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Review

Azadirachta indica (Neem) as a Potential Natural Active for Dermocosmetic and Topical Products: A Narrative Review

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Abstract: *Azadirachta indica* (Neem) is a large tree that is native to India and is traditionally used due to its several properties, mainly to treat skin diseases, as well as its "herbicidal" activity. Its bark, leaves, seeds, fruits and flowers are widely used in medicinal treatment due to the presence of active secondary metabolites with biological effects, mainly limonoids and tetranortriterpenoids, such as azadirachtin. Thus, *A. indica* was studied in a variety of conditions, such as anticancer, antiseptic, anti-inflammatory and chemopreventive agents, as well as a biopesticide. Furthermore, differentiated cell tissue in *A. indica* cultivation was reported to produce active metabolites for different purposes. However, only a few studies have been developed regarding its potential use in cosmetics. For instance, most studies explained the antimicrobial properties in health conditions, such as acne, dandruff and personal health care. Here, we summarized not only the most common cosmetic claims to treat acne but also mitigating other skin disorders related to inflammatory and oxidant processes in recent in vivo studies and patents to aid researchers and industrialists to select *A. indica* derivatives as novel cosmetic ingredients.

Keywords: acne treatment; antiaging effect; anti-dandruff; natural cosmetics; Neem biocompounds; oral care; skin disorders; skin-whitening

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1. Introduction

Azadirachta indica A. Juss, traditionally named Neem (Meliaceae), has been widely known for centuries as a source of active ingredients to develop products for health providers in remote areas. Thus, primary healthcare in developing countries has included treatments with this tree or its parts [1]. For instance, Indian traditional medicine reported cases of success that were not always scientifically tested [2]. *A. indica* is considered a multipurpose medicinal tree. Outstanding for its wide distribution in nature, as well as its low toxicity, Neem can be considered a natural source of cosmetic raw material for large-scale production. This tree is biologically close to Mahogany and all its parts (root, gum, leaves, flowers and fruits) can be used in agriculture, medicine and cosmetology since

the seeds and leaves have a higher concentration of secondary metabolites, which are more accessible through different extraction processes. Therefore, its beneficial effects can be attributed to one or more phytochemicals, including flavonoids, for instance. In general, it has a better effect through the synergism of its constituents [3].

The concentration of phytocomponents may vary according to the mode of harvest, storage, moisture content, light, temperature and variations in pH. In recent literature, antiseptic, anti-inflammatory and chemopreventive activities were described [4]. Moreover, other pharmacological features, such as antidiabetic, hypolipidemic, hepatoprotective, antipyretic, antifertility, hypoglycemic, cardioprotective, antiulcer, neuroprotective, antioxidant, microbicidal, nematicidal and antileishmaniasis properties were described [1]. In addition, more recent scientific reports remarked on the possibility of using it as a biopesticide [1,5,6]. However, there are few studies describing its use in topical products for human health. For instance, it was evaluated in terms of the in vitro SPF (sun protection factor) values of creams containing *A. indica* oil in a recent study. The SPF values were higher than the commercial cream, thus, revealing this plant as an alternative for the production of multifunctional sunscreens. Moreover, such cream was inoffensive to representative skin flora [7]. Likewise, until the present date, there have been only a few efforts of in vivo testing with cosmetic samples containing *A. indica* ethanolic extract with satisfactory safety results [8].

As an interesting technological development, plant biotechnology is currently used to produce targeted compounds with extraction advantages beyond the traditional ones [9]. Furthermore, some reports highlighted the importance of culturing *A. indica* to enhance the production of useful targeted compounds [9,10]. Although there are some previous studies about *A. indica* general uses, this review not only focused on the main dermatological effects of *A. indica* but also on the new possibilities claimed in patents to produce dermocosmetics with enhanced attributes. Hence, we aimed to aid in the scientific selection of novel ingredients derived from plant resources and meeting the increasing consumers' demand for naturally derived ingredients, including in the cosmetic market.

2. Phytochemical Composition

The major representative phytochemical compounds are oxidized tetranortriterpenoids, such as azadirachtin A (azadirachtin), azadirachtin B (3-tigloylazadirachtol), azadirachtin D (1-tigloyl-3-acetyl-11-hydroxy-meliacarpin), azadirachtin H (11-demethoxycarbonyl azadirachtin), azadirachtin I (1-tigloyl-3acetyl-11-hydroxy-11-demethoxycarbonyl meliacarpin), azadirachtanin, azadiriadione, azadirachtolide, deacetylnimbin, epoxyazadiradione, isoazadirolide, margosinolide, nimbin, nimbolin A, nimbandiol, nimocinol, nimbinene, nimbocinone, nimbocinolide, nimocin, nimbolide, salannin and related derivatives [3,11–13]. Many of these have specific physiological functions, mainly in the defense against harmful environmental factors, such as light, predators, microorganisms and insects. Moreover, the different parts of Neem trees were used to isolate more than 135 phytocompounds. At least nine limonoids are triterpenes. Limonoids named azadirachtin A–G (produced in leaves) are the most studied and they have activities that allow them to be used as potent insecticides. Moreover, the bark is rich in lignans [3].

Other biological activities found in Neem oil studies are attributed to the following compounds: salannin, nimbin, meliantriol, meliacin, tignic acid, gedunin (also present in leaves), nimbidin, nimbidic acid, nimbidinin, nimbolide (found in leaves), valassin, meliacin, deacetylnimbin, linoleic acid, stearic acid, palmitic acid, oleic acid, azadiradione, hexadecanoic acid, caryophyllene oxide, linalool oxide, mahmoodin, margolone, azadirone, nimbolin, nimbinene and nimbosterol. Neem kernels have 30–50% oil, which is mainly used in soaps, biopesticides and pharmaceuticals [3,11–13].

Furthermore, it can be mentioned that the following nonisoprenoid compounds are present: proteins (amino acids), sulfurous compounds, carbohydrates (polysaccharides), polyphenolics such as flavonoids and their glycosides, rutin, dihydrochalcone, quercetin, carotenoids, catechin, ferulic acid, β-sitosterol, steroids (produced in leaves and/or bark),

coumarin and tannins (produced in the bark), aliphatic compounds, ellagic acid, lupeol, saponins (leave), alkaloids (leave), resins, gums, margisine, cyclic trisulphide, steroids and ketones [3,4,13,14]. Hence, Table 1 shows the main secondary metabolites that are used in cosmetics and topical products with the corresponding reported biological activities.

Table 1. Phytochemical structures of *Azadirachta indica* (Neem) parts with their cosmetic or topical applications.

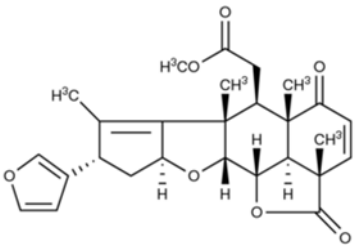
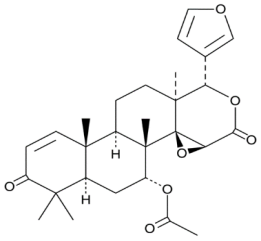
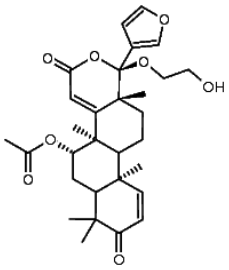
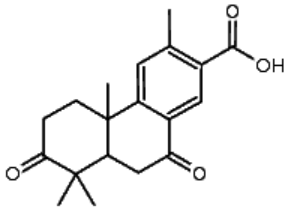
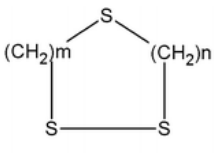
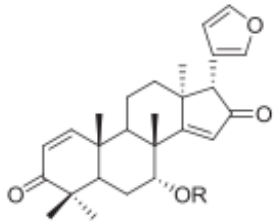
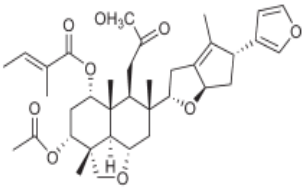
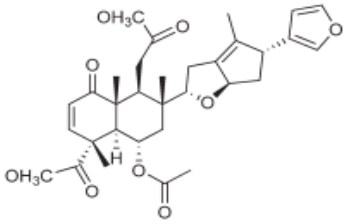
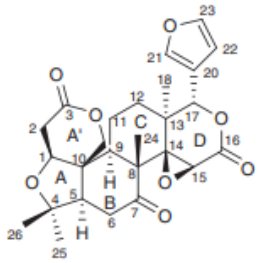
Phytochemical	Neem Part	Structural Formula	Biological Activity	References
Nimbolide	Oil from leaves and seeds, fruit		Psoriasis, antibacterial	[15,16]
Gedunin	Oil from leaves and seeds		Antifungal	[15,16]
Mahmoodin	Oil from seeds		Antibacterial	[15]
Margolone	Oil from seeds and stem bark		Antibacterial	[15,16]
Cyclic trisulphide	Oil from seeds and leaves	 m = 1 - 4 n = 2 - 3 **Cyclic Trisulphide**	Antifungal	[16]

Table 1. *Cont.*

Phytochemical	Neem Part	Structural Formula	Biological Activity	References
4 azadiradione-type limonoids	Leaf extract		Melanogenesis inhibition	[17]
2 salannin-type limonoids	Leaf extract		Melanogenesis inhibition	[17]
5 nimbin-type limonoids	Root extract		Melanogenesis inhibition	[17]
Limonoid	Leaves extract		Anti-inflammatory	[18]

3. Recent Developments in Cell Culture to Produce Molecules for Cosmetic Use

The biotechnological aspects of differentiated plant cell tissue in *A. indica* cultivation, as well as the biosynthesis of interesting secondary metabolites under different nutritional and environmental conditions, are well described in the literature. Recent efforts have focused on modifying the nutritional necessities of cells, optimizing the culture medium composition, and evaluating the amount of both the elicitor and precursor, which could improve not only cell growth but also secondary metabolites formation. Such studies intended to produce enhanced azadirachtin and related limonoids (AZRL) [5]. However, this review focused on the production of possible cosmeceutical ingredients derived from *A. indica*.

The rheological properties with their main parameters, such as the Reynolds number, apparent viscosity, volumetric power and phase state of *A. indica* cell cultures, were evaluated in 250 mL shake flasks that were 5 cm in diameter. The morphological aspects were evaluated and it was demonstrated that the isodiametric round shape of their huge individual cells, as well as their tendency to produce cell clusters, determined the capacity of such cell cultures to store secondary metabolites with high commercial interest in terms of developing both pharmaceuticals and cosmetics [19]. Moreover, the culturing of plant cells of *A. indica* was developed to continuously produce its principal secondary metabolites in recent years [9,10]. For instance, azadirachtin, mevalonic acid and squalene were

successfully extracted from an *A. indica* cell suspension culture by using a green analytical method for ultrasound-assisted extraction with ethanol as solvent [9].

Another recent study investigated the effect of different culture types, as well as some concentrations of both cytokinins and auxins on cell biomass production, cell growth and azadirachtin production with further accumulation in cell suspension, as well as callus cultures of *A. indica* [10].

Furthermore, an *A. indica* culture as a cell suspension was carried out in shake flasks and bioreactors using a modified culture medium, as well as precursors and elicitors. They achieved an increase from 0.77 to 4.52 mg limonoids/g dry cell weight (DCW), with the consequent limonoids volumetric production increasing more than threefold using a two-stage culture (the one-stage process produced 13.82 mg limonoids/L, while the two-stage one produced 41.44 mg/L) in the bioreactors [20]. Thus, hydrodynamic stress under different agitation speeds was evaluated in *A. indica* cultivations using shake flasks. Some interesting parameters, such as limonoid concentration, cell viability and growth, reactive oxygen species (ROS) production and glutathione peroxidase (GPx) enzymatic activity, confirmed the effect of mechanical stimulus on the production of specific secondary metabolites. In addition to this, the most suitable agitation was 400 rpm with no cell integrity damage and a maximum biomass concentration of 10.53 ± 1.03 g DW containing 82.66 ± 32.96 mg limonoid/g DW [21].

4. In Vitro Tests with Dermocosmetic Applications

4.1. Antimicrobial Activity

Plants have substances called secondary metabolites that can develop antibacterial, antifungal and/or antioxidant activities (such as glycosides, alkaloids, flavonoids, saponins, among others), and can be also part of a defense mechanism against pathogens [14,22]. Such phytochemicals are extracted according to the molecule polarity and the characteristics of the vegetable part used. Thus, studies with methanolic extracts from *A. indica* leaves inhibited the action of *Bacillus*, while oils from seeds, bark and leaves could inhibit the growth and/or viability of Gram-negative and Gram-positive bacteria. Among Gram-positive bacteria, we highlight the strains of *M. pyogenes*, *Streptococcus mutans* and *Staphylococcus aureus*, which are commonly found on the skin's surface [4].

Moreover, β-sitosterol is recognized for its wide spectrum in treating skin diseases [4]. In addition to the compounds mentioned above, nimbolide and nimbidin were found in *A. indica*, which showed antibacterial activity against the following species: *Staphylococcus coagulase*, *S. aureus*, *Staphylococcus* sp. and *Serratia*. Moreover, various concentrations of an aqueous extract of Neem leaves inhibited the growth of *Bacteroides intermedius*, *B. gingivalis*, *Streptococcus viridans* and *S. salivarius* in vitro [23]. Furthermore, nimbidin isolated from bark showed antifungal activity [4,14].

A. indica was classified as the most effective medicinal plant for dermatophytosis in traditional treatment due to its components. A study revealed the minimum fungicidal concentration (MFC) and the minimum inhibitory concentration (MIC) for the leaf and seed extracts of *A. indica* against various dermatophytes, such as *Trichophyton mentagrophytes*, *T. rubrum* and *Microsporum nanum*. The growth curve of such dermatophytes was affected in the presence of *A. indica* extracts, whose effect was unexpected when compared with the dermatophytes without extracts [24].

Moreover, gedunin has antifungal activity and deoxygedunin has moderate antibacterial action, both of which were isolated from Neem seed oil [4]. *A. indica* leaf and seed grain extracts were effective against the human fungi *Candida*, *Geotrichum*, *Epidermophyton*, *Trichophyton*, *Microsporum* and *Trichosporon* [14].

In addition to this, the antifungal properties of *A. indica* leaf extracts were attributed to the inhibition of dermatophytes. The effect was attributed due to the presence of quercetin [14]. Thus, various concentrations of petroleum ether extracts of the Neem leaves inhibited the growth of *Microsporum canis*, *M. gypseum*, *Epidermophyton floccosum*, *Trichophyton concentricum*, *T. rubrum* and *T. violaceum* [24]. Furthermore, the leaf extracts

and seed oil inhibited the growth of *E. floccosum T. mentagrophytes, M. canis* and *T. rubrum*, where the extract from Neem leaves had the highest antifungal activity of both [25].

Sulfur-containing compounds isolated from the steam distillation of fresh and ripe Neem leaves revealed antifungal activity against *Trichophyton mentagrophytes* [4]. In addition, some *A. indica* extracts that contained flavonoids with known antioxidant activity were evaluated. For instance, rutin was one of the flavonoids found in *A. indica* leaves, where the antibacterial action of quercetin combined with rutin was superior in relation to the same isolates [14].

Studies demonstrated that tetranortriterpenoid and some other phytocompounds, such as azadiradione, 6-deacetylnimbin, salannin, nimbin and epoxyazadiradione, were the main antifungal compounds from *A. indica*. However, the triterpenoids from Neem oil in its pure form (separately) showed basically no antifungal activity, while the combined effect was demonstrated against the three fungi tested, which may be indicative of such compounds having additive or synergistic effects [26].

Furthermore, nimbolide showed the greatest zone of inhibition against some bacteria species, such as *S. epidermis, S. aureus, P. aeruginosa, K. pneumoniae* and *S. aureus* (MR —methicillin resistant), in agar diffusion assay. The highest inhibition level was found against *K. pneumonia*, as well as the synergistic action of nimbolide, when combined with the pre-existing antibiotics cephalexin (97% pure) and cefazolin (98% pure). Thus, a greater antimicrobial effect in the synergy with these antibiotics compared to the other tested compounds (such as deacetylnimbin) was shown [27].

4.2. Antioxidant Activity

It is known that ROS and free radicals are involved in cancer, DNA damage and even aging [1], and the addition of antioxidants in dermocosmetics can minimize these effects. Thus, some extracts that contained flavonoids with known antioxidant effects and other extracts derived from *A. indica* flowers and young leaves showed strong antioxidant potential [4]. Moreover, the aqueous fraction of the Neem bark had greater antioxidant activity than the leaf extract because of the higher concentration of phenolic compounds [28].

The topical application of *A. indica* leaf ethanolic extract was tested on hairless mice exposed to UVB irradiation to prevent the formation of wrinkles. To carry out this study, dry Neem leaves (10 g) were used, which were pulverized and extracted three times with 1 L of 50% ethanol over 24 h at room temperature. Then, the extract was analyzed via liquid chromatography, using methanol as the mobile phase. Moreover, a significant amount of rutin was found in the dried leaf extract, showing a high capacity to eliminate free radicals, which indicated the antioxidant activity. Finally, it was found that mice exposed to UVB irradiation and received the treatment with *A. indica* leaf extract had less wrinkle formation than the other groups, indicating a possible antiaging effect of the *A. indica* extracts [29].

4.3. Skin-Soothing and Melanogenesis Inhibition Activities

A detailed investigation of two species from Meliaceae plants, namely, *Azadirachta indica* (Neem, AI) and *Azadirachta indica* var. siamensis (Siamese Neem, AIS), isolated and categorized 81 limonoids, 11 flavonoids and 1 diterpenoid, and revealed inhibitory activity against melanogenesis in B16 and tumor promoter assay (TPA)-induced inflammation in mice [17].

As it is well known, skin color depends mainly on the pigment melanin. The biosynthetic pathway named melanogenesis is responsible for melanin production in human skin. Furthermore, a form of hyperpigmentation condition named melasma was often reported as brown or gray patches on facial skin. Thus, there is an increasing demand for dermocosmetics to diminish skin hyperpigmentation, e.g., those containing melanogenesis inhibitors. Many of the AI and AIS compounds have revealed inhibitory activity against melanogenesis in B16 4A5 (mouse melanoma cells) stimulated with [30] or without α-melanocyte-stimulating hormone (α-MSH) [31]. Hair and skin pigmentation are a response to the stimulation of α-MSH. Among the AI and AIS compounds, some of them

(4 azadiradione-type limonoids, 5 nimbin-type limonoids and 2 salannin-type limonoids) showed higher melanogenesis inhibition (79.1–108.1% cell viabilities) than the reference arbutin (4-hydroxyphenyl β-D-glucopyranoside; 100.1% cell viabilities), which was used as a depigmentation alternative in skin whitening for the cosmetic sector. Likewise, tyrosinase, which catalyzes the oxidation of L-DOPA to L-DOPA quinone, as well as the hydroxylation of L-tyrosine to L-(3,4-dihydroxyphenyl) alanine (L-DOPA), tyrosinase-related protein-1 (TRP-1), TRP-2 and tyrosinase, are involved in melanin biosynthesis. Furthermore, a nimbin-type limonoid from an AIS root extract, which was reported as the most abundant limonoid, was the most effective melanogenesis inhibitor [17].

Furthermore, the TPA-induced inflammatory ear edema in mice was studied to investigate the limonoids from AI and AIS extracts. A single application of TPA induced skin inflammation that was mainly defined by edema, polymorphonuclear leukocyte infiltration and erythema. The tested limonoids showed higher anti-inflammatory activity (50% inhibitory dose, ID50 = 0.19–0.75 μmol/ear) than indomethacin (ID50 = 0.91 μmol/ear) [17].

5. In Vivo Tests (Subjects) for Cosmetic Applications

Some preclinical trials that investigated the antidiabetic, anti-inflammatory, analgesic, antinociceptive and insecticidal/pesticidal activities of *A. indica* extracts using different parts are described in the literature [1]. Thus, we discuss the first attempts with volunteers for new cosmetics development in the next paragraphs.

Neem oil was used in an emollient cream to assess its photoprotective effect, rheological characterization, toxicity and effect on the natural cutaneous flora health. Volunteers were used as donors of their skin's bacterial flora, rubbing it with sterile swabs and grown on agar with nutrients and incubated for 24 h at 37 °C. As a result, the rheological characterization was similar to the commercial creams. Such Neem formulation had no cytotoxicity in 3T3 cell lines, as well as no damage to the natural cutaneous flora. In addition to this, when titanium dioxide was added to this cream, it showed greater photoprotective activity than the commercial product [7].

A randomized double-blind study with a commercial nutritional supplementation using turmeric polyherbal formulation decreased facial redness compared with the turmeric or placebo in volunteers in a study within 4 weeks. The turmeric polyherbal tablets included 500 mg of a blend of the following certified organic herbs: *A. indica* (Neem) leaf, *H. indicus* (anantamul) root, *C. longa* (turmeric) root, *R. cordifola* (manjistha) root, *C. asiatica* (brahmi/gotu kola) leaf, *Glycyrrhiza glabra* (licorice) root, *Tinospora cordifolia* (guduchi) stem, *P. amarus* (bhumyamalaki) herb and *P. emblica* (amalaki) fruit. Thus, the beneficial effects of this formulation were attributed to a synergic effect of each plant, such as anti-inflammatory, antioxidant, photoprotective and anti-aging properties, which enhance both the prevention and treatment of different skin conditions with different origins [32].

A polyherbal cream was developed as a vaginal cream containing *A. indica* (Neem) dry seed extract, *Sapindus pericarp* extract and quinine hydrochloride, which gave the cream a spermicidal action, as well as antimicrobial properties. The mentioned ingredients were incorporated into a water-soluble cream base and stabilized with the addition of antioxidant and preservative components. Then, an in vivo test was performed with intravaginal application in both rabbits and monkeys. Finally, such cream was tested and considered devoid of both sensitization and irritation with the Draize test using a 21-day duration cumulative skin sensitivity test in volunteers [33].

In a more recent study, a polyherbal gel was developed with ethanolic extracts of the following species: *A. indica, Piper betle, Adhatoda vasica, Pongamia pinnata* and *Ocimum tenuiflorum* to evaluate its antimicrobial action. Moreover, its drug content, physical appearance, viscosity, pH, spreadability, washing ability and skin irritation were tested. To do so, twenty healthy volunteers were submitted to the patch test, which was applied in the forearm of each participant to detect the possible reactions to formulations A, B, C (with 0.1, 0.3 and 0.5% ethanolic extracts, respectively) and the control after 48 h. It was concluded that the combination of ethanolic extracts did not demonstrate any serious

adverse reaction, and thus, they are considered safe in those conditions. In addition, the most effective concentration tested was 0.5% [8].

Furthermore, a 6-week study was performed to evaluate the effectiveness of Neem extract dental gel with a positive control containing chlorhexidine gluconate (0.2% *w/v*) mouthwash. It showed that the Neem extract dental gel exhibited a higher reduction in the bacterial presence, as well as the plaque index than the control group [34].

6. Alternative Dermatological Effects

Other dermatological properties of *A. indica* included its use as a wound healer. The healing activity of methanolic extracts from leaves of both *A. indica* and *Tinospora cordifolia* was evaluated using models of excision and incision of wounds in rats. It was concluded that the extracts had healing activity both in the excision and in the incision, with the first presenting greater healing potential than the second. There was greater resistance to traction of the scar tissue, as well as a higher proportion of wound contraction [35].

Organic Neem oil has been used to treat other skin disorders, such as acne, psoriasis, eczema, mycosis and warts. Indeed, the Indian Siddha medicine has been using Neem oil and leaves since ancient times to treat skin diseases, mainly psoriasis [36,37].

In addition, new technologies involving Neem and its dermatological effects are emerging with interesting results that could be used for the improvement of new dermocosmetics. For instance, the antidermatophytic activity of leaves and seeds of *A. indica*, as well as the effect on growth patterns in dermatophytes, were performed. After the extraction of compounds from Neem leaves and seeds, the fungi *Trichophyton mentagrophyte*, *Microsporum nanum* and *T. rubrum* were inoculated. The MIC and MFC values were determined, where the first is considered the lowest extract concentration that did not reveal any viable growth after incubation (21 days), while the second is the lowest extract concentration that inhibits the fungal growth in the solid medium. The growth pattern of the tested fungi with Neem extract on an agar medium was compared with the control. Thus, it was identified that the extract of Neem leaves and seeds had high antidermatophytic properties [24].

On the other hand, the potential of *A. indica* in microspheres with a mucoadhesive polymer, which were prepared using an ionotropic gelation method, was evaluated as an oral controlled drug delivery system. The in vivo pharmacokinetic studies using rabbits revealed an increase in mean residence time by 75% and relative bioavailability by 1.5 times, meaning that it is a good option for oral controlled drug delivery systems [38].

Furthermore, the Neem constituents were deemed effective against several cancer types, including both connective tissue and skin cancer using in vivo and in vitro models. Animal studies showed anti-cancer activity from nimbolide. For instance, nimbolide isolated from Neem at 5 and 20 mg/kg significantly reduced the growth of colorectal cancer xenografts in mice. Xenografts are tissues that are transplanted from one species to another. Moreover, a significant decreased in tumorigenic protein, such as those related to proliferation, survival, invasion, metastasis and angiogenesis, were also reported in the mice xenograft treated with nimbolide [39].

An ethosomal formulation with soy lecithin (300 mg), ethanol (35%), luliconazole (100 mg) and Neem extract was more effective against *Candida parapsilosis* than *Aspergillus niger* in fungal culture tubes. In the same study, the in vitro drug permeation test with Wistar albino rat skin model demonstrated the release of 83.45 $\pm$ 2.51% in 24 h. Thus, luliconazole and Neem extract showed synergistic effects against fungal infections [40].

Interestingly, *A. indica* was reported as a nutritional strategy for psoriasis in a recent study since it is rich in nimbidin. A significant reduction in the PASI (psoriasis area and severity index) score was observed after 12 weeks of consuming three capsules/day of *A. indica* in an RCT (randomized controlled trial) of 50 patients. According to the authors, this may have been due to the inhibition of prostaglandin synthetase by nimbidin, which is a secondary metabolite found in the *A. indica* essential oil [41]. Hence, Figure 1

summarizes the mentioned biological activities of *A. indica* parts with the corresponding dermocosmetic attributes.

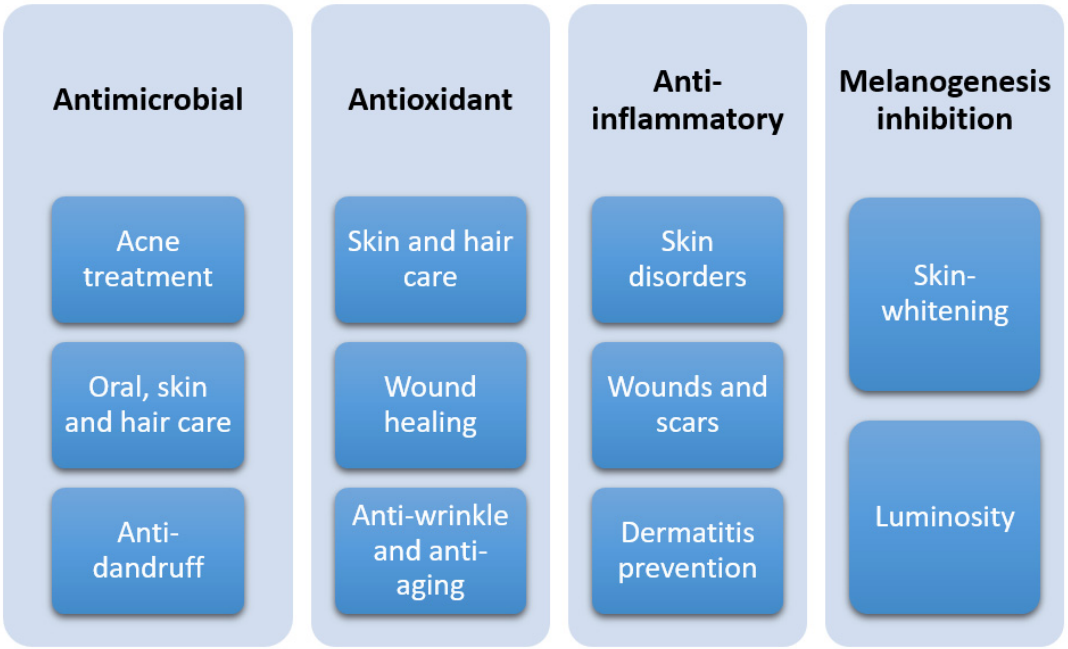


Figure 1. Main *A. indica* biological activities with their dermocosmetic attributes.

7. Toxicology Issues

Regarding the toxicological profile of *A. indica* species, recorded data refers to the extracts of the leaf and bark, the limonoids and the oil from seeds [42]. The first and principal data came mainly from scientific studies conducted on animals, as they were in a period before the current evolution of alternative methods in scientific research. Furthermore, most of this data was registered at REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals), which is an institution regulated by the European Union. These data are freely accessible and can be used to understand the toxicological profile of *A. indica* and its phytochemical components, exempting the need to repeat safety tests that have already been performed [43].

Considering the *A. indica* safety for human health, the principal tests for Neem and its components described by the Organization for Economic Cooperation and Development (OECD) found the following results: ocular irritation, skin sensitization and irritation, acute oral toxicity, subacute oral toxicity, acute dermal toxicity, reproductive toxicity, teratogenicity, inhalation toxicity, mutagenicity and genotoxicity. Concerning the ocular irritation classification, a study revealed that when 0.1 mg of an ethanolic extract of Neem seed or an aqueous solution of 1 or 5% sodium nimbidinate was applied in the eyes of male New Zealand albino rabbits, no severe eye damage or irritation reaction was observed [44]. Moreover, a complementary study under the same conditions mentioned above with a 10% aqueous solution of sodium nimbidinate revealed that the substance applied to the eyes of guinea pigs did not show any irritating reaction [45]. Therefore, there is no scientific evidence for the potential eye irritant action of *A. indica*.

Some toxicological studies of different parts of *A. indica* were recently described [1]. In addition, a complementary study showed that Margosan-O®, an approved Neem-based insecticide, which included a concentrated ethanolic extract of Neem seeds at 3000 ppm azadirachtin (±10%), caused low-to-moderate primary skin irritation when intradermally administered to albino rabbits [46]. Additionally, there is a record of the classification of the substance azadirachtin (CAS number 11141-17-6, with 95% purity) in category 1B of the GHS (Globally Harmonized System of Classification and Labeling of Chemicals) for skin

sensitization. This category indicates that the substance, under the conditions evaluated, is a skin sensitizer [47].

Acute oral toxicity studies showed that methanolic extracts of Neem leaf and peel, administered orally in rats, showed an LD50 (50% oral lethal dose) of about 13 g/kg of the animal, leading to the death of mice by terminal seizure [48]. Moreover, the study noted that the animals that survived had gastrointestinal spasms, hypothermia and apathy, and refused water and feed. These data on acute oral toxicity in rodents corroborated another study in which Neem seed oil was orally administered to rats and rabbits, and the measured LD50 of 24 h was 14 and 24 mL/kg, respectively [49].

According to a study that assessed the subacute oral toxicity in rodents, adult Holtzman rats received a single daily dose of an alcoholic solution of nimbidin at concentrations of 25, 50, and 100 mg/kg in their diets for 6 weeks. The trial revealed that the rodents did not show any severe toxic symptoms during the study period [50]. Moreover, acute dermal toxicity data published on the administration of concentrated ethanolic extract Margosan-O® in albino rabbits indicated that the substance had an LC50 (lethal concentration 50%) greater than 2 mL/kg [46].

Concerning reproductive toxicity, a study evaluated the administration of an aqueous extract of *A. indica* wood ash in male albino mice in three different doses (5, 50 and 100 mg/kg body weight). The parameters evaluated were the following: gonadosomatic index, sperm count, sperm motility, sperm morphological analysis, serum quantification of follicle-stimulating (FSH) and luteinizing (LH) hormones, testosterone assay and histopathological observations of the testicles. The results concluded that even without a toxic effect on testicular weight, testosterone, and the hormones FSH and LH, there was a significant reduction in motility and sperm count [51]. Furthermore, another study evaluated the effect on reproductive toxicity in quails (*Coturnix coturnix* japonica L.) fed with Neem seeds incorporated in the feed in the proportions of 0, 5, 10, 20 and 40% for 60 days. The study concluded that there were significant changes in sperm concentration, seminal volume, vigor, motility and sperm viability [52]. Thus, in both studies, the authors concluded that *A. indica* should impair reproduction, presenting a considerable degree of reproductive toxicity.

Furthermore, another study evaluated the teratogenic profile of nimbidin. It assessed the effects of the oral administration of a 10% aqueous solution of nimbidin at 25, 50 and 100 mg/kg in fertile rats mated for 13 days. The evaluated parameter was the presence of severe anatomical fetal abnormalities. This study concluded that even the highest applied doses did not cause any deformity in the organs of the born pups. Thus, there was no subsequent adverse effect on breeding performance. In conclusion, nimbidin did not have a potential teratogenic action [50].

Toxicological data identified for *A. indica*, as well as its phytochemical components, indicated that these substances were susceptible to applications in dermocosmetic formulations since there was no evidence of risks to human health for the intended application. However, there were no scientific reports on the safety of *A. indica* and its components regarding carcinogenicity and phototoxicity, for example. Thus, additional toxicological tests are still necessary to expand the coverage of the safety of these substances. Furthermore, all dermocosmetic information, even if supported by toxicological data of the individual components, must present evidence of the toxicological safety of the integral formula, previous safety of potential synergism or potentiation of toxic effects associated with the substance. In contrast, a survey of toxicological data on the safety of *A. indica* for the environment revealed that this substance and its components could be quite aggressive to the environment [42,53], unlike the toxicological records identified for their safety concerning human health.

Additionally, a recent review on the latest in vivo toxicity of *A. indica* extracts is presented in two major sections: (a) aquatic species toxicity and (b) mammalian (rats, rabbits, etc.) toxicity, with the corresponding final application as a pesticide or a possible new therapeutic drug, respectively [54].

Above all, scientific reports showed and recognized the medicinal properties of *A. indica*, even naming it the "village pharmacy" or the "doctor's tree". Hence, *A. indica* is an excellent candidate for exploratory tests for dermocosmetic effectiveness.

8. Patents Applications

In this section, our research group used three free databases, namely, Espacenet®, Google Patents® and PatentScope®, to search patents within a 10-year period (2010–2020). To standardize the web research, we used the keywords "*Azadirachta indica*" with two refined options, namely, title and title + abstract, on the same day (16 July 2020). The main results are summarized in Table 2.

Table 2. Patents found in 3 databases with the keywords *Azadirachta indica* in the title or title + abstract.

Keywords	No. of Patents		
	Espacenet®	Google Patents®	PatentScope®
Azadirachta indica (title)	24	5	4
Azadirachta indica (title + abstract)	204	27	23

In summary, we found patents regarding interesting topics about inducing methods for hairy roots of *A. indica* cell cultures, novel methods to prepare extracts, herbal compositions with different health claims and further pharmaceutical and cosmetic products. Outstandingly, azadirachtin, nimbolide and salannin from Neem are well described in biopesticides preparations and repellent formulations. Other remarkable innovations also described the powdered plant in association with other plants to eliminate bacteria from clothes, as well as insects with an additional advantage on the clothes' softness. Some documents concerning medical applications of herbal mixtures containing *A. indica* claimed the enhancement of the immune systems, as well as cancer treatment, in particular for skin-related malignancies. Furthermore, different drugs were related to ameliorate metabolic syndrome with inhibition of the increment of body fat, as well as anti-inflammatory compounds for dental care.

Regarding cosmetic applications, the main patents that involved the use of different *A. indica* parts and extracts are summarized in Table 3. Some cosmetic formulations were developed to ensure the claimed effect reported in each document. We remark that the synergic effects of the combination of different herbs, their parts or their extracts were the most common strategy found in the formulations to achieve good results. Only two products were developed with a sole *A. indica* seed extract, which were shampoos that claimed to provide a desquamation treatment, as well as anti-dandruff properties [55,56]. The most common cosmetic effect claimed was to treat acne, as well as mitigate other skin disorders related to inflammatory processes.

Table 3. Phytochemical structures of *Azadirachta indica* (Neem) parts with their cosmetic or topical applications.

A. indica Part(s)	Other Main Plants in Formulations	Claims in Cosmetics	Patent
Seed extract with n-hexane	NI *	Bacteria-killing agent in the skin	CN109805037A [57]
Extract	Mentha canadensis extract and holy basil	Inhibits the expression of inflammatory cytokines (IL-8, IL-6) caused by fine dust, thus alleviating skin irritation and skin inflammation; acne prevention and alleviation; flushing alleviation; and dermatitis prevention and alleviation	WO2018021777A1 [58]

Table 3. *Cont.*

A. indica Part(s)	Other Main Plants in Formulations	Claims in Cosmetics	Patent
Leaf extract 1–10%	NI *	Skin rejuvenation and fungi and bacteria treatment	MX2013004928A [59]
4–7% of bark aqueous extract	0.5–4% extract of Berberis aristata, Jasminum officinale, Glycyrrhiza glabra, Picrorhiza kurroa, Rubia cordifolia, Pongamia pinnata, Saussurea lappa, Stellata wild and Terminalia chebula; 6–9% Curcuma longa and Trichosanthes diocia	Topical wound healing formulation	NZ578363A [60]
0.1–5% aqueous extract	0.1–5% Cymbopogon martyinii oil, 0.1–5% aqueous extract of A. indica or Phyllanthus emblica or both	Anti-dandruff composition (shampoo)	WO2020020539A1 [61]
5–8% folium extract	0.1–0.5% Sophorae flavescentis, 3–10% Hammamelis extract, 5–10% Chinese radix Rehmanniae extract, 3–5% radix Scutellariae extract, 3–5% heartleaf Houttuynia herb extract and 5–8% Glycine soja seed extract	Acne-removing compound, removes redness and eliminates swelling in the skin and acne bacillus on the surface of a human body cannot generate drug resistance to the acne-removing compound preparation	CN110613658A [62]
Flower and fruit extract	Coconut extract, shea butter fruit oil, Sambucus chinensis extract and Eucheuma gelatinae extract	Lipstick, good moistening and antioxidant effect, and can be protected so that it can be stored for one year without bacteria breeding	CN110101628A [63]
Folium extract	Herba violae extract, Lactobacillus/pear juice fermented product filtrate and Polygonum cuspidatum extract.	Multiple-effect facial mask that has good repair effects for acne and the bad symptoms, including rough skin, scars, hyperpigmentation, etc., accompanying the acne	CN108904432A [64]
Folium extract	Lactobacillus/pear juice fermented product, Phellodendron bark extract, white willow bark extract, a western pear extract, Scutellariae root extract and Herba houttuyniae extract	Antioxidant components and inhibits monophenyl oxidase, where the acne-removing effect was remarkable	CN108888678A [65]
Leaf extract	Houttuynia cordata extract, wild soybean seed extract, Phellodendron bark extract and Scutellaria baicalensis extract	Acne-removing skincare product treatment was mild, non-irritant, easy to absorb, not greasy, safe and efficient	CN108434032A [66]
Leaf extract	Dioscoreae bulbiferae bark extract, Sophorae flavescentis extract and Salviae miltiorrhizae extract	Inhibits acne inflammation	CN108158972A [67]

Table 3. *Cont.*

A. indica Part(s)	Other Main Plants in Formulations	Claims in Cosmetics	Patent
Folium extracts	Sea sludge extracts, amur cork tree bark extracts, white willow bark extracts, China Rehmannia extracts and Scutellaria extracts	Acne-removing function with a remarkable oil control effect.	CN105770160A [68]
Seed extract	Gleditsia australis extract, Sapindus mukurossi extract and Camellia extract	Cleaning skin with a role in inhibiting and killing bacteria and fungi, capable of eliminating smells and resisting corrosion, skin friendly and soluble in water	CN105769710A [69]

* NI—no information available.

9. Trends in Dermocosmetic Formulations

In this section, we suggest dermocosmetic formulations according to the principal benefits related to the biochemical composition of *A. indica*.

- Acne: Suppression of the ability of the *Propionibacterium acnes* pathogen to induce ROS and pro-inflammatory cytokines. Antibacterial action of *A. indica* oil was demonstrated in vitro against pathogenic bacteria by inhibiting bacterial cell membrane synthesis. *A. indica* contained nimbolid (antibacterial), gedunin (antifungal), mahmoodin (antibacterial), margolone (antibacterial) and cyclic trisulphide (antifungal) [3].
- Anti-aging: *A. indica* stimulated collagen production, promoted soft and supple skin, aided in reducing old scars and supported healing [12].
- Anti-dandruff: *A. indica* produced antibacterial, antifungal, pain-relieving and another specific phytocompounds to avoid dandruff [70].
- Oral care: Reduction in plaque and treatment of gingivitis and periodontitis (dental gel) [3]. Dental gel with Neem extract reduced plaque and bacteria more than the commercial mouthwash (chlorhexidine gluconate 0.2% *w/v*). Furthermore, Neem could reverse incipient carious lesions and inhibit Streptococcus mutans (bacterium causing tooth decay) [4].
- Health and personal care products: *A. indica* could be used for nail care (nail oils and polish), hair care (hair oils and shampoo), oral hygiene (toothpaste and Neem twigs), skin cleanser, soaps and insect repellent (spray and lotion) [4,14,71,72].
- Pediculosis treatment: shampoo with *A. indica* seed extract was more active than permethrin-based shampoo against head lice [73].
- Scabies treatment: *A. indica* oil was used in soap production and was indicated to scabies.
- Skin disorders: *A. indica* could treat lice and scabies. Neem in a paste combination with Curcuma longa treated scabies and 97% of the volunteers were cured after application (3 to 15 days) with no adverse effects. Neem was used for diabetic foot, dry psoriasis and wounds [4,12].
- Skin-whitening: *A. indica* inhibited 47% of tyrosinase with no anti-wrinkle effect, with it being best for young skin [74].
- Wound healing: the presence of tannins could favor the healing of wounds [4].

10. Perspectives and Conclusions

For centuries, the production and commercialization of herbal medicines have faced many challenges worldwide, mainly in developing countries. Thus, some issues must be considered as problems to be solved to offer plants or extracts as qualified raw materials for several industries, such as quality, processing and harvesting issues, and even mainly clini-

cal trials [2]. Fortunately, plant biotechnology has emerged to aid in secondary metabolite production in in vitro controlled conditions, mainly via cell suspension cultures [9].

On the other hand, the most accepted biopesticides are those containing Neem derivatives, e.g., azadirachtin, which is currently established as an essential insecticidal ingredient [3,5,6,75]. It acts as a repellent, antifeedant and repugnant agent that also induces sterility in insects by interrupting sperm production, as well as preventing oviposition in males. Therefore, more alternative strategies for pest resistance are evolving to develop more specific nanocarriers aiding in environmental conservation [75].

Most recently, nanotechnology-based plants are used in many scientific domains, such as controlling pollutants and biomedical fields [76]. For instance, metal oxide nanoparticles were shown to enhance both the antimicrobial activity and photocatalytic potential of the aqueous leaves extract of *A. indica* in a recent review [77]. Regarding skin disorders, a simple green route to synthesize silver nanoparticles containing the aqueous extracts of *A. indica* leaves had greater antibacterial and free-radical scavenging efficacy. Moreover, a hydrogel with such nanoparticles was described as a low-toxic, eco-friendly delivery vehicle with interesting potential in wound healing due to the presence of phenolic compounds, flavonoids, terpenoids and terpenes using topical wounds in rats [78]. Therefore, formulating topical products with *A. indica* nanoparticles can be a novel choice to produce safer topical products with proven dermocosmetic attributes.

Indeed, some industries are developing many efforts to establish "green chemistry" to reduce or avoid both the use and creation of substances that are dangerous to the environment and even human health. Therefore, such new technologies enhance the production of targeted biocompounds that are to be incorporated as cosmeceutical ingredients with some advantages, such as a less cultivation time, high concentration/extraction of targeted compounds and less environmental damage.

Finally, plant derivatives, mainly *A. indica* (Neem) byproducts, can be carefully used in cosmetics for skin and hair care after all the specific safety and efficacy assessments to offer skin benefits described in our review with good cutaneous tolerance.

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