

Development and evaluation of an anti-dandruff shampoo based on aqueous extract of *Azadirachta indica* (neem) leave

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Abstract

This study focused on the development of an anti-dandruff shampoo based on an aqueous extract of *Azadirachta indica* (neem) leaves, a plant known for its antifungal and anti-inflammatory properties.

The extraction was performed by maceration, followed by qualitative and quantitative phytochemical screening. Shampoo formulations were developed with 2% neem extract and evaluated physicochemically (pH, viscosity, foamability), microbiologically and dermatologically. A single-blind pilot study was conducted on 30 adult women suffering from mild to moderate dandruff.

Phytochemical screening revealed richness in flavonoids, tannins, and phenolic compounds. Formulation F3 showed optimal characteristics, including a suitable pH (5.7), good microbiological stability, and satisfactory skin tolerance. After four weeks of use, participants who used the neem shampoo showed a significant improvement in symptoms: reduced flaking, relief of itching, and improved scalp condition. No adverse effects were observed.

These results confirm the anti-dandruff efficacy of the shampoo, attributed to the bioactive properties of neem extract. The product thus presents a promising natural alternative to conventional treatments, with a good safety profile and appreciable cosmetic acceptability.

Keywords: *Azadirachta indica*; Shampoo; Anti-dandruff; Formulation; Quality control

1. Introduction

Dandruff, a common scalp condition characterized by flaking and itching, affects a significant portion of the global population [1]. The origin of dandruff is multifactorial; it can be linked to excessive sebum production, proliferation of *Malassezia* fungi, as well as genetic factors, stress, climatic conditions, and certain hygiene habits [2]. Although various synthetic anti-dandruff agents are available, concerns regarding potential side effects and environmental impact have led to growing interest in natural alternatives. *Azadirachta indica*, commonly known as neem, is a plant with a long

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history of medicinal use in traditional systems, particularly for its antimicrobial, anti-inflammatory, and antifungal properties [3]. According to Nokam *et al.*, the aqueous extract of neem leaves contains several functional groups identified by phytochemical screening tests. These compounds include flavonoids, tannins, saponins, anthraquinones, alkaloids, and glycosides. These metabolites are suspected to be responsible for its anti-inflammatory and gastroprotective effects [4]. These properties make it a promising candidate for the treatment of dandruff, often associated with proliferation and inflammation of the scalp. The justification for this research is to leverage the well-documented therapeutic potential of neem in a practical and effective topical application, offering a natural and potentially safer alternative to conventional anti-dandruff products. The main objective of this study is to formulate and characterize an anti-dandruff shampoo incorporating an aqueous extract of *A. indica* leaves and to evaluate its physicochemical properties and *in vivo* efficacy against dandruff.



Figure 1 *Azadirachta indica* tree (present study, Mokolo locality / Far North Cameroon)

2. Materials and Methods

2.1. Preparation and Characterization of *Azadirachta indica* Aqueous Leaf Extract

2.1.1. Plant Material

Fresh *Azadirachta indica* leaves were collected in the locality of Mokolo in the Far North region of Cameroon, identified, and authenticated at the National Herbarium of Cameroon by botanist Ngansop T. Eric in comparison to specimen n° 19223/SRFCam.

2.1.2. Extraction

The leaves were air-dried, reduced to powder, and subjected to aqueous extraction by maceration for 24 hours with 250 g of powder in 5 liters of distilled water [5]. The extract was filtered and dried in an oven at 55°C for 72 hours.

2.1.3. Phytochemical Screening

Using standard methods, a qualitative and quantitative phytochemical analysis was carried out to identify and quantify the main bioactive compounds such as flavonoids, phenolic compounds, tannins, and saponins, known for their antimicrobial and anti-inflammatory properties [6-10].

2.2. Formulation of Anti-Dandruff Shampoo

2.2.1. Excipient Selection

Appropriate surfactants, conditioning agents, thickeners, preservatives, and fragrances were selected based on their compatibility and desired shampoo properties (Table 1).

Table 1 General formula for a liquid shampoo [11].

Excipients	Proportions (%)
Surfactants (cleansing agents)	15 to 25
Foam stabilizers	1 to 4
Thickeners	2 to 5
Opacifiers	1 to 2
Preservatives	0.1 to 0.5
Special treatments	Quantity as needed
Colorants and fragrances	Sufficient quantity
Water	Sufficient quantity

2.2.2. Formulation Development

Various shampoo formulations were prepared incorporating different concentrations of *Azadirachta indica* aqueous leaf extract. Optimization was performed to obtain the desired consistency, foam, and cleaning properties.

Preparation of aqueous and oily phases.

- **Preparation of the aqueous phase:** In a 600 mL beaker, two-thirds of the total amount of distilled water was used to disperse hydroxyethylcellulose, benzoic acid, potassium sorbate, and allantoin. Stirring was performed using a whisk until a homogeneous mixture without lumps was obtained. Then, the surfactants were incorporated one by one: first the anionic surfactants (Sodium Lauryl Sulfate and Sodium Lauryl Ether Sulfate), then the amphoteric surfactant (Cocoamidopropyl betaine). Stirring continued until a homogeneous gel formed. Glycerin was added at the end of this step, giving the product a pearlescent appearance.
- **Preparation of the oily phase:** In an Erlenmeyer flask, lavender, peppermint, and tea tree essential oils were combined. This lipophilic phase was then homogenized by moderate manual stirring.

Mixing of the different phases.

The oily phase was slowly poured into the aqueous phase under vigorous stirring, yielding a homogeneous mixture with a creamy texture. Simultaneously, the remaining distilled water (1/3) was used to disperse the viscosifying agent (xanthan gum) to prevent lump formation. This mixture was gradually introduced into the main phase under constant stirring until a viscous consistency characteristic of a shampoo was obtained. The aqueous extract of *Azadirachta indica* leaves was incorporated at 2% of the total volume of the formulation under stirring.

pH control and adjustment.

The pH of the shampoo was measured using a pH meter and adjusted, if necessary, to a value between 4.5 and 6.

2.2.3. Control Formulation

A placebo shampoo without the extract was prepared for comparison purposes.

2.2.4. Evaluation of the Formulated Shampoo

Physicochemical and microbiological properties:

- The pH was measured using a calibrated pH meter.
- Viscosity was determined using a viscometer.
- Foaming power and foam stability were evaluated by agitating a fixed volume of shampoo solution and measuring the height and stability of the foam over time in a graduated cylinder [12].
- The absence of microbial contamination by bacteria, yeasts, or molds was verified in accordance with ISO 21149 and 16212 standards [13,14].
- Skin tolerance was performed by the elbow crease test: on 30 adult female volunteers, a small amount of shampoo (approximately 0.5 mL) was applied to the elbow crease, an area of approximately 3 cm², previously sterilized with alcohol, without dressing or occlusion. The product remained in contact with the skin for 30

minutes, then was rinsed with distilled water. Visual observation of the treated area was performed immediately after rinsing (T0), then at a distance (T1), and the next day (T24), to detect any signs of irritation or intolerance (erythema, pruritus, edema, dryness, etc.). The absence of a significant reaction in all participants indicates good skin tolerance of the tested product [15].

2.2.5. Evaluation of in vivo efficacy and safety (Pilot Study)

Study Design

A small-scale, single-blind, placebo-controlled pilot study was conducted on 30 adult female volunteers suffering from mild to moderate dandruff, after obtaining informed consent and ethical approval.

Treatment Protocol

Volunteers were randomly assigned to use either the *Azadirachta indica*-based shampoo or the placebo shampoo for a period of 4 weeks. On wet hair, 3 to 5 milliliters of shampoo were applied, left for 3 minutes before rinsing. Application was repeated after 2 days within the week. No other lotion, cream, or anti-dandruff shampoo was allowed during the study period.

Evaluation Parameters

Dandruff severity was assessed by visual inspection using a standardized scoring system (macroscopic desquamation, erythema, itching) at the beginning of the study, weekly, and at the end of the study. Scalp health was also monitored.

3. Results

3.1. Characterization of *Azadirachta indica* Aqueous Leaf Extract

The aqueous extract yielded approximately 12.8% (w/w) dry matter. Phytochemical screening revealed the presence of flavonoids, tannins, saponins, steroids, terpenes, phenols, and glycosides. High concentrations of flavonoids (2.27 ± 0.005 mg QE/g) and total phenols (1.470 ± 0.005 mg GAE/g) were observed.

3.2. Physicochemical Properties of the Formulated Shampoo

Table 2 Composition and properties of the obtained formulations

Composition	F1	F2	F3
Distilled water	SQF	SQF	SQF
Sodium Lauryl Sulfate	10%	12%	10%
Sodium Lauryl Ether Sulfate	10%	12%	10%
Cocoamidopropylbetaine	5%	5%	5%
Glycerin	3%	3%	3%
Neem extract	2%	2%	2%
Provitamin B5	1%	1%	1%
Benzoic acid	0.6%	0.6%	0.6%
Xanthan gum	0.5%	0.1%	0.3%
Tea tree essential oil	0.3%	0.3%	0.3%
Peppermint essential oil	0.2%	0.2%	0.2%
Quality control			
pH	5.9	6.7	5.7
Viscosity	5000 cP	2000 cP	3000 cP
Density	1.26	2.02	1.10

Foam index	9	5	7
Microbiology	Conforms	Conforms	Conforms
Skin tolerance	Good	Good	Good
Appearance	Homogeneous	Heterogeneous	Homogeneous
Color	Brown	Brown	Brown
Odor	Mentholated	Mentholated	Mentholated
Texture	Compact	Fluid	Gel-like

F1: Formulation 1; F2: Formulation 2; F3: Formulation 3; SQF : Sufficient Quantity For; cP : centipoise

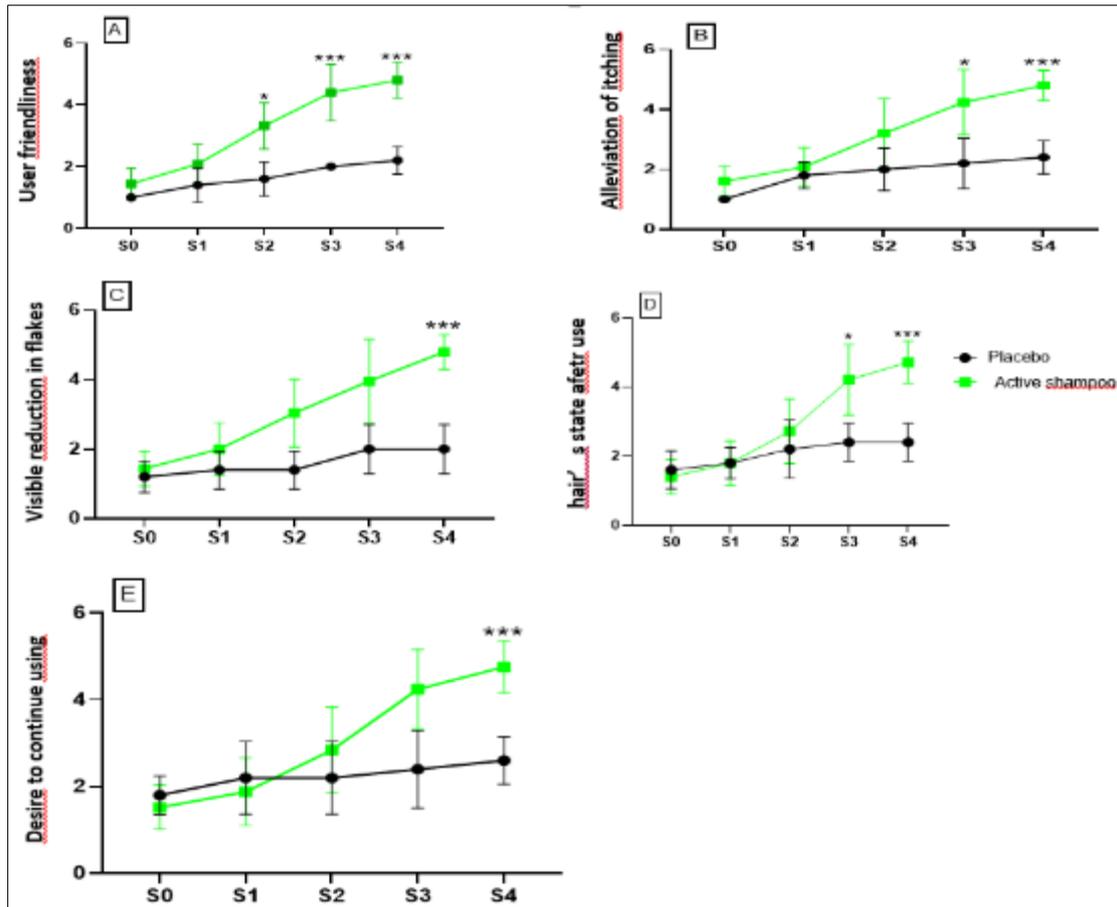
The optimized shampoo based on *Azadirachta indica* (Formulation F3) had a pH of 5.5 ± 0.2 , suitable for scalp application. The viscosity was found to be in the range of 3000 cP, offering good fluidity and spreadability. Excellent foamability and foam stability were observed, comparable to commercial shampoos. Table 2 summarizes the composition and physicochemical properties of the developed formulations. Figure 2 presents the shampoo before and after conditioning.



Figure 2 a) Formulated shampoo; b) Conditioned shampoo

3.3. Evaluation of *in vivo* Efficacy and Safety

The results of the efficacy test of the formulated shampoo in volunteer patients are presented in Figure 3 below. There was a significant increase in ease of use (Figure 3 A) in patients treated with the shampoo compared to those treated with placebo from week 2 ($p < 0.05$), and continuously until the end of week 4 ($p < 0.001$). This trend is similar for all parameters investigated, where at the end of week 4 of the study, an increase ($p < 0.001$) in pruritus relief, a visible reduction in scales ($p < 0.001$), and an improvement in hair condition ($p < 0.001$) were observed in patients treated with the active shampoo compared to placebo, demonstrating the efficacy of the *A. indica* extract-based formulation. Furthermore, an increase in participants' willingness to continue the study was observed, indicating an overall positive appreciation based on the results observed after shampoo use. No adverse reactions or significant scalp irritation were reported by volunteers using the *A. indica*-based shampoo, indicating good tolerability. Figure 4 visually illustrates the improvement in dandruff condition after 4 weeks of treatment with the formulated shampoo. We note an absence of scales, inflammation, and a shine of the scalp and hair.



* p < 0.05; *** p < 0.001: significant differences in the active shampoo-treated group compared to the ; Placebo-treated group.

Figure 3 Efficacy of the *A. indica* extract-based shampoo formulation



Figure 4 Dandruff condition after 4 weeks of treatment with the active shampoo

4. Discussion

This study aimed to develop a formula for an anti-dandruff shampoo based on an aqueous extract of *Azadirachta indica* (neem) leaves for the treatment of dandruff.

The aqueous extraction yield obtained was 12.8% (32 g of dry extract from 250 g of dry powder). This result is comparable to that reported by Nokam *et al.*, who observed a 15.8% yield from a similar extraction of Cameroonian neem leaves [4].

Qualitative phytochemical screening revealed the presence of saponins, phenolic compounds, sterols and triterpenes, and cardiac glycosides. These findings are consistent with those of Nokam *et al.*, who identified the same classes of metabolites in aqueous neem extracts [4]. The absence of alkaloids contrasts with some studies and may be due to varietal specificity, geographical location, or the extraction protocol used.

Quantitative analysis showed high levels of total polyphenols (1.470 mg GAE/g dry extract) and total flavonoids (2.277 mg QE/g dry extract). These values are higher than those reported by Kumasi *et al.* for aqueous extracts of Indian neem (0.98 mg GAE/g and 1.280 mg QE/g dry extract) [16], but lower than those of Khandagale *et al.* for ethanolic extracts (2.1 g GAE/g and 520 µg QE/g dry extract) [17]. These variations could reflect differences in geographic origin (Cameroonian vs. Indian biotypes), cultivation conditions (soil, climate), and extraction methods (aqueous maceration vs. organic solvents).

Quality control tests on our shampoo formulated with 2% *Azadirachta indica* extract showed a pH of 5.70—optimal for the scalp as it helps preserve the hydrolipidic barrier; a pseudo-plastic viscosity facilitating application without compromising user experience; and notable microbiological stability marked by the absence of pathogens in the formulation, complying with Good Manufacturing Practices and thus confirming the product's safety.

The anti-dandruff efficacy of our shampoo was demonstrated by a reduction in flakes (96% vs. 45% for the placebo) and relief from itching (92% vs. 45% for the placebo). These results are statistically significant ($p < 0.01$) and outperform the rates reported for other natural actives by Kumasi *et al.* [16]. The satisfaction rate (88%) supports the product's cosmetic acceptability, particularly due to its menthol fragrance and smooth texture. Such a significant efficacy difference suggests a real therapeutic effect of neem. This result is consistent with the literature, which highlights neem's antifungal activity against *Malassezia*, the primary fungus responsible for dandruff [17]. Indeed, our findings align with previous observations that neem extracts exhibit strong *in vitro* inhibition of *Malassezia furfur*, which explains the significant reduction of flakes in the treatment group. Furthermore, the notable decrease in pruritus in the treated group corresponds to neem's known anti-inflammatory and soothing properties. These beneficial effects are corroborated by several studies reporting that neem reduces skin inflammation and calms irritation thanks to its bioactive compounds [18–19]. In summary, our clinical observations align with published data on neem extracts: their anti-dandruff effectiveness can be attributed to a combination of antifungal, anti-inflammatory, and scalp-protective actions.

5. Conclusion

This study successfully developed and evaluated an anti-dandruff shampoo formulated with an aqueous extract of *Azadirachta indica* leaves. The formulated shampoo exhibited desirable physicochemical properties, promising *in vivo* anti-dandruff efficacy, and no reported adverse effects in a preliminary study. These results suggest that the aqueous extract of *A. indica* leaves incorporated into a shampoo offers an alternative to conventional dandruff treatments.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

Approval was given by the Research Ethics Committee of the Faculty of Medicine and Biomedical Sciences (FMBS), University of Yaoundé I and by the Regional Research Committee for Human Health of the Center (RRCHHC).

Statement of informed consent

Informed consent was obtained from all participants included in the study.

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