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Formulation and Antimicrobial Activity of Triclosan-Based Conditioning Medicated Shampoo

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Abstract

The human scalp is susceptible to microbial build-up due to elevated natural oil (sebum) level and so needs to be thoroughly and frequently washed with appropriate cleansing agents such as shampoos. This study investigated the antimicrobial efficacy of a conditioning medicated shampoo which was formulated using triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol as the antimicrobial agent and a blend of anionic and non-ionic surfactants to provide cleansing and conditioning effects, respectively. The multifunctional shampoo was designed to cleanse, condition the hair and principally eliminate fungal and bacterial microbes which can cause scalp disorders and diseases. The shampoo was tested against three selected fungal species (*Candida* spp, *Aspergillus* spp, *Malassezia globosa)* and two bacterial species (*Staphylococcus aureus* and *Escherichia coli*) at dilution concentrations (%) of 90,80,70,60,50,40,30, 20 and 10. The results of the antimicrobial resistance and susceptibility tests revealed that the shampoo was highly effective against the three fungi at all concentrations but the higher concentrations (50-90% dilution) had slightly greater efficacy than the lower concentrations (10-40 % dilution). *E coli* showed susceptibility to the medicated shampoo at all concentrations. The shampoo formulation showed no activity against *S. aureus* at all dilutions (10-90%) as there was no zone of inhibition. The medicated shampoo is evidently effective against *Malassezia globosa,* the fungus that causes dandruff, *Aspergillus* spp, *Candida* spp as well as *E coli* at all dilution levels. The in-use application of the shampoo revealed that it possessed

very good lathering and cleansing ability as well as conditioning attributes which were observed in the soft, silky lustrous appearance of the hair after washing. Thus, the shampoo can be used to maintain a clean, healthy scalp and hair.

Keywords: Antimicrobial agent, medicated shampoo, triclosan, bacterial and fungal species, non-ionic surfactant, conditioning agent

INTRODUCTION

The maintenance of clean, healthy scalp and hair, through regular washing, is an important aspect of personal grooming. A clean, well-groomed hair is pleasant to behold, enhances appearance and boosts the confidence of the wearer. The human scalp can become a reservoir for microorganisms due to the high sebum level if not frequently and properly washed with appropriate cleansers. A build-up of microorganisms can lead to itching, inflammation, scalp disorders or diseases like dandruff and ringworm which are common amongst the human populace. However, regular washing of the hair with the appropriate cleansing agents such as shampoos, will minimize the incidence of these diseases. Dandruff is the unusually high shedding of dead skin cells from the scalp [1]. It is caused by a yeast-like fungus, *Malassezia globosa* (formerly known as *Pityrosporum ovale)* which lives on the scalp, feeding on skin oils [2-4]. Dandruff is a common scalp disorder affecting almost half of the world's population at the post pubertal age and of any sex and ethnicity [5]. The symptoms are an itchy scalp and flakiness which can also be accompanied by redness and irritation [6-8]. The white, dry flakes associated with dandruff are visually observable and distinguishable on the scalp, in the hair and clothing of the sufferer. The presence of these abnormal scaly materials can constitute an embarrassment and also cause low esteem-problems in those affected by the disorder, thus the treatment is necessary for physiological, psychological and social reasons. Although dandruff is not caused by poor hygiene, infrequent washing of the hair can exacerbate the condition. *Tinea capitis*, also known as 'scalp ringworm' [9] is a cutaneous fungal infection of the scalp [10]. Ringworm of the scalp typically presents as a single or multiple patches of hair loss that may be accompanied by inflammation, scaling, pustules, and itching. [9] *Tinea capitis* is predominant in pre-pubertal children, more often boys than girls [11].

The term 'antimicrobial agent' is generally used to describe a wide variety of chemical compounds, drugs and physical agents that are used to destroy, prevent or

slow the growth of microorganisms [12][13]. They include antibacterial, antiviral, antifungal and antiparasitic agents [12]. Antimicrobial agents are therefore, generally used to prevent infections and diseases caused by pathogens. An ordinary shampoo is a cleansing agent that contains anionic surfactants [14] and effectively removes oil and dirt from the scalp and hair while a medicated shampoo, provides an additional benefit of substantially eliminating or reducing the microbial load on the scalp which minimizes the incidence of scalp disorders and diseases. A conditioning medicated shampoo is multifunctional as it cleanses, eliminates microbes and also imparts conditioning benefits to the hair.

Triclosan, (also known as Irgasan DP 300) is a chlorinated aromatic compound that has functional groups representative of both ethers and phenol [15]. A polychlorophenoxy phenol, triclosan is a white powdered solid with a slight aromatic /phenolic odour [15]. It is chemically known as 5-chloro-2-(2,4-dichlorophenoxy) phenol [16] [17]. Triclosan is used as an antibacterial and antifungal agent in a variety of consumer products such as toothpastes, mouthwashes, soaps, deodorants and surgical cleaning treatments [18-20]. Some studies [21] suggest that antimicrobial hand soaps containing triclosan provide a slightly greater bacterial reduction on the hands compared to plain soaps due to its bacteriostatic and fungistatic action. This is also applicable to the medicated shampoo, which is expected to inhibit, reduce or eliminate microbes from the scalp and hair, thereby reducing the incidence of scalp diseases better than the ordinary shampoo.

A number of antifungal chemical substances have been found to be effective against scalp disorders. These include ketoconazole, selenium disulphide, zinc pyrithione [6][21], which have been found to be particularly effective against dandruff. Other effective anti-dandruff agents include salicylic acid, sulphur, coal tar as well as flower, leaf, root and stem extracts of plants [22] [23] An anti-dandruff shampoo is specifically designed to treat dandruff. Assortments of anti-dandruff shampoos, containing the aforementioned antimicrobial agents are commercially available in the Nigerian market. There is however, a far less supply of medicated shampoos, which have a broader spectrum of activity as they do not only control dandruff but other scalp disorders as well. There is a dearth of literature report on the use of triclosan as an antibacterial agent or medicament in the formulation of medicated shampoos.

In this study, a conditioning medicated shampoo was formulated using triclosan as an antimicrobial agent. The antifungal and antibacterial efficacy of the shampoo was evaluated using selected microorganisms. The conditioning effect of the shampoo on the hair as well as its cleansing efficacy and foamability were assessed in comparison with a popular brand.

2.0 MATERIALS AND METHODS

2.1 Materials

The chemicals and reagents used for the biochemical tests, shampoo formulation and antimicrobial studies were all obtained from assured importers / suppliers of pharmaceutical grade chemicals.

2.2. Methods

2.2.1 Production of Medicated shampoo

The formula presented in Table 1 was adopted after series of trial formulations were carried out experimentally to ensure stability, clarity, homogeneity, cost-effectiveness and optimal performance of the conditioning medicated shampoo.

Table 1: Formula for Production of Conditioning Medicated Shampoo

Chemical Component	**Function**	**Weight %**	**Weight (g)**
A. Sodium lauryl sulphate	Anionic surfactant	12.00	60.00
Sodium chloride	Thickener	3.00	15.00
Coconut Diethanolamide	Conditioner, foam booster viscosity booster	0.50	2.50
B. Triclosan	Antimicrobial agent	0.30	1.50
Ethanol	Solvent	1.00	5.00
C. Colourant (blue)	Colour	0.005	0.025
Perfume	Fragrance	0.20	1.00
Distilled water	Diluent	82.995	414.96
Total		**100.00%**	**500.00g**

Procedure for Production of Medicated Shampoo

Sodium chloride (15g) was dissolved in about one-third part of the distilled water (30ml). Sodium lauryl sulphate (60g) was gradually added to the salt solution with stirring until it completely dissolved to give a clear homogeneous solution. The remaining amount of water was then added to the solution. Diethanolamide (1.5g) was added with stirring . Triclosan (1.5g) was dissolved in 5g of ethanol and the triclosan solution (B) was added to the shampoo (A) with stirring. The colourant and fragrance were added with stirring to give a clear, homogeneous, light blue viscous liquid, which is the medicated shampoo.

3.2.2 Isolation of Microorganisms from Human Hair

Sterile swab sticks were used to collect the samples by rolling the sticks on the scalp of ten female students of Ebonyi State University, Abakaliki. The samples were inoculated into test tubes containing 5mls of nutrient broth medium and incubated at a temperature of 37^0C for 24hours. Mannitol salt agar was used for the detection of *Staphylococcus aureus* in the sample. MacConkey agar was used for isolation of *Escherichia coli* while for isolation of fungi organisms in the samples, potato dextrose agar (PDA) was used [24]. All suspected growth *of S. aureus, E.coli*, Aspergillus and Candida spp were sub-cultured onto nutrient agar slant from which biochemical tests were carried out.

3.2.3 Identification and Confirmation of Microorganisms

Standard microbiology technique and biochemical tests were used to identify and confirm the presence of the various microorganisms used in the study. These tests include catalase, indole, oxidase and citrate tests [24].

3.2.4 Evaluation of Cleansing and Foamability Properties of the Medicated Shampoo

The cleansing and foaming properties of the medicated shampoo which are important product features were evaluated by in-use application of the shampoo. The shampoo was used to wash half the hair of twenty (20) female students. The performance of the shampoo in these two attributes was assessed in comparison with a popular commercial shampoo brand, which was used to wash the other half of the hair.

3.2.5 Evaluation of Shampoo Conditioning Attributes

The conditioning effect of the shampoo on the hair was evaluated after the hair had been washed. Conditioning properties encompass all desirable benefits imparted to the hair such as increased body to the hair (for fly-way hair), improved gloss or lustre, improved softness and silkiness, mending of split ends, reduced static charge and improved combability and manageability [25-27].

3.2.6 Antimicrobial Susceptibility and Resistance Patterns

This test is usually carried out to determine the susceptibility or resistance of organisms to chemical formulation agents, which in this case is the medicated shampoo. This test was carried out using the method described by Cheesbrough [24]. The test organisms were sub-cultured onto fresh nutrient broth. Broth cultures were then incubated at 37^{o}C until a turbidity of 0.5 Mcfarland standard was obtained. The turbidity of the actively growing broth culture was then adjusted with normal saline to obtain 0.5 McFarland turbidity standards. This was streaked on the surface of solid Mueller Hinton agar plates using sterile swab sticks. Wells of 8mm in diameter and about 2cm apart were punched in the culture media with sterile cork borer and the dilutions were used to fill the holes. The plates were incubated at 37^{o}C for 24 hours (bacteria) and 72 hours (fungi). Zones of inhibition around the wells were measured in millimeters using a ruler.

3. RESULTS AND DISCUSSION

3.1 Isolation of Microorganisms from Human Hair

Four different species of microorganisms were isolated from the human scalp. These include two fungal species **(***Aspegillus* spp *and Malassezia globosa) and* two bacterial specie (*Staphylococcus aureus* and *Escherichia coli*). The third fungal specie, *Candida* spp was a clinical sample obtained from Federal Teaching hospital, Abakaliki, Nigeria.

3.2.Biochemical Tests

The results of the biochemical tests confirmed the presence of *Staph aureus, E. coli, Candida* spp, *Aspergillus* spp *and Malassezia globosa* used in the study.

3.3 Cleansing and Foamability Properties of Shampoo

The formulation of the multifunctional shampoo was skillfully carried out in order to ensure the stability and optimal performance of the product in terms of cleaning, conditioning and antibacterial action. The shampoo showed excellent lathering and cleansing ability when used to wash the hair and scalp as it removed oil, dirt and dandruff scales effectively and efficiently. The washing process was accompanied by formation of rich lather, which is a desirable attribute, since consumers generally have the perception that copious foam formation in a shampoo is evidence of good cleansing power. These desirable product features are due to the combination of an anionic surfactant (sodium lauryl sulphate), which has good cleansing property and a non-ionic surfactant (a fatty acid alkanolamide) which boosts foam formation in the shampoo [25][26]. The alkanolamides are often used in conjunction with the anionic surfactants as coactive agents (or co-surfactants) and are indeed the most commonly used coactive agents in toiletry formulations [23]. They are traditional coactive ingredients in shampoo and foam bath formulations in which they act as foam boosters, foam stabilizers, viscosity modifiers and conditioning agents. Hard water and the presence of abundant soils reduce foam activity; this occurs often during the initial application of a shampoo to soiled hair. The addition of a fatty acid alkanolamide can have a pronounced effect in stabilizing and boosting the foam level and improving the lather consistency. In addition, the alkanolamide enhances the solubility of the primary surfactant, disperses lime-soap curds and increases shampoo viscosities. Most commercial alkanolamides are usually the mono- or diethanolamide adducts such as oleic monoethanolamide, lauric diethanolamide [26] and coconut diethanolamide

3.4. Conditioning Effect

Hair conditioners are preparations that impart certain desirable attributes to the hair. These include increased body to the hair (for fly-way hair), improved gloss or lustre, improved softness and silkiness, mending of split ends, reduced static charge and improved combability and manageability [25-27]. Hair naturally has an oily layer, which tends to collect dirt. Shampooing removes not only the dirt from the hair but also most of the protective layer of natural oil (sebum) that surrounds the hair, which has to be replaced, or else the hair appears dry, dull and tangled [25][28]. Hence the need to add conditioners that will be substantive to the hair leaving it conditioned, soft and manageable. Modern formulations of shampoo usually contain a conditioning agent in addition to the cleaning agent. Sodium lauryl sulphate, an anionic surfactant and cleansing agent used in the shampoo formulation, has the tendency of stripping the natural oils from the hair in the process of washing, thereby leaving the hair dry

and brittle. However, this was not the case with the conditioning medicated shampoo, which imparted noticeable silkiness and luster to the hair. This is due to the incorporation of an alkanolamide which has conditioning effect on the hair as well as other benefits of boosting foamability and increasing shampoo viscosity. The conditioning property of alkanolamides can be attributed to their substantivity to hair and skin.

3.5. Antimicrobial Susceptibility and Resistance Patterns

The antimicrobial susceptibility test was used to determine the degree of efficacy of the medicated shampoo against fungal and bacterial species at various concentrations (dilution levels). The medicated shampoo, was tested against two selected species of bacteria *Staphylococcus aureus* and *Escherichia coli)* and three fungal species (*Malassezia globosa, Aspergillus* spp, *Candida* spp*).* The results of the antimicrobial susceptibility patterns are presented in Table 2 and Fig.1.

Table 2. Results of Antimicrobial susceptibility and resistance patterns of the shampoo at various concentrations

Name of microorganism	**Percentage Dilution (%)**								
	90	80	70	60	50	40	30	20	10
	Inhibition zone Diameter, IZD (mm)								
Aspegillus spp	30	30	30	30	30	24	22	20	20
Candida spp	28	28	28	28	26	25	25	20	20
Malassezia globosa	28	22	22	22	14	14	12	11	12
Staphylococcus aureus	0	0	0	0	0	0	0	0	0
Escherichia coli	20	19	17	12	18	10	10	10	10

The results revealed that the medicated shampoo was effective against all the fungal isolates *(Aspergillus* spp, *Candida* spp and *Malassezia globosa)* at all concentrations (10-90%). It also displayed antimicrobial activity against one of the two bacterial isolates, *Escherichia coli* at all concentrations. This implies that the shampoo is

effective against all the fungal species as well as *E coli* at both high dilution (low concentration) and low dilution (high concentration) levels. The shampoo showed no activity against *S. aureus* at all concentrations (10-90%) as there was no zone of inhibition. This implies that the shampoo is ineffective against the particular strain of *Staph. aureus* tested.

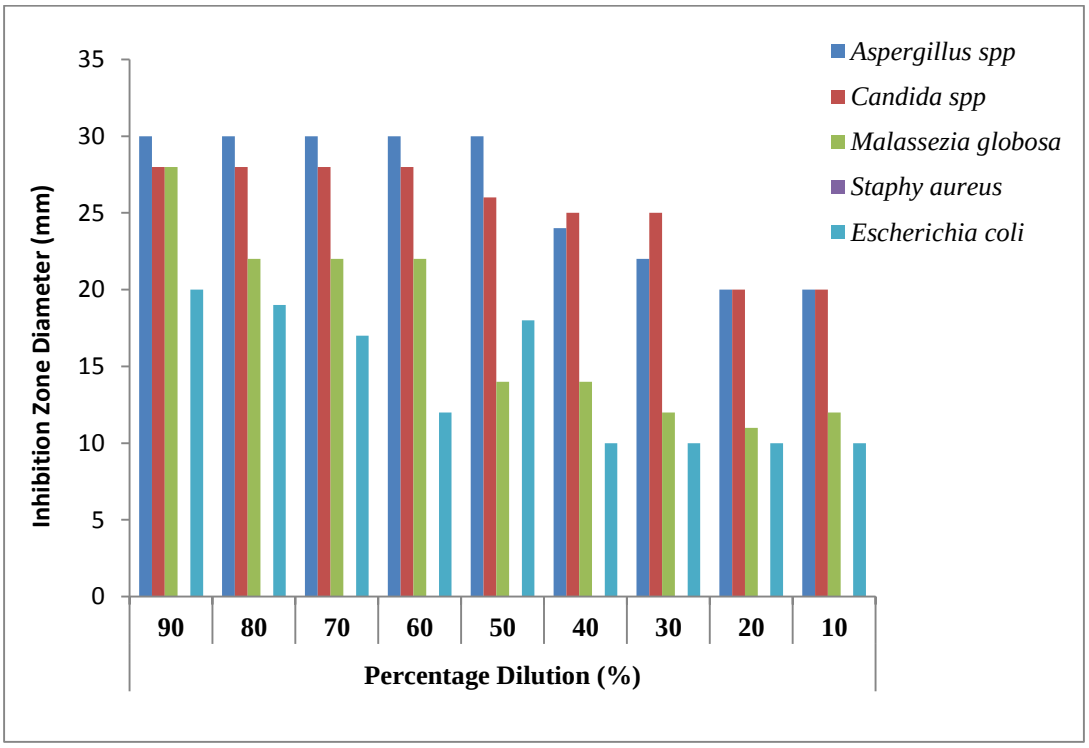

Fig.1 Plots of Inhibition zone diameter versus percentage dilution (%) for different fungal and bacterial species

Higher antimicrobial activity was observed against the fungal isolates as is evident in their larger zones of inhibition which is largely in the range of 20-30mm (for *Aspegillus* spp and *Candida* spp) ; 12-28 (for *Malassezia globosa)* while those for *E coli* and *Staph* were 10- 20 mm and 0 mm respectively. It is evident that the shampoo has greater efficacy against fungal species than bacterial, which is a desirable phenomenon since most scalp and skin disorders like ringworm, dandruff, psoriasis and sebborrheic dermatitis are caused by fungi [1][11].

The highest zones of inhibition values were observed at shampoo concentrations (%) of 90, 80,70, 60 and 50 for the fungal isolates and for *E coli* as shown in Fig 1. Activity against, *Malassezia globosa* , the fungal microbe that causes dandruff, was

also markedly higher at higher shampoo concentrations (low dilution levels) of 60-90% which suggests that the shampoo is more effective against dandruff as well as other microbial species at higher concentrations (low dilution levels) . This therefore implies that over-dilution of the shampoo beyond 60% is likely to reduce its efficacy during the process of washing the scalp and hair. *Candida* spp is not found on the human scalp and hair but in and around the genitals, gastrointestinal tract and oral cavity [29]. Thus, the effectiveness of the shampoo against this fungus suggests that the shampoo could be used to prevent as well as control infections caused by candida such as candidiasis (which occurs within and around the genitals) by regularly washing the area with the medicated shampoo.

CONCLUSION

This study has proven that the formulated triclosan-based medicated shampoo when appropriately diluted is effective against the three selected fungal species (*Candida* spp, *Aspergillus* spp, *Malassezia globosa)* and one bacterial species *Escherichia coli*. Consequently, it can be used for the prevention and treatment of scalp disorders and diseases such as dandruff caused by *Malassezia globosa* fungus and scalp ringworm, caused by *Tinea capitis* . The shampoo had pronounced conditioning effects on hair as well as good cleansing and lathering ability. Thus, cleanliness of hair and scalp and protection from scalp diseases and disorders can be achieved through thorough and frequent washing with the triclosan-based conditioning medicated shampoo.

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