Bioprospecting and new cosmetic product development: A brief review on the current status

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Abstract

Life in the earth is impossible without plants. They not only cater to our basic needs like food, shelter and clothing but also extend their support in curing the imbalances of the body. Plants possess their medicinal properties because of the reserved secondary metabolites. These metabolites are used from ages for treating the illness. Exploring such medicinal plant leads for their application in cosmetic industry opens a wide scope in the area of prospecting research. The article makes an attempt to review the needs of personal care industry for prospecting the herbs and the modern day consumer needs. It also throws an insight into the understanding of the bio-targets for the leads to act upon, the biochemical approaches for screening the potent herbs and the herbs known in the literature for their respective cosmetic application. The article opens the way for exploiting the biotechnological and chemi-informatics tools to commercialize the active molecules and thereby conserving the biodiversity.

Key words: secondary metabolites, medicinal plant, biotechnological, chemi-informatics, biodiversity.

1.0. Introduction:

Plants are the wonderful gift of nature for the welfare of all other creatures. We consume the plant products for our survival, use their core wood for making our shelter, through the transpiration process they help in the raining and through photosynthesis they consume the carbon dioxide and releases oxygen thereby purifies the air too. They are used for these basic needs and their applications are extended in the remediation of the diseases to the humans, animals and now even to the polluted environment. Search of such potential plants or plant based products/molecules to add value to the health of the globe is called bioprospecting.

Bioprospecting can literally be defined as the systematic search and sustainable use of chemical and genetic components of biodiversity for creating economic and social benefits. In earlier days, prospecting is mainly focused on the forest ecosystem, whereas now-a-days various forms of biodiversity like insects, algae, microbes of different (soil/marine) ecosystem have also been explored.

In a biodiversity, the autotrophs dominate because of the self sustaining ability, where the heterotrophic organisms depend on autotrophs for the survival. These autotrophic organisms synthesise their own food with the help of sunlight. Photosynthesis results in the synthesis of sugar molecules which paved the way for the yield of other essential molecules for survival (primary metabolites like proteins, lipids & nucleic acids). Once after the synthesis of primary metabolites, the excessive energy in the autotrophs is stored...
in the form of secondary metabolites. The autotrophs utilize these secondary metabolites to protect themselves from the biotic and abiotic stress factors.

The quantity & variety of secondary metabolites vary between organisms. As the complexity of the organism increases, the molecular diversity in secondary metabolites also increases. Plants/herbals are the complex self sustaining organisms among the autotrophs and are the richest sources of the variety of chemical molecules across all the species. With respective to herbal resources, bioprospecting means the extraction and screening of chemical compounds from plants to develop useful leads for potential drugs.

1.1. Need for prospecting:
1.1.1. Pharma industries: Pharma industries prospect the herbal extracts for various reasons. The candidate drugs from the natural origin are wide in their chemical diversity and highly effective in curing the diseases i.e., plant therapeutics, are not dependent on one single compound for curing the diseases, it’s a mixture of active molecules. Even though the mechanism of action of herbal actives is relatively known, these multiple molecules in a herb act on multiple sites to treat the diseases with less or no side effects compared to a synthetic one. If the causative factor for any pathogenic condition is microbes or the host malignant cells, then the process of developing resistance against these multiple molecules of natural origin is not so easier when compared to single synthetic molecules.

1.1.2. Food industries: following the pharma, food industries have also started the prospecting research in order to address the needs of modern community. Example: as per the WHO, one third of the Indian population is diabetic and the diabetic population is not ready to sacrifice the sweetness out of their life. But the intake of chocolates/ any sweets tends to increase the blood glucose level which is detrimental to their health. Hence, the business gap is identified and filled by the production of chocolates/ sweets for the diabetic patients. These chocolate products not only have the artificial sweetener (with zero calories) which imparts the sweetness to the product but also contains the anti-diabetic functional active ingredients which reduces the blood glucose level. It is the drug in food format. In the edible food products, the actives from the natural origin are preferred over the synthetic ones because of the same advantages. Thus, the food industries conduct bioprospecting research for delivering the better value added/functional food products with natural actives.

1.1.3. Cosmetic industries: nowadays even the cosmetic industries are also in line in going back to nature. From ancient history, it is known that the rulers have promoted and funded vaidyas for finding the anti-aging principles and beautifying recipes for keeping them young, beautiful & immortal. There started the application of natural products for the cosmetic benefits. Those recipes and formulae developed by vaidyas are not meeting the current day consumer needs. The market demand on personal care products nowadays are so vast like skin whitening, anti-aging, antiacne, antidadruff, hair growth promotion and cellulite reduction as some representative examples. Hence, the cosmetic industries are also interested in prospecting research in search of lead molecules and actives from natural origin which act on the bio-targets to deliver the beauty from within.

1.2. Traditional system of medicine:
Once the searching of leads from nature is decided, it is always useful to look into the traditional knowledge / traditional system of medicine. Traditional knowledge is the knowledge and practices of indigenous and local communities embodying traditional life style. It is sometimes the undocumented wisdom passed down by word of mouth over many generations of holistic traditional scientific utilization of the natural resources and environment.

Traditional system of medicine can be defined as health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. In such cases, India is one among the richest custodians of such traditional knowledge. The Indian systems of medicine covers ayurveda, siddha, yoga , naturopathy, unani and homeopathy which originated in India and abroad, but which got adopted and adapted in India in the course of time.

1.2.1. Ayurveda: The word ayurveda can be read as ayu & veda. Ayu stands for life and veda stands for science or knowledge. Hence, ayurveda can be understood as science of life. Ayurveda teaches the ways of preventing diseases, maintaining good health and treatment therapies. Ayurveda believes that any imbalance in physical (body) and mental (mind) elements that reduces the body’s resistance will result in diseases. It recommends the herbal formulae, life style change and the diet to boost the body’s defense mechanisms to correct the imbalance.

1.2.2. Siddha: Siddha system of medicine uses herbs and green leaves as medicines. When the disease condition is chronic, siddha practioners used metals, minerals and salts in the calcined forms to treat them. The herbal drugs used for curing the diseases may be a single herb or combination of herbs. Generally, the basic concepts of the siddha medicine are almost similar to ayurveda. The only difference appears to be that the siddha medicine recognizes predominance of vatham, pitham and kapham in all the stages of life like childhood, adulthood and old age respectively, whereas in
ayurveda it is totally reversed: kapham is dominant in childhood, vatham in old age and pitham in adults.

Siddha / ayurvedic herbs and formulae exhibit wide spectrum of therapeutic activity. One such example is guggul. It is recommended in ayurveda for more than 25 ailments because of its anti-inflammatory, anticoagulant, hypolipidemic and antibacterial activity. It was found to have anti-inflammatory activity in both polar and non-polar solvents indicating that several constituents with different physical and chemical properties exerting the same neuropharmacological actions.

1.2.3. Unani: The unani system of medicine originated from greece and roman literature was translated into arabic & persian languages. Thus, the arabs and persians introduced unani system of medicine in India. Various therapies employed in unani system are

- Ilaj-bid-dava: using the drugs from herbal, mineral and animal origin.
- Ilaj-bit: regimental therapy like cupping, diaphoresis, turkish bath, massage, purging, exercises etc.,
- Jarahat: surgery

Unani medicines mainly act as immunomodulators besides their combating function on disease causing factors.

1.2.4. Homeopathy: This system of medicine is developed by samuel hahnemann (1755–1843) and is being practiced in many parts of the world. It is governed by two main principles. One is “like cures like” in which the patients with particular signs and symptoms can be helped by a homeopathic remedy that produces these signs and symptoms in healthy individuals. The second principle is that these remedies retain biological activity after repeated dilution which is beyond avagadro’s number.

In order to apply such knowledge available in the traditional system of medicine for discovering the cosmetically beneficial leads, one needs to have a good biochemical understanding of the cosmetic substrates like skin and hair. Hence, the current review is directed in understanding the biochemical targets and the herbal remedies known in the literature to attain the properties like skin whitening, anti--acne, hair growth promotion, hair colours and cellulite reduction. It is concluded with the tips for commercializing such identified novel molecules in a sustainable way from the natural origin.

2.0. Skin whitening

Skin, being the outermost organ acts as an important barrier in protecting our body. Melanin is the important pigment of the skin whose protective role should not be underestimated. Melanin protects the skin by absorbing the harmful ultraviolet radiations [1]. It also acts as an endogenous antioxidant by combating the free radicals generated due to UV irradiation.

Fig 1: Irradiation of UV on to skin enhances the biogenesis of melanosomes in the melanocytes. Synthesized melanosomes acts as a vehicle for the transport of melanin from the melanocytes to keratinocytes. The delivered melanosomes releases the melanin in the nucleus of the keratinocytes, where in the melanin caps the DNA and renders protection from UV radiation.

Melanogenesis is initiated or accelerated upon exposure to UV radiation by the feed forward stimulation of tyrosinase enzyme, which results in skin darkening. The dermal DNA photodamage, infiltration of neutrophils, expression of matrixmetalloproteases (MMPS) and interleukin-10 (IL-10) seems to be more in white skin compared with black skin [2]. Thus, melanin protects the skin from sunburn, photo-aging and carcinogenesis by absorbing and scattering the detrimental UV rays [3]. On the other hand, over exposure to UV radiation will result in the pathological increase of melanin (hyperpigmentation disorders) as seen in the clinical conditions like melasma, solar lentigines and ephelides [4].

2.1. Biochemistry of melanogenesis:

The body biochromes namely oxyhemoglobin (red), reduced hemoglobin (blue), carotenoids (yellow) and most importantly the production, distribution, type and quantity of melanin (brown) decides the skin colour of the individual [5]. The specialized cells in the epidermis that produce such brown pigments are called melanocytes [6]. The population of melanocytes among the keratinocytes in the basal layer of the skin is in the approximate ratio of 1:10. Even though, the number of melanocytes among the different racial/ethnic group is same, the difference arises in the melanocyte density at different sites of the body [7].

Melanocytes possess a dedicated membrane bound ovoid organelle called melanosome to synthesize and store the pigment melanin. Melanocytes transport the melanosomes through their elongated dendrites to the neighbouring keratinocytes [8] as shown in fig 1. These keratinocytes with
Fig 2: The pathway indicates the biosynthesis of melanin from the precursor tyrosine. The regulatory enzymes involved in this biochemical pathway of eumelanin and pheomelanin synthesis includes tyrosinase, DOPA chrome tautomerase (DCT) and tyrosinase related protein 1 (TYRP1).

The ingested melanosomes proliferates from the suprabasal epidermal layer to the skin surface. The ingested melanosome forms a critical barrier/shield on the DNA by forming supranuclear caps to exhibit photoprotection [9].

There are two types of melanin pigments produced in the body. They are pheomelanin and eumelanin. The light red or yellow coloured, alkali soluble sulfur containing pigments predominantly seen in red hair or in freckles phenotype is pheomelanin [10]. Eumelanin is of two types either in dark black or dark brown colors which are insoluble, found in the dark skin and black hair [11]. Human skin contains all the pigments in varying ratios, which determines the race / ethnic groups. The ratio is unique to the individuals and hence forms the body image.

Regulation of melanogenesis is a complex process and it involves the expression or suppression of more than 125 genes directly or indirectly [12]. The regulation involves various steps which includes the expression of enzymes essential for the synthesis of melanin, structural proteins for the formation of melanosome, the membrane bound transporters for the import of substrate tyrosine to the spot of synthesis and the signaling proteins for the trafficking of melanosomes thereby resulting in the distribution of melanin in the skin [13].

Tyrosinase is the key enzyme which catalyses the initial rate limiting step of melanin biosynthesis. It catalyses the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and the subsequent oxidation from DOPA to DOPA quinone [14]. The DOPA quinone spontaneously cyclizes to form DOPA chrome. DOPA chrome is the precursor for the formation of dark/brown melanin called eumelanin [15]. The enzyme DOPA chrome tautomerase (DCT) acts on DOPA chrome and forms 5,6-dihydroxyindole-2-carboxylic acid (DHICA), the DHICA is acted upon by tyrosinase related protein 1 (TYRP1) and forms eumelanin as shown in fig 2 [16].

The DOPA chrome loses its carboxylic acid group, oxidised and polymerises to yield eumelanins [17]. This reaction is also catalysed by TYRP1. In presence of aminoacids like glutathione/cysteine, the DOPA quinone is converted into the cysteiny1 DOPA and further oxidation results in pheomelanin [18]. Mutations in any of or all the key regulatory enzymes like tyrosinase/DCT/ TYRP1 will dramatically affect the quality and quantity of the melanin synthesized [19].

2.2. Skin whitening actives- screening approaches:
There are various approaches used by the scientific community to screen the natural or synthetic molecules with skin lightening property. The tyrosinase enzyme isolated from the microbial/mushroom/ cell line sources were incubated with the molecule of interest for examining the skin whitening property through the tyrosinase inhibition [20]. The s.bikiniensis culture or the B16 melanoma cells were used as a model system to screen the potent molecules with pigmentation inhibition efficiency [21]. The co-culture of melanocytes and keratinocytes from mouse or human skin acts as a skin equivalent model which mimics the in-vivo system for testing the skin whitening agents [22]. The establishment of brownish guinea pig model through the exposure to UV radiation or by the injection of α-melanocyte stimulating hormone (α-msh) was the in-vivo model used to screen the molecules with potent skin whitening property [23].

2.3. Skin whitening actives-a survey:
The potent skin whitening actives used by the cosmetic industries and well known in the literature are reviewed in this section.

2.3.1. Licorice extracts:
Licorice (Glycyrrhiza glabra) contains the battery of secondary metabolites that suppresses the skin pigmentation system [24]. The actives in the licorice extracts are glabridin, glabrene, isoliquiritigenin, licurside, licochalcone a, liquiritin and its iso form. Most of these compounds exhibit the skin whitening property through one way or the other. The compounds like glabridin, glabrene and others exhibit the tyrosinase inhibition in the melanoma cells, whereas liquiritin has no effect on tyrosinase; however it causes the skin whitening through alternate route [25].

2.3.2. Mulberry:
The mulberry plant (Morus alba) is the reservoir of various class of compounds. It contains the phenolic compounds such as gallic acid, quercetin and the fatty acids like linoleic
acid and palmitic acid. The active component in these leaves are Mulberries F (moracin m-6,30-di-o-beta-d-gluco-pyranoside), which showed the tyrosinase inhibitory activity and the inhibition of melanin formation in melanoma cells [26]. These compounds are the potent antioxidants which inhibit the autooxidation of unsaturated fatty acids and nullify the toxic effect of superoxide radicals.

2.3.3. Aloesin:
The plant genera aloe is rich in the metabolite aloesin. Aloesin inhibits the tyrosine hydroxylase and DOPA oxidase activities of tyrosinase from human, mushroom and murine sources in a competitive fashion. The tyrosinase inhibition and the photoprotection properties of the aloesin in the in-vitro & in-vivo system are in a dose dependent manner [27]. The noncompetitive and competitive inhibitors such as arbutin and aloesin shows synergestic effect on tyrosinase inhibition.

2.3.5. Hesperidin:
The citrus fruits, its peel and the membrane are rich in the bioflavonoid called hesperedin. The studies documented reveals that the hesperidin inhibits the melanogenesis without cytotoxicity in the b16 melanoma cells and human primary melanocytes. Hesperidin protects the fibroblast and the collagen fibrils from the UV induced oxidative damage [28]. Thus, hesperidin actively exerts skin whitening property and thereby improves the skin tone [29].

2.3.6. Bioflavonoids & polyphenols:
Bioflavonoids can be divided into flavones, flavonols, isoflavones, and flavanones. Flavones such as eriodictyol, naringenin have the structure similar to hydroquinone, where they exhibit a competitive inhibition on the active site of tyrosinase. The cranberry juice and grape seeds are the richest sources of proanthocyanidins or procyanidins. They possess potent antioxidant activity than vitamin C or E [30]. The ellagic acid and ginseng p-coumaric acid are the potent tyrosinase inhibitors.

3.0. Anti-acne:
Acne is a common skin disorder that affects almost all the individuals at least once in their lifetime. The occurrence of acne peaks in the teenage but generally affects the people in the age group of 20-40 years. Acne can have important negative psychological consequences for the affected individual, with diminished self-esteem.

3.1. Mechanism of acne formation:
The levels of steroid hormones are peak in teenage which results in the enhanced production of sebum by increasing the size and activity of sebaceous glands. This excessive sebum acts as an excellent culture medium for the growth of the obligate anaerobic skin pathogen Propionibacterium acnes [31]. The triglycerides of sebum is acted upon by the enzyme from the P.acnes called lipase.

Fig 3: The healthy hair shaft with balanced sebaceous secretion with no inflammation. Infection with the organism Propionibacterium acnes triggers the inflammation resulting in the inflamed hair shaft with excessive sebaceous secretion.

The increase in sebum also results in a relative deficiency of linoleic acid within the follicle and stimulates the follicular keratinization. Thus, the principal factors like excess secretion of sebum, microbial infection, altered follicular keratinization and inflammation results in the pathogenic state called acne vulgaris. The severity of inflammation in the acne spot is highly influenced by the microbial factors like Propionibacterium acnes and Staphylococcus epidermidis than any other. Hence, Propionibacterium acnes and Staphylococcus epidermidis are the main target sites for antiacne drugs.

3.2. Anti-acne actives – screening approaches:
All the antibiotics and antibacterial actives currently in the market are not highly effective against the above said pathogens. Hence, paves the scope for screening the actives with potent anti-acne activity. The approaches used are the traditional methods for screening the antibacterial activity. i.e., Kirby bauer method to find the zone of inhibition exerted by the active against bacterial growth. Once the active exerts the effective zone, the effective concentration required to
inhibit the bacterial growth is found by minimum inhibitory concentration (MIC) test. The enzyme lipase extracted from the microbial sources acts as the molecular targets for the anti-acne drugs. Hence, the lipase inhibition assay is also carried out to identify the potent drugs using high throughput screening methods.

3.3. Anti-acne actives- a survey:
The effective anti-acne herbs well known in the literature are reviewed in this section.

3.3.1. Azadirachta indica:
Azadirachta indica (neem) leaves exhibit the antiacne activity against the organisms like P. acnes and S.epidermidis. Inflammatory mediators like reactive oxygen species (ROS) and pro-inflammatory cytokines (interleukin-8 and tumor necrosis factor-α) were released by P. acnes, which play a significant role in acne pathogenesis. The anti-inflammatory role of Azadirachta indica in the acne environment is proved by treating the polymorphonuclear leukocytes (PMNL) and monocytes with the culture supernatant of P. acnes in the presence and absence of herb. It was found that Azadirachta indica significantly suppresses P. acnes induced ros and pro-inflammatory cytokines [33]. Based on such promising results, the herb is used in antiacne creams.

3.3.2. Momordica charantia:
Momordica charantia (bitter melon) is one of the very common Indian herb having various medicinal properties used in traditional system for treating skin diseases like psoriasis, leprosy etc., antimicrobial activity of bitter melon fruit against P. acnes and S.epidermidis was examined. The antiacne potential of Momordica charantia may be due to its antibacterial and anti-inflammatory efficacy.

3.3.3. Eucalyptus globulus:
The antiacne potential of Eucalyptus globulus and the chemical constituents in the eucalyptus volatile oil responsible for the antiacne efficacy has been examined by Athikomkulchai et al., (2008). The study reveals that the possible inhibitory effect against acne may be due to the presence of the major component in the volatile oil namely gamma-terpinene. It is a monoterpen that plays an important role for its antibacterial activity [34]. It might cross the cell membranes, thus penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity.

3.3.4. Psidium guajava:
The essential oil of guava exhibits antibacterial activity against wide range of bacteria including Propionibacterium acnes. The antimicrobial activity of Psidium guajava determined by disk diffusion method was compared to tea tree oil (tto), doxycycline and clindamycin antibiotics. The zone of inhibition was significantly higher than those of tto but less when compared to doxycycline or clindamycin [35]. From these studies, it can be concluded that Psidium guajava may be beneficial in treating acne especially when they are known to have anti-inflammatory activities.

3.3.5. Juglans regia:
The medicinal property of the walnuts and the leaves of the cosmopolitan tree Juglans regia 1. Is well known. The insecticidal and bactericidal properties of the leaf extracts of J. Regia have been reported. The antiacne activity of Juglans regia was confirmed by testing its efficacy against P. acnes and S.epidermidis. The organisms were isolated from the acne lesions of female and male human volunteers and cultured for the growth of acne causing bacterium in presence of the herbal extract [36]. The results evidenced that the bacterial growth inhibitory property was higher in walnut leaf extract. Thus, the plant extract acts as the potential antiacne agent.

4.0 Hair growth:
Hair is an important feature of body image due to the unique styling interest of the individuals. The loss of hair will affect psychologically with the symptoms of social withdrawal due to embarrassment and depression. In order to address the hair loss issues with herbal remedies, the basic understanding of the hair growth cycle and its deviation in the disease condition called alopecia needs to be understood [37].

4.1. Hair growth cycle:
Hair growth cycle is similar to the cell cycle. Each hair seen in the comb has to pass through the different phases of hair growth cycle, which are described in the below section.
The excessive androgenic hormones (5α-dihydrotestosterone (DHT)) bind to the hair root and acts via the second messengers. This alters the hair growth cycle phases. In alopecia, the period of anagen phase is so short that the emerging hair does not reach the skin surface. The presence of pore is the only evidence for the functioning follicles. In addition, the latency period between telogen hair shedding and anagen regrowth becomes longer, leading to a reduction in the number of hairs present on the scalp [42].

4.2. Hair growth promoting herbs – screening approaches:
The hair growth promotion efficacy of the herbs can be screened by attacking two targets. One is the hair follicle proliferating potential of the herbs in the in-vitro cultured model of the hair follicles [43]. The other is reducing the turnover of the androgen DHT in the hair follicles by inhibiting the enzyme 5α-reductase. It is an enzyme that converts the androgenic hormone testosterone to DHT. The prevalence of DHT and the 5α-reductase in the bald scalp is high compared to a normal scalp [44]. Through in-vitro follicle culture and the inhibition of 5α-reductase is monitored for the high throughputs screening of the herbs with potent hair growth promoting property.

4.3. Hair growth promoting herbals- a survey:
The herbs with potent hair growth promoting property known in the literature and used traditionally are reviewed in this section.

4.3.1. Hibiscus rosasinensis:
This is a glabrous shrub widely cultivated in the tropics. It is well documented in the ayurvedic and siddha system of medicine. The leaves and flowers of Hibiscus rosasinensis have hair growth promoting and antigreying properties. In India, the herbal products in the market intended for hair growth include the extract of various parts of Hibiscus rosasinensis. The leaf extract of this plant has a potential effect on maintaining the hair growth, are scientifically validated in the in-vivo and in-vitro model systems [45].

4.3.2. Cuscuta reflexa:
It is commonly distributed in tropical, temperate region and found throughout the Indian subcontinent. It is a leafless, twinning, parasitic dodder with slender long yellow stems. It is commonly known as amarbel. The Cuscuta reflexa stem enhances the hair growth by transforming the hair follicle from telogen to anagen phase on a periodic basis [46].

4.3.3. Asiasari radix:
The root/ rhizome of Asiasarum heterotropoides (belongs to the family aristolochiaceae) is called Asiasari radix. The plant extract has hair growth promoting potential, which is exerted due to its regulatory effects on the expression of genes that codes for cell growth factor [47].
5.0. Hair colours:
The hair graying is the natural phenomena happen when the individual is chronologically aged. The graying of hair becomes prevalent even in the young age these days due to various reasons. The graying of hair affects the self-esteem of the individuals’ results in the poor presentation of themselves in the personal and professional life. Even though the synthetic hair dyes are well known for their side effects, they are used to address these issues. Hair colouring with various coloured dyes becomes the integral part of unique hair styling which is prevalent among the youngsters these days. Prospecting the nature for the colours/dyes to style the hair with nil side effects reveals the scope. Hence, the current section describes the colouring of hair using natural herbal active lawsones and reviews the other colours available in nature for the hair applications.

5.1. Mechanism of hair colouring using lawsones:
The lawsones are the secondary metabolites present in the leaves of the plants such as Lawsonia alba, Lawsonia spinosa and Lawsonia inermis (henna). Lawsonone is responsible for imparting the red colour to hair when applied as paste. The compound is also found in the plant Juglans regia (walnut). When the henna paste is applied on the hair shaft, the lawson molecule penetrates inside the hair shaft and binds with the keratin fibre. When the light bounce on the henna applied hair, the lawson molecule in the outer layer of the hair shaft shimmers / reflects the light in such a way to present the hair to be in red colour [50]. The hair colouring mechanism of lawsones is shown in fig 6.

The use of henna for dyeing the hair and nail is known in the practice for more than 5000 years. The content of lawson in the henna makes it a substantive dye for keratin in the acidic medium. The dye is formed within the hair and not as a coating as in the case of other metallic dyes. Henna is quite innocuous and very few cases of allergy have been reported for its use in hair and nail.

5.2. Natural hair colours – screening approaches:
The various parts of the herbs or trees were collected and immersed in the hydrophobic and hydrophilic solvents to extract the colouring material based on its solubility. The extracts were spotted on the thin layer chromatography. Based on the colour and intensity of the bands, the coloured materials are eluted through column chromatography and concentrated. The concentrated colors were applied to the greyed hair / black hair and the integrity of the colour to hair shaft is measured using minolta spectrophotometer [51].

5.3. Natural colours for hair- a survey:
The current section reviews the colours from the natural origin which have the scope to be used in hair styling.

5.3.1. Indigotin:
The dye indigotin is a blue to mauve colour, extracted from the fermented leaves of the plant Indigofera tinctoria (was also known as Pigmentum indicum). The indigo dye is found in the numerous indigofera species which is a glucoside component namely indican. This dye is quite colourful and mixed with other color components to produce a wide range of colorants. It is also used in the treatment of mouth and infected ulcers.

5.3.2. Juglon:
The plant species like Juglans cinerea, J. Regia, J. Nigra (butternut, walnut, black walnut) are the resources for the class of napthoquinone compounds including juglone and juglandin. Juglon is a yellow-brown colour and referred as juglandic acid or nucin. The hulls of the nut contain dark colorant which is used in the colour ranging from pink, brown to dark brown.
5.3.3. Lapachol:
Lapachol is a yellow crystalline material belonging to the class of naphthoquinone compound isolated from the heartwood of the plant *Tabebuia avellanedae* belonging to the family bignoniaceae. It is reported for the medicinal properties like anti-ulcers, anti-inflammatory, antiseptic, antiviral, bactericidal, fungicidal, insecticidal, pesticidal and viricidal.

5.3.4. Pratol:
The natural colorant pratol is extracted from the plant *Trifolium pretense* (clover). Pratol is chemically named as 7-hydroxy-4’-methoxyflavone, which is a dull, golden yellow in shade. Clover has been traditionally used in treating the eczematous skin conditions, boils and pimples. It is known from the literature that the occurrence of pratol in the yellow sweet clover botanically named as *Melilotus officinalis*. The flower heads are used externally in the treatment of ulcers, burns, sores and skin complaints.

5.3.5. Sanguinarine:
Sanguinarine is a quaternary ammonium salt belonging to the group benzylisoquinoline alkaloids extracted from the plants like *Sanguinaria canadensis* (bloodroot), *Argemone mexicana* (mexican prickly poppy), *Chelidonium majus* and *Macleaya cordata*. It is also found in the root, stem and leaves of the opium poppy. Sanguinarine, an alkaloid extracted from dried rhizome *sanguinaria canadensis*, is used as an anti-plaque agent in toothpaste and mouthwash preparations.

6.0. Cellulite reduction:

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6.0. Cellulite reduction:

The term “cellulite” is originated from the French medical literature. The synonymous meaning for cellulite is adiposis, edematosa, dermopanniculosis, deformans, status protrusus cutis, and gynoid lipodystrophy [52]. It is most commonly seen in the post pubertal females and rarely seen in males [53]. Cellulite is the orange peel or cottage cheese type dimpling of skin seen most commonly on the thighs and buttocks. Cellulite can be located in any area of the body that contains subcutaneous adipose tissue. This excessive deposit of fat on one or more susceptible spots gives irregular physical appearance resulting in the distress of the individuals.

6.1. Physiology of cellulite:
The main factors purport to explain the physiology of cellulite includes sexually dimorphic skin architecture, altered connective tissue septae and vascular changes [54]. The anatomy of the adipocytes or layer of adipose tissue organized beneath the skin is different for the man & woman as indicated in the fig 7. The shape of the adipocytes in the females facilitates the elastic expansion and prone them to cellulite accumulation.

6.2. Cellulite reduction – screening approaches:
As it is known that adipose tissue is a specialized cell type which stores the excessive fat beneath the skin. The excessive fat in the blood is in the form of lipoproteins (a fat transporter protein) that transports the triacylglycerol from liver and intestine to the adipose tissue. Unfortunately adipose tissue lacks the triacylglycerol transporter protein on the cell surface. Instead they are equipped with the membrane bound enzyme called lipoprotein lipase which cleaves the triacyl glycerol into free fatty acids and glycerol as shown in fig 8.

The liberated free fatty acids and glycerol enter inside the adiposites and esterified to triacylglycerol to be stored in [56]. Hence, by blocking the activity of the enzyme lipoprotein lipase results in preventing the storage of excess fat which reflects as reduced cellulite [57]. Thus, lipoprotein
lipase becomes the target for screening the herbs with potent cellulite reducing property.

6.3. Cellulite reducing - herbs survey & scope:
There are lots of physical and mechanical methods like endermologie, liposuction and subcision are available these days for cellulite reduction. Because of the cost and complications involved in these treatments, topical therapies are preferred. Although, there are numerous topical treatments are available over the counteract pharmacies, spas and boutiques, there are no large scale clinical studies demonstrating the effectiveness of these therapies [58]. Currently, two agents namely aminophylline and retinoids have been critically evaluated. In the herbal plat form, it has been reported that the extract of the plant Hibiscus sabdariffa has shown potent lipoprotein lipase inhibition [59] the clinical efficacy of the herbal extract needs to be evaluated. This knowledge gap opens up the scope of prospecting the herbs to reduce the cellulite.

7.0. Conclusion
Various herbal extracts were screened for their cosmetic benefits using multiple biochemical approaches in order to establish the inherent potency of the herb. Once the extract/compound passes via robust biochemical filters, the commercialization comes in picture. The quantity of herbal material required to commercialize the personal care products with the incorporated potent herbal extract is high.

It is unethical to exploit the wealth of nature for commercialization, will result ultimately in the disturbed ecosystem. As mahatma gandhi stated “nature can feed your need; not your greed”, is applicable here. The utilization of the herbs available from the nature for commercialization will come to end upon the extinction of the herbal species one day. Hence, there arises the use of biotechnological tools for the sustainable growth.

- The mass cultivation of the specific herbal material based on the geographical and seasonal conditions through contract farming will result in the bulk availability of herbal species for commercialization.
- Tissue culture of the potent herbal species and isolating the active component from the cells will yield the purified active which can also be incorporated in cosmetic formulations for delivering the expected benefit.
- Rapid advances in structural biology and computer technology, structure-based computer-aided drug design (CADD) using docking techniques, virtual screening and library design, along with target/structure focusing combinatorial chemistry, has become a powerful tool to minimize the time period required for screening the novel potent candidates [60]. Based on these studies, the synthesis of such compound will result in the availability of molecules in bulk for commercialization.

8.0. Scope of prospecting in cosmetics:
The discussions in the earlier sections on skin whitening, antiacne, hair growth promoters, herbal hair colours and the cellulite reduction are encouraging in prospecting the herbs with potent cosmetic benefits. The light needs to be thrown in the areas like antidandruff, antiwrinkling, antilice, skin moisturisation and sebum control where in the personal care industry is running short of the effective leads [61]. Hence, there is a wide scope for prospecting the herbs with potent cosmetic benefits.

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