 to 5 µ filter) in order to form the gel formulation. The mixture can be mixed vigorously using mechanized homogenizer to get the uniform gel product.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0015]** The accompanying figures, in which like reference numerals refer to identical or functionally-similar elements throughout the separate views and which are incorporated in and form a part of the specification, further illustrate the present invention and, together with the detailed description of the invention, serve to explain the principles of the present invention.

**[0016]** FIG. 1 illustrates a perspective view of a process for preparing an herbal extract formulation using herbaceous plants, *Ageratum* spp., in accordance with the disclosed embodiments; and

**[0017]** FIG. 2 illustrates a high level flow chart of operations illustrating logical operational steps of a method for preparing herbal extract formulation using herbaceous plants, *Ageratum* spp., in accordance with the disclosed embodiments.

## DETAILED DESCRIPTION

**[0018]** The particular values and configurations discussed in these non-limiting examples can be varied and are cited merely to illustrate at least one embodiment and are not intended to limit the scope thereof.

**[0019]** The embodiments now will be described more fully hereinafter with reference to the accompanying drawings, in which illustrative embodiments of the invention are shown. The embodiments disclosed herein can be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art. Like numbers refer to like elements throughout. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items.

**[0020]** FIG. 1 illustrates a perspective view of a process 100 for preparing an herbal extract formulation 190 using herbaceous plants, *Ageratum* spp. (including *Ageratum conyzoides,* (Synonym: *Ageratum coeruleum*) and *Ageratum houstonianum*) 110, in accordance with the disclosed embodiments. *Ageratum* spp. are annual herbaceous plants belonging to the family Asteraceae. They are common weeds and grow rapidly. They are known by several names such as goat weed, chick weed, white weed, etc. *Ageratum* spp. are found in many countries ranging from Southeastern North America to Central America, Asia and Africa; but the center of origin is in Central America and the Caribbean. *Ageratum* spp. are also found in several countries in tropical and sub-tropical regions, including India, Brazil, Vietnam, Nigeria, Kenya, Congo, Cameroon, etc. in abundance.

**[0021]** *Ageratum* spp. are very valuable herbs as they contain many bioactive compounds including flavonoids, alkaloids, cumarins, essential oils, chromenes,

benzofurans, terpenoids and tannins that have medicinal, antimicrobial and insecticidal properties. The extracts of *Ageratum conyzoides* 110 and other *Ageratum* species have been used to cure a wide range of ailments such as muscle pains, inflammations, dysentery, indigestion, constipation, wounds in humans. The use of *Ageratum* spp. 110 in stimulation of hair follicles is novel. It has not hitherto been known that *Ageratum* spp. 110 have a simulative action on hair growth and hair fall control.

**[0022]** A Common Plant Extraction Approach (CPEA) is adapted to prepare the herbal extract from *Aageratum* spp. 110. The Common Plant Extraction Approach (CPEA) described herein can be a principle extraction technique that is well known in the art for extracting the compounds 180 of plants. Note that other extraction processes can be alternatively employed in the place of suggested without losing the scope of invention. The leaves and soft stems of *Ageratum* spp. 110 preferably 60 to 90 days old plants are initially sliced into pieces 120 (i.e., approx. 1 cm) and shade dried at room temperature (approx. 28-30°C) for 48 hrs to 72 hrs. The sliced pieces 120 can be also dried at 45°C using a fluid flash dryer for 24 hrs. Further, the dried plant slices 120 are ground to course powder using a mechanized pulverizing machine 130 in order to thereby extract the compounds 180 using the ethanol solvent 160, methanol solvent 170 and other solvents separately. The solvent extraction process described herein can be performed at a 1:10 (W/V) ratio using a soxhlet apparatus in order thereby separate the green colored solvent extracts using a rotoevaporator. Note that the solvents utilized for extracting compounds herein should not be construed in any limited sense. Those skilled in the art can understand that similar kind of solvents such as hexane, ethyl acetate, acetone, chloroform, dichloromethane, etc. can also tried for preparing herbal extraction and developing the hair growth stimulant formulation.

**[0023]** The compounds extracted at the methanol solvent extraction process 150 can be such as, for example, but not limited to, N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, 1-deoxy-d-mannitol, 15-hydroxypentadecanoic acid, 2-chromenone, 3',4',5,6,7,8-hexamethoxyflavone, 3,5-di-tert-butylbenzoic acid, 3,7,11,15-tetramethylhexadec-2-en-1-ol, 4-tert-butyl-2,6-dimethylacetophenone, 6-vinyl-

7-methoxy-2,2-dimethylchromene, Anhydro-d-mannosan, Beta-Funebrene, Caryophyllene, Coumarin, Hexa-o-methylmyricitin, Hydrocoumarin, Linoleoyl chloride, Methyl cis-11,14,17-icosatrienoate, Methyl linoleate, Neophytadiene, p-Octylacetophenone, Phenol, 2,4,6-tris(1,1-dimethylethyl), Phytol, Precocene I and Squalene.

**[0024]** Table 1 illustrates the list of compounds extracted by treating the plant material 120 using the methanol solvent 170.

| # | Compound |
|---|---|
| 1 | N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine |
| 2 | 1-deoxy-d-mannitol |
| 3 | 15-hydroxypentadecanoic acid |
| 4 | 2-chromenone |
| 5 | 3',4',5,6,7,8-hexamethoxyflavone |
| 6 | 3,5-di-tert-butylbenzoic acid |
| 7 | 3,7,11,15-tetramethylhexadec-2-en-1-ol |
| 8 | 4-tert-butyl-2,6-dimethylacetophenone |
| 9 | 6-vinyl-7-methoxy-2,2-dimethylchromene |
| 10 | Anhydro-d-mannosan |
| 11 | Beta-Funebrene |
| 12 | Caryophyllene |
| 13 | Coumarin |
| 14 | Hexa-o-methylmyricitin |
| 15 | Hydrocoumarin |
| 16 | Linoleoyl chloride |
| 17 | Methyl cis-11,14,17-icosatrienoate |
| 18 | Methyl linoleate |
| 19 | Neophytadiene |
| 20 | p-Octylacetophenone |
| 21 | Phenol, 2,4,6-tris(1,1-dimethylethyl) |
| 22 | Phytol |
| 23 | Precocene I |
| 24 | Squalene |

**Table 1**

**[0025]** Similarly, the compounds extracted at the ethanol solvent extraction process can be such as, for example, but not limited to, N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, (Z)-,Beta-farnesene, 1-(3,5-ditert-butyl-4-hydroxyphenyl)ethanone, 2-(1,3-benzodioxol-5-yl)-5-hydroxy-3,6,7,8-tetramethoxy-4h-chromen-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-4H-1-benzopyran-4-one, 2,4,6-tri-tert-butylphenol, 2-chromanone, 2H-chromen-2-one, 3',4',5,6,7,8-hexamethoxyflavone, 3,5-di-tert-butylbenzoic acid, 3,7,11,15-tetramethylhexadec-2-en-1-ol, 3-thujanol, 4-tert-butyl-2,6-dimethylacetophenone, 6-demethoxyageratochromene, 6-vinyl-7-methoxy-2,2-dimethylchromene, 9,12-octadecadienoic acid (Z,Z)-, All-trans-squalene, Alpha-benzopyrone, Alpha-caryophyllene, Alpha-linolenic acid, Alpha–octadecanoic acid, Alpha-tocopherol-β-D-mannoside, Beta-sesquiphellandrene, Butylphosphonic acid, hexyl 4-(2-phenylprop-2-yl)phenyl ester, Caryophyllene, Coumarin, Delta-cadinene, Dotriacontane, Gamma-mourolen, Gamma-sitosterol, Germacrene D, Gitoxigenin, Hexacosane, Hexadeconoic acid, Hydrocoumarin, Methyl cis-11,14,17-icosatrienoate, Methyl linoleate, Methyl palmitate, Neophytadiene, N-hexatriacontane, n-tertacontane, N-tetracosane, N-tetratriacontane, N-triacontane, Phytol, Precocene I, Precocene II, Squalene, Stigmasterol, Stimasta-4,2,2-dien-3,β-ol and Tetracontane, Tetradecanoic acid.

**[0026]** Table 2 illustrates the list of compounds extracted by treating the plant material 120 using the ethanol solvent 170.

| # | Compound |
|---|---|
| 1 | N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine |
| 2 | (Z)-,Beta-farnesene |
| 3 | 1-(3,5-ditert-butyl-4-hydroxyphenyl)ethanone |
| 4 | 2-(1,3-benzodioxol-5-yl)-5-hydroxy-3,6,7,8-tetramethoxy-4h-chromen-4-one |
| 5 | 2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-4H-1-benzopyran-4-one |
| 6 | 2,4,6-tri-tert-butylphenol |
| 7 | 2-chromanone |

| | |
|---|---|
| 8 | 2H-chromen-2-one |
| 9 | 3',4',5,6,7,8-hexamethoxyflavone |
| 10 | 3,5-di-tert-butylbenzoic acid |
| 11 | 3,7,11,15-tetramethylhexadec-2-en-1-ol |
| 12 | 3-thujanol |
| 13 | 4-tert-butyl-2,6-dimethylacetophenone |
| 14 | 6-demethoxyageratochromene |
| 15 | 6-vinyl-7-methoxy-2,2-dimethylchromene |
| 16 | 9,12-octadecadienoic acid (Z,Z)- |
| 17 | All-trans-squalene |
| 18 | Alpha-benzopyrone |
| 19 | Alpha-caryophyllene |
| 20 | Alpha-linolenic acid |
| 21 | Alpha–octadecanoic acid |
| 22 | Alpha-tocopherol-β-D-mannoside |
| 23 | Beta-sesquiphellandrene |
| 24 | Butylphosphonic acid, hexyl 4-(2-phenylprop-2-yl)phenyl ester |
| 25 | Caryophyllene |
| 26 | Coumarin |
| 27 | Delta-cadinene |
| 28 | Dotriacontane |
| 29 | Gamma-mourolen |
| 30 | Gamma-sitosterol |
| 31 | Germacrene D |
| 32 | Gitoxigenin |
| 33 | Hexacosane |
| 34 | Hexadeconoic acid |
| 35 | Hydrocoumarin |
| 36 | Methyl cis-11,14,17-icosatrienoate |
| 37 | Methyl linoleate |
| 38 | Methyl palmitate |
| 39 | Neophytadiene |
| 40 | N-hexatriacontane |

| | |
|---|---|
| 41 | n-tertacontane |
| 42 | N-tetracosane |
| 43 | N-tetratriacontane |
| 44 | N-triacontane |
| 45 | Phytol |
| 46 | Precocene I |
| 47 | Precocene II |
| 48 | Squalene |
| 49 | Stigmasterol |
| 50 | Stimasta-4,2,2-dien-3,β-ol |
| 51 | Tetracontane |
| 52 | Tetradecanoic acid |

**Table 2**

**[0027]** The green colored solvent extract from ethanol/methanol/other solvents are removed using vacuum evaporation. Among the compounds of plants, *Ageratum* spp. four compounds, 9,12-octadecadienoic acid (Z,Z)-, Alpha-linolenic acid, Hexadeconoic acid and Hydrocoumarin are identified as 5 Alpha-reductase inhibitors. The 5 alpha reductase enzyme metabolizes the testosterone (male hormone) into dihydrotestosterone (DHT), which is known to induce scalp hair loss in man. The 5 alpha reductase inhibitors present in the extracts of *Ageratum* spp. can attach with 5 alpha reductase enzyme and prevent it to convert the testosterone into DHT. Thereby decrease and stop the hair loss.

**[0028]** Table 3 illustrates the list of four compounds identified as 5 Alpha reductase inhibitors present in both ethanol and methanol extracts of the hair growth product.

| # | **Compound** |
|---|---|
| 1 | 9,12-octadecadienoic acid (Z,Z)- |
| 2 | α-linolenic acid |
| 3 | Hydrocoumarin |
| 4 | Hexadeconoic acid |

**Table 3**

**[0029]** The compounds obtained at both the ethanol solvent extraction process 140 and the methanol solvent extraction process 150 (and other solvent processes) are further pooled together in order to form the herbal extract 180 for simulating the hair growth. The green colored solvent extracts are separated and the ethanol/methanol/other solvents are removed using vacuum evaporation. Among the compounds of plants, *Ageratum* spp. six major compounds, N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, 3',4',5,6,7,8-hexamethoxyflavone, Phytol, Precocene, Caryophyllene, Squalene are identified as common components present in both ethanol and methanol extracts of the hair growth product.

**[0030]** Table 4 illustrates the list of six major compounds identified as common components present in both ethanol and methanol extracts of the hair growth product.

| # | **Compound** |
|---|---|
| 1 | N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine |
| 2 | 3',4',5,6,7,8-hexamethoxyflavone |
| 3 | Phytol |
| 4 | Precocene |
| 5 | Caryophyllene |
| 6 | Squalene |

**Table 4**

**[0031]** The herbal extract contains the above 5 alpha reductase inhibitors and other major compounds of plants, *Aageratum* spp. 110 in combination with an acceptable carrier or diluent can be further prepared into an herbal extract formulations 190 such as, aqueous (liquid), gel, oil or shampoo formulation for topical application.

**[0032]** The herbal extract of *Ageratum* spp. with the above mentioned compounds can be dissolved in the sterile distilled water (at 1-5 g/l) and the aqueous suspension

can be filtered (through 1 to 5 μ filter, pH adjusted to neutral) in order to form a liquid formulation 192 of the herbal extract. Similarly, the herbal extract is dissolved in vegetable oil/PG/PL and the suspension is filtered (through 1 to 5 μ filter) in order to form an oil formulation 194. The herbal extract can be dissolved in a small quantity of ethanol and filtered (through 1 to 5 μ filter) in order to form the gel formulation 196. The mixture can be mixed vigorously using mechanized homogenizer to get the uniform gel product. The herbal extract can be dissolved in an acceptable carrier or diluent in order to get the shampoo formulation 198. The mixture can be mixed vigorously using mechanized homogenizer to get the uniform shampoo product. The herbal extract formulation 190 disclosed herein can be utilized to effectively control the hair loss due to scenarios such as, but not limited to, genetic predisposition, endocrine disorders, medication, radiation, chemotherapy, exposure to chemicals, nutritional factors, generalized or local skin diseases, stress, child birth, Alopecia areata and mechanical damage, Trichotillomania, hair styling treatment, hair braids and weaves. The herbal extract formulation 190 prevents the cease of production of hair due to physiological phase changes such as, Anagen, Catagen and Telogen in humans.

**[0033]** FIG. 2 illustrates a high level flow chart of operations illustrating logical operational steps of a method 200 for preparing an herbal extract formulation 190 using herbaceous plants, *Ageratum* spp. 110, in accordance with the disclosed embodiments. Note that in FIGS. 1-2, identical or similar blocks are generally indicated by identical reference numerals. Initially, the plants, *Ageratum* spp. 110 with 60 to 90 days of age can be identified and the leaves and soft stems of the *Ageratum* spp. 110 can be sliced into pieces (approx. 1 cm size) 120, as illustrated at block 205. The sliced pieces 120 of the plants, *Ageratum* spp. 110 can be further shade dried at room temperature or dried at 45°C using a fluid flash dryer, as depicted at block 210. A mechanized pulverizing machine 130 can be employed to grind the dried plant slices 120 into coarse powder, as illustrated at block 215. The plant material can be further extracted using the ethanol and methanol solvents and other solvents 160 and 170 separately, as depicted at blocks 220 and 230. The solvent extraction process 140 and 150 described herein can be performed at a 1:10 (W/V) ratio using a soxhlet apparatus. The green colored

solvents can be further extracted and separated by removing ethanol/methanol/other solvents 160 and 170 using a rotoevaporator, as illustrated at blocks 225 and 235.

**[0034]** The compounds obtained at both the ethanol solvent extraction process 140 and the methanol solvent extraction process 150 can be pooled together or separately in order to form the herbal extract 180 for simulating the hair growth, as depicted at block 240. As described earlier, six major compounds including N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, 3',4',5,6,7,8-hexamethoxyflavone, Phytol, Precocene, Caryophyllene, Squalene are identified as common marker components in the hair growth product. Further, four compounds, α-linolenic acid, 9,12-octadecadienoic acid (Z,Z)-, Hexadeconoic acid and Hydrocoumarin are identified as 5 α-reductase inhibitors in the ethanol extract of *Ageratum* spp. and these compounds probably responsible for hair loss preventing activity of the hair growth product. As illustrated at block 245, the concentrated herbal extract 180 can be further formulated with an acceptable carrier or diluent in order to formulate as liquid, oil, gel or shampoo for topical application. The purified form of herbal extract 180 can be dissolved in the sterile distilled water (at 1-5 g/l) in order to filter the aqueous suspension (through 1 to 5 μ filter, pH adjusted to neutral), as illustrated at block 250. The concentrated material can be further added with acceptable carrier or diluent in order to form the aqueous (liquid) formulation 192 of the herbal extract, as illustrated at block 255.

**[0035]** Similarly, the herbal extract 180 can be dissolved in vegetable oil/PG/PL in order to filter the suspension (through 1 to 5 μ filter), as illustrated at block 260. The concentrated extract 180 can be further added with the acceptable carrier and diluent (e.g., coconut oil) in order to form the oil formulation 194, as depicted at block 265. The concentrated material 180 can be dissolved in a small quantity of ethanol in order to filter the suspension (through 1 to 5 μ filter), as illustrated at block 270. The concentrated material 180 can be further added with the acceptable carrier and diluent in order to form the gel formulation 196, as depicted at block 275. The mixture can be mixed vigorously using mechanized homogenizer to get the uniform gel product 196. The concentrated material 180 can be further added with the acceptable carrier and

diluent in order to form the shampoo formulation 198, as depicted at block 275. The mixture can be mixed vigorously using mechanized homogenizer to get the uniform shampoo product 198. Note that the aqueous (liquid), oil, gel and shampoo formulations 192, 194, 196 and 198 of the herbal extracts are packed separately in PP bottles aseptically. Such an herbal preparation using plants, *Ageratum* spp. can be utilized as an efficient hair care solution in a wide range of hair growth related problems of humans in a cost effective manner.

**[0036]** The clinical trials on the with respect to the formulations 192-198 have proved that the formulations do not have any adverse events and deviations. Under standard lab conditions, the formulations 192-198 did not indicated a potential for dermal irritation and/or allergic contact sensitization.

**[0037]** Three different formulations such as aqueous (liquid), gel and oil developed using the herbal preparation of *Ageratum* spp. were tested for hair growth promotion in animal studies using C57BL/6 mice model. The experimental design is as follow:

| **GroupDescription** | |
|---|---|
| Group-I | Negative control |
| Group-II | Placebo – 1 (Gel excluding herbal extract) |
| Group-III | Placebo – 2 (Aqueous excluding herbal extract) |
| Group-IV | Placebo – 3 (Oil excluding herbal extract) |
| Group-V | Formulation - 1 (Gel with herbal extract) |
| Group-VI | Formulation – 2 (Aqueous with herbal extract) |
| Group-VII | Formulation – 3 (Oil with herbal extract) |
| Group-VIII | Standard Market Product |

**[0038]** The hair growth potential of the given formulations was evaluated using C57BL/6 mice and for which, approval from ethical committee was obtained. Primary skin irritation test for all formulations was done for 48 hours. The hairs were removed from the mice using conventional method (Paraffin wax strip) and hair growth

formulations were applied in mice on regular intervals of 24 hours. The following are the observations made.

- Colour change.
- Raising of hair follicle.
- Excision of skin for H & E staining.
- Immuno- staining to differentiate the keratinocytes.
- Hair density measurements.

**[0039]** Among the eight groups, Group I acted as a negative control and Group VIII acted as a positive control. The positive control group received (standard market product) as test compound according to the standard procedures. All the three formulations alone excluding the active principle were kept as Placebo (Group II, III, and IV). Hair depilation was made by paraffin wax strips. All the test formulations were applied at 24 hours intervals and the animals were kept under observations on changes in skin colour. Since the chosen mice displayed change in the skin colour with reference to hair growth, the indication on skin colour implies the efficacy of the test formulations. It has been observed that both negative control and placebo groups displayed normal hair growth profile, whereas mice in the groups of V, VI, VII and VIII have displayed advancement in hair growth pattern. The colouration from pink to grey and then to dark was significantly advanced in Group VII and it was comparable with Group VIII. Similarly, the H&E staining of sections also demonstrated the advancement in hair follicle regeneration and immunostaining has suggested the early keratinization in test formulations and standard market product treated mice.

**[0040]** Interestingly, the hair density was maximum in Group VIII, followed by VII and V and the size of hair and hair bulb nature were also significant changed in the above Groups mice compared to negative control and Placebo treated mice. Among the three formulations (gel, aqueous and oil), the gel and oil formulations were more effective than the aqueous formulation. Although, the aqueous formulation induced localized hair growth profile, because of non-adherence to the skin, the expected results

were not obtained. Overall, the study suggested, that the gel and oil formulations displayed effective hair growth profile comparable to that of positive control, standard product.

**[0041]** Similarly, the 10% aqueous herbal extract prepared using *Ageratum* spp. was tested against dandruff causing fungal pathogens, *Pityrosporum ovale* and *Pityrosporum folliculitis* under in vitro condition by well diffusion technique. The experimental design is as follow:

| Treatment | Description |
|---|---|
| 1 | Sterile water - Negative control) |
| 2 | Ketaconazole (100 µg/ml) – Positive control |
| 3 | Herbal extract (10% @ 50 µl/well) |
| 4 | Herbal extract (10% @ 100 µl/well) |

**[0042]** The agar plates were prepared and the dandruff pathogens, *Pityrosporum ovale* and *Pityrosporum folliculitis* were inoculated over the agar surface. Four wells were made using sterile cork borer and 10% aqueous herbal extract of *A. conyzoides* was added at 50 and 100 µl in respective well. Commercial fungicide, Ketaconazole at 100 µg/ml (Positive control) and sterile water (Negative control) were also added in respective wells. The plates were incubated at room temperature for seven days and observed for the inhibition of fungal growth.

**[0043]** Prominent zone of inhibition of both the dandruff pathogens, *P. ovale* and *P. folliculitis* was observed around aqueous herbal extract of *A. conyzoides* at 50 and 100 µl added wells and it was higher than the commercial fungicide, Ketaconazole added well. No growth inhibition was recorded around sterile water added well. In summary, the study suggested that the aqueous herbal extract of *A. conyzoides* exhibited superior antifungal activity against the dandruff pathogens, *P. ovale* and *P. folliculitis* than the commercial antidandruff agent, Ketaconazole.

**[0044]** It will be appreciated that variations of the above-disclosed and other features and functions, or alternatives thereof, may be desirably combined into many other different systems or applications. Also that various presently unforeseen or unanticipated alternatives, modifications, variations or improvements therein may be subsequently made by those skilled in the art which are also intended to be encompassed by the following claims.

## CLAIMS

I/We claim:

1. An herbal preparation for stimulation using herbaceous plants, *Ageratum* spp., comprising:

at least one primary compound of a herbaceous plant, *Ageratum spp.* can be extracted using a common plant extraction approach (CPEA) wherein said at least one primary compound can be extracted using an ethanol solvent and a methanol solvent separately; and

an herbal formulation can be prepared by pooling said at least one primary compound of said herbaceous plant, *Ageratum spp.* at said ethanol solvent and said methanol solvent in order to thereby obtain an herbal extract by adding an acceptable carrier for simulating follicles of hair wherein said herbal extract can be effectively used for hair growth and control of hair fall, dandruff and infections thereof.

2. The herbal preparation of claim 1 wherein said at least one primary compound comprises at least one of the following concentrated materials: N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, 3',4',5,6,7,8-hexamethoxyflavone, Phytol, Precocene, Caryophyllene, Squalene, α-linolenic acid, 9,12-octadecadienoic acid (Z,Z)-, Hexadeconoic acid and Hydrocoumarin.

3. The herbal preparation of claim 1 wherein said methanol solvent extraction process extracts at least one of the following compounds: N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, 1-deoxy-d-mannitol, 15-hydroxypentadecanoic acid, 2-chromenone, 3',4',5,6,7,8-hexamethoxyflavone, 3,5-di-tert-butylbenzoic acid, 3,7,11,15-tetramethylhexadec-2-en-1-ol, 4-tert-butyl-2,6-dimethylacetophenone, 6-vinyl-7-methoxy-2,2-dimethylchromene, Anhydro-d-mannosan, Beta-Funebrene, Caryophyllene, Coumarin, Hexa-o-methylmyricitin, Hydrocoumarin, Linoleoyl chloride, Methyl cis-11,14,17-icosatrienoate, Methyl linoleate, Neophytadiene, p-Octylacetophenone, Phenol, 2,4,6-tris(1,1-dimethylethyl), Phytol, Precocene I and Squalene.

4. The herbal preparation of claim 1 wherein said ethanol solvent extraction process extracts at least one of the following compounds: N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine, (Z)-,Beta-farnesene, 1-(3,5-ditert-butyl-4-hydroxyphenyl)ethanone, 2-(1,3-benzodioxol-5-yl)-5-hydroxy-3,6,7,8-tetramethoxy-4h-chromen-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7,8-tetramethoxy-4H-1-benzopyran-4-one, 2,4,6-tri-tert-butylphenol, 2-chromanone, 2H-chromen-2-one, 3',4',5,6,7,8-hexamethoxyflavone, 3,5-di-tert-butylbenzoic acid, 3,7,11,15-tetramethylhexadec-2-en-1-ol, 3-thujanol, 4-tert-butyl-2,6-dimethylacetophenone, 6-demethoxyageratochromene, 6-vinyl-7-methoxy-2,2-dimethylchromene, 9,12-octadecadienoic acid (Z,Z)-, All-trans-squalene, Alpha-benzopyrone, Alpha-caryophyllene, Alpha-linolenic acid, Alpha-tocopherol-β-D-mannoside, Beta-sesquiphellandrene, Butylphosphonic acid, hexyl 4-(2-phenylprop-2-yl)phenyl ester, Caryophyllene, Coumarin, Delta-cadinene, Dotriacontane, Gamma-mourolen, Gamma-sitosterol, Germacrene D, Gitoxigenin, Hexacosane, Hexadeconoic acid, Hydrocoumarin, Methyl cis-11,14,17-icosatrienoate, Methyl linoleate, Methyl palmitate, Neophytadiene, N-hexatriacontane, n-tertacontane, N-tetracosane, N-tetratriacontane, N-triacontane, Phytol, Precocene I, Precocene II, Squalene, Stigmasterol, Stimasta-4,2,2-dien-3,β-ol, and Tetracontane.

5. The herbal preparation of claim 1 wherein said herbal extract can be dissolved in the sterile Distilled water (at 1-5 g/l) and the aqueous suspension can be filtered (through 1 to 5 μ filter, pH adjusted to neutral) in order to form a liquid formulation of said herbal extract.

6. The herbal preparation of claim 1 wherein said herbal extract is dissolved in the vegetable oil or PG/PL and the suspension is filtered (through 1 to 5 μ filter) in order to form an oil formulation of said herbal extract.

7. The herbal preparation of claim 1 wherein said herbal extract can be dissolved in a small quantity of ethanol in order to form a gel formulation of said herbal extract.

8. The herbal preparation of claim 1 wherein said acceptable carrier comprises a diluent.