Bulletin of Environment, Pharmacology and Life Sciences

Bull. Env. Pharmacol. Life Sci., Vol 9[5] April 2020 :23-32 ©2020 Academy for Environment and Life Sciences, India Online ISSN 2277-1808 Journal's URL:http://www.bepls.com CODEN: BEPLAD Global Impact Factor 0.876 Universal Impact Factor 0.9804 NAAS Rating 4.95

ORIGINAL ARTICLE



An appraisal of the effectiveness of native salt (sea salt) in the treatment of fungal nail infection (*Onychomycosis*)

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ABSTRACT

This research work focused on the effect of sea salt as an anti-fungi agent in the treatment of Onychomycosis, which is a painful nail condition caused by fungi infection. Since most anti-fungi drugs and medicines are not effective in killing or destroying the disease-causing fungi over a long period of time, the objective of this research was to ascertain the effectiveness of sea salt (native salt) in the treatment of Onychomycosis. Different concentrations of sea salt was prepared and the minimum inhibitory concentration (MIC₇₂) that hindered the growth of the fungi was determined using the spread plate method with sterile disc impregnated with the determined concentrations of native salt. The minimum inhibitory concentration (MIC₇₂) for fungi infected nail scrappings from Eboh, Jakpa and Delta Steel Company (DSC) was found to be $19.91 \pm 0.31\%$, $24.32 \pm 0.48\%$ and $24.66 \pm 0.73\%$ respectively, with no fungi growth in the Airport area samples. The results from this appraisal revealed that sea salt was effective in the treatment of the nail condition Onychomycosis. The study method is simple, natural, non-oral with short response time for inhibiting the development of the fungi pathogens.

Keywords: anti-fungi; fungi infection; fungi - Tinea unguium; minimum inhibitory concentration (MIC);Onychomycosis; sea salt.

Received 29.01.2020

Revised 18.03.2020

Accepted 04.04.2020

INTRODUCTION

In the last few decade, there has been a growing trend of people infected with the nail condition -*Onychomycosis* (mycotic nail). *Onychomycosis* refers to all fungal infections of finger and toenails caused by a species of fungi called *Tinea unguium*[1]. Persons daily exposed to substances (e.g. waters) containing dermatophytes, yeast, or non-dermatophyte molds are the most susceptible[2,3]. The condition can cause nails to be brittle, thickened, crumbly, distorted with no luster (dull). There can also be a dark colouration caused by build-up of debris (dirt) under the nails and sometimes the infected nails may be separated from the nail bed (Plate 1) [4]. The infected persons may feel intense pain on the fingers or toes, which could result in nail breakage with very offensive odour been released from the opening on the affected areas. The condition could persist for years, 6-8 years or beyond even when treated with appropriate anti-fungi medications, synthetic chemicals or substances. However, most medications are not viable in killing or crushing the fungi species over a significant period of time [5].

Due to the pains, ugly look, offensive smell or foul odour, infected persons tends to hide their fingers and toenails from others by wearing plastic hand gloves, applying nail vanish (polish) or covering the region with cloth and covered shoes / wears. By this process of hiding their nails, they make the finger or toe environment moister, creating an enabling condition for the fungi to thrive / grow. Since fungi typically grows and bloom in warm, moist environment, it makes the condition even more painful and the smell worse [6]. The infection usually occur in people whose hands are daily exposed to water or substances containing the organisms for a long period of time, for example dishwashers in restaurants, hair dressers (salonists), house cleaners, laundry workers, nursing mothers etc. [7].

Onychomycosis has been seen to be something more than a minor domestic issue and in spite of an infected individual's high sterile status and living condition, *Onychomycosis* still spreads and persists. The

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occurrence rate of *Onychomycosis* is enhanced by specific factors, some of which incorporate age, hereditary inclination, social class, occupation, heat, dampness, climate and living condition. Certain components, which worsens the condition are unavoidable and these mainly incorporate activities the infected people cannot circumvent/keep away from since they depend on them for their daily livelihood or are in consistent contact with the polluted waters[8,9].

Some possible factors that causes *Onychomycosis* include:

1. **Age:** The risk of developing *Onychomycosis* increases with age, this might be on the grounds that older individuals have slower developing nails and experience issues when dealing with their nails[10].

2. Sports: Some sports may enhance powerlessness to pathogenic fungi, which will increase the danger of the parasitic disease. Individuals who are athletes, swim or ski, for instance, are bound to have the ailment than the individuals who do not.

3. Immunodeficiency: Like other different types of infection, a weakened or low immune system increases the probability or risk of developing *Onychomycosis*.

4. Genetic factors: A few people are normally more vulnerable than others in regard to contagious fungal diseases. Although the exact genes responsible have not been well defined, some researchers propose that an individual is bound to develop fungal contaminations if there is a family history of the infection [10, 11].

5. Other factors: Persistent contact with water, wearing of synthetic latex gloves, handling of sweet products (candy, confectionary, pastry). Others include excessive use of cleaning or washing agents, smoking, rehashed microtrauma to nails and exposed professionals (for example beautician, manicurist, podologist) [12].

Onychomycosis is a very difficult disorder to manage and treatment is usually long-termed with minimal success. Some treatment comprises of topical medicines that are applied directly to the nails or with the use of topical medications, for instance Griseofulvin, Ketoconazole, Itraconazole, Terbinafine and Fluconazole. Topical therapy is mainly effective for very mild cases. These antifungals inhibit the growth of vulnerable fungi, leading to the death of fungi cells. However, the application of topical therapy could sometimes be ineffective with a high probability of 50-85% relapse (which means that the condition of the nail tends to return to its previous state) [13,14].

Onychomycosis can have undesirable consequences on an individual's social and work related functions, affecting a reasonable aspect of the affected person life style. The affected persons may feel uncomfortable in public or work environment, feeling ashamed, unclean and reluctant to expose their hands and feet. Sufferers are often apprehensive that they may transmit the disease to relatives, companions, or colleagues, and these feelings can lead to low self-esteem. Sometimes employers are unwilling to hire persons identified with the infection, especially in businesses requiring the services of food vendors, beauty care, beverage manufacturing, and laundry services amongst others[15, 16, 17].

The discomfort experienced by infected persons occasioned by prolonged standing, washing, writing or typing prevents them from carrying out their task and this is a tangible barrier to work success and achievement. *Onychomycosis* can make workers take frequent time off work especially if treatment is prolonged and ineffective. If no cure is achieved after a prolonged period, patients may get discouraged and discontinue such therapy and resign themselves to bearing the pains, disfigured nails, discomfort and shame. Persons with human immunodeficiency virus (HIV) having *Onychomycosis* can pose a more serious health challenge. Besides having the contaminated nails as an unpleasant and deteriorated condition, there is the possibility of transferring high levels of the pathogens in the infected nails to others [8].

Salt is important at certain level to the health of people and animals and is used universally as seasoning. It is widely used in cooking or added to perishable items as preservative. It is also employed in some refrigeration processes, dyeing, manufacture of soap and glass (prisms and lenses) [18]. Practically most salts originate naturally from the sea although commercially available sea salts differ in their composition chemically and most do not have the same constituents as found in natural seawater, mainly due to fractionation during production process. In seawater, salt usually have the following composition: 55.5% chloride, 7.7% sulphate, 30.8% sodium, 1.1% potassium, 1.2% calcium and 3.7% magnesium [19].

Sea salt (native salt) had been known to inhibit the growth of the fungi. Thus, it can be used in the remediation of the nail condition (*Onychomycosis*) caused by dermatophytes (fungi), *Candida*, and non-dermatophytic (molds) [20]. This study evaluated the inhibitory potentials of sea salt in preventing the growth of fungi - *Tinea unguium* as a simple, natural and non-oral application. This was with the view of proffering solution to sufferers of the nail condition (*Onychomycosis*).



Plate 1: Fingernail of a person infected with *Onychomycosis*: Source: Piraccini and Alessandrini, [21]

MATERIAL AND METHODS STUDY AREA

In this experimental research, fungi species from infected persons and water samples from their residence were collectedfrom four (4) designated sampling points (Warri (Eboh), Effurun (Jakpa), Bendel estate (Airport road) and Udu Local Government Area [Delta Steel Company (DSC) complex] in communities adequately distributed throughout the lower Niger Delta region, in Delta State, Nigeria (Figure 1). Twelve samples in triplicate was collected from each location making a total of one hundred and forty four (144) samples. Warri is an area with a land mass of 17,698 km² that experiences moderate humidity of 92%. The climate is characterized by rainy and dry season. The dry season starts from November and ends in April while rainy season starts from May and ends in October, having a short time off rain called August break. The area has a mean annual temperature of 32.8°C with temperatures ranging from 36°C to 37°C, usually witnessed during the dry season. In some areas, the vegetation is swamp rainforest, enriched with timber, fruit trees and palm [22].



Figure 1: Map of Warri and its environs showing sampling location (marked in red) TEST CHEMICAL

Sea salt (native salt) was used for the fungi toxicity bioassay. From the stock solution (100%), the solutions for exposure were diluted serially for both the preliminary and actual test. The concentrations for the range-finding assay was 1, 10 and 100 % after which 80, 40, 20, 10 and 5 % were serially diluted for the actual (definitive) assay.

Sampling Location	Latitude	Longitude		
Jakpa	N05º 561 60.311	E005º 751 47.311		
Airport	N05° 54107.811	E005°761 42.411		
Eboh	N05°521 38.111	E005 ° 731 21.311		
DSC	N05 º 281 73.411	E005 º 461 18.911		

 Table 1: Global positioning system (GPS) for the sampling locations

TEST SPECIES (*Tinea unguium***)**

The fungi species used for the study was *Tinea unguium*, which is the fungus that affects the finger and toenails of persons suffering from *Onychomycosis*. The test organisms which were collected from nails of infected persons were exposed to the test chemical for the period of the test.

TOXICITY BIOASSAY FOR FUNGI (*Tinea unguium***)**

(i)COLLECTION OF TEST ORGANISMS AND WATER SAMPLES

The test organisms were collected from the nails of infected persons in Warri and its environ in Delta State, Nigeria. Twelve (12) pure samples in triplicate were randomly collected by a cross-sectional mapping of the area, which include: Warri (Eboh), Effurun (Jakpa), Bendel estate (Airport road) and Udu Local Government Area [Delta Steel Company (DSC) complex]. For the analysis of physico-chemical parameters, water samples from the boreholes where the infected persons resided was also sampled, duly preserved and tested for some physico-chemical parameters, metals and microbial (fungi) [23].

(ii)ACCLIMATION OF THE TEST ORGANISMS

The sample from the infected nails was gently collected by soaking or impregnating the nail in sterile normal saline (0.85% NaCl) to extract the test organism from the finger using a cuticle remover. After extraction, it was cultured on sterilized and cooled potato dextrose agar (PDA) using the spread plate method and incubated for 3 days at $28 \pm 2^{\circ}$ C. Discrete colonies were then sub-cultured on another sterile PDA plate and incubated to get a pure culture. The pure cultures were then inoculated unto PDA slants, incubated for 3 days at $28 \pm 2^{\circ}$ C and stored in the refrigerator at 4° C until required for use.

EXPERIMENTAL BIOASSAY PROCEDURE

The experimental procedure for *Onychomycosis* bioassay was carried out using the disc or spread plate standard protocol as described by Ameen, *et al.*, [3]. The experiment commenced with the preliminary test by obtaining the least concentration of the test salt that gives no effect and the maximum concentration that inhibits the growth of the fungi species. This was done to establish the exposure concentration for the actual bioassay. Toxicity end point indicator considered was mortality and growth inhibition. The minimum inhibitory concentration (MIC) is regarded as the least concentration of a substance or chemical that will prevent the growth of exposed microorganisms for a period.

In this method, the filter paper was cut into the shape of a disc (Figure 2). The agar was prepared according to the manufacturer's recommendation. Twenty (20) mL of the cooled molten agar (medium was cooled to about 45°C) and poured into each sterile petri dish, including the anti-fungi free control. The agar was allowed to cool and solidify. The fungus (pure culture) was streaked (inoculated) on the solidified potato dextrose agar (PDA) plates. The cut and sterilized filter paper was dipped into each concentration in triplicate. Using a sterile forcep, the filter paper disc impregnated with the test solution was then placed on the streaked (inoculated) plates and the plates incubated at a temperature of 37°C for the test duration. The anti-fungi free control plates/ dishes, which contained no test chemical was labelled as 0% [24].

ASSESSMENT OF RESPONSE

The effect of the test chemicals on the fungi species was assessed after three (3) days (72 hours). The end point indicator for the acute microbial toxicity was estimated using growth inhibition and mortality. After three (3) days, the potato dextrose agar was removed from the incubator and the zone of clearing was measured using a meter rule. Curve concentration value was plotted against the zone of clearing to obtain the growth inhibition. The test organism was considered dead when the zone of clearance is at its maximum range. That is the zone of inhibition (clearing) represents the area where mortality and growth was inhibited by the antimicrobial test chemical (sea salt). However, after incubation, it was observed that organisms control (anti-fungi free) plate grew to a maximum without inhibition. The data obtained was used to obtain the minimum inhibitory concentration (MIC₇₂) at 72 hours that is the least concentration causing inhibition of the fungi specie.





Figure 2: Experimental set up for fungi toxicity bioassay

STATISTICAL ANALYSIS

The susceptibility of the fungi to sea salt was performed with the Probit method of analysis for median minimum inhibitory concentration MIC₇₂ at 72 hours. The level of significance between the treated groups was considered at a probability level of 5%. Line graphs was used to illustrate the test endpoint.

RESULTS

The effectiveness of sea salt in inhibiting the growth of the nail fungi was assessed in this study (Plate 2). The growth of the fungi was subdued on exposure to the test chemical used in the assessment (Plate 3). Table 2 contained the mean results for the zone of clearing in the exposed organisms while Table 3 depicts the average concentrations of physico-chemicals, anions, cations and microbial analysis of groundwater samples.



Plate 2: Fingernail of a person infected with Onychomycosis: Source - this study



Plate 3: Fingernail of a person infected with *Onychomycosis* treated with sea salt already healing: Source - this study

TOXICOLOGICAL EFFECTS OF THE TEST CHEMICAL ON FUNGI

The results obtained from the bioassay from the sampled areas showed that sea salt was effective in inhibiting the growth of fungi as depicted in Figure 3 - 5. The nail fungi samples were sub-cultured to obtain the pure culture of the fungi. However, the samples from Airport area did not show any growth after been plated for 3 days with Potato Dextrose Agar (PDA), which implied that the infected nails may not be very active with the fungi. Some of the infected persons in that area claimed to have used some form of treatment or the other. Thus, the lack of growth during the sub-cultured stage may also be as a result of the treatment the infected persons have received or the nails have been subjected to. The results for the varying concentrations 5, 10, 20, 40 and 80 % had values for the zone of clearing in % as shown in Table 2.The results for fungi analysis in all the water samples except the Airport Road, were not fungi-free as slight growth of the fungi was observed when plated with Potato Dextrose Agar (PDA) (Table 3). The minimum inhibitory concentration (MIC) obtained for sea salt for Eboh, Jakpa and DSC was 19.91 \pm 0.31%, 24.32 \pm 0.48% and 24.66 \pm 0.73% in the respective order (Figures 3 - 5).

Concentrations, (%)	% zone inhibition (clearing)					
	Jakpa	Airport	Eboh	DSC		
5	25 ± 0.12	No growth	40 ± 0.40	20 ± 0.20		
10	40 ± 0.23		45 ± 0.32	35 ± 0.00		
20	45 ± 0.31		50 ± 0.40	45 ± 0.42		
40	55 ± 0.80		55 ± 0.62	60 ± 0.56		
80	70 ± 0.00		60 ± 0.00	85 ± 0.60		







Figure 4: Percentage zone of clearing for sea salt against log concentration in Jakpa



Figure 5: Percentage zone of clearing for sea salt against log concentration in DSC

PHYSICO-CHEMICAL EXAMINATION OF THE WATERS IN THE STUDY AREA

The results for the physico-chemical, anions, cations and microbiological parameters for the water collected from the sample locations are showed in Table 3. Water analysis was done to ascertain the water quality in the residence of the infected persons. The pH of the water samples were acidic to moderately acidic with values ranging from 4.18 ± 0.06 (Jakpa) to 5.40 ± 0.03 (DSC). This fell short fall of the DPR and WHO limits of 6.5 - 8.5 pH units. The water samples were 'fresh' as evident in the concentrations obtained for Total dissolved solids (TDS) ($32 \pm 2 - 236 \pm 23$ mg/L) and salinity ($17.51 \pm 1.5 - 82.59 \pm 5.9$ mg/L). The heavy metals levels were generally low and within the DPR / WHO allowable limits. The waters from Eboh, Jakpa and DSC except Airport road recorded fungi counts of 13 ± 1.0 , 17 ± 1.0 and 11 ± 1.0 MPN/100 mL respectively. This is contrary to the WHO limit which states that no pathogenic microorganisms should be detected in potable waters (Table 3).

Tuble of Mean results of p		physico ene	inicul un		iogical analy	sis of groundwater sumples		
Parameters	WHO's Max Acceptable Limit	WHO's Max Allowable Limit	DPR Standard	FME Standard	DSC	Jakpa	Eboh	Airport Road
Physico-								
chemical								
parameters								
pН	5.5-6.5	6.5-9.2	6.5-8.5	6.5-8.5	5.40 ± 0.03	4.18 ± 0.06	5.00 ± 0.08	4.51 ± 0.05
Temperature °C	N/A	N/A	25	25	29.9 ± 0.12	29.8 ± 0.14	29.5 ± 0.10	29.45 ± 0.09
Total dissolved Solid (TDS) ,	1500	1500	N/A	2000	32 ± 2	127 ± 12	236 ± 23	64 ± 9
mg/L								
Electrical Conductivity, μS/cm	250	N/A	N/A	N/A	65 ± 5	255 ± 14	472 ± 29	129 ± 11
Turbidity, NTU	5.00	5.00	N/A	10	3.34 ± 0.05	1.17 ± 0.01	0.37 ± 0.00	0.26 ± 0.01
Total Suspended Solid (TSS), mg/L	N/A	N/A	N/A	40	4.0 ± 0.10	2.45 ± 0.05	<0.01	< 0.01
Salinity, mg/L	200	600	N/A	600	17.51 ± 1.5	25.60 ± 2.3	82.59 ± 5.9	20.2 ± 1.8
Anions								
Bicarbonates, mg CaCO3/L	500	500	N/A	N/A	0.16 ± 0.01	<0.01	<0.01	<0.01
Nitrate, mg/L	10	N/A	N/A	N/A	0.52 ± 0.02	0.15 ± 0.01	0.21 ± 0.01	0.10 ± 0.01
Sulphate, mg/L	200	400	N/A	N/A	1.43 ± 0.02	2.43 ± 0.03	3.18 ± 0.06	2.13 ± 0.02
Metals								
Calcium, mg/L	75	200	N/A	N/A	4.0 ± 0.10	12.0 ± 0.30	8.0 ± 0.20	8.0 ± 0.20
Total Iron, mg/L	0.30	1.00	N/A	1.00	< 0.001	0.11 ± 0.01	0.27 ± 0.02	0.53 ± 0.05
Lead, mg/L	N/A	0.05	0.05	0.05	< 0.001	< 0.001	< 0.001	< 0.001
Copper, mg/L	0.05	1.50	1.50	1.50	0.014 ± 0.001	0.004 ± 0.001	0.015 ± 0.001	0.023 ± 0.002
Cadmium , mg/L	N/A	0.01	N/A	N/A	< 0.001	< 0.001	< 0.001	< 0.001
Zinc, mg/L	5.0	15.0	1.00	1.00	< 0.001	0.724 ±	0.151 ±	0.309 ±
Magnesium, mg/L	50.0	150	N/A	N/A	2.00 ± 0.10	4.0 ± 0.40	4.0 ± 0.40	4.0 ± 0.40
Microbiological								
Parameter								
Fungi (MPN/100ml)	Nil	Nil	Nil	Nil	11 ± 1.0	17 ± 1	13 ± 1	Nil

DISCUSSION

The nail (finger and toe) condition *Onychomycosis* present a cosmetic issue to a suffering individual especially if their profession requires interaction with the public for example food vendors, salonists, front desk officers, actors etc. Globally, this condition is receiving growing concerns as the number of infected persons are increasing daily. This long-term infection is usually very difficult to eliminate permanently with the likelihood or tendency of reoccurrence even after prolong therapy. In administering therapy to infected persons, Griseofulvin is extensively used with a prolonged period of treatment usually 6 to 12 months and the chances of recurrences are very common. Some topical antifungal chemicals such as Ciclopirox and Tavaborole are not effective in destroying the fungi responsible for onychomycosis with less than 20% clearing achievement. Although, some studies have suggested Terbinafine to be the most effective agent, the nail are usually not normal at the termination of the therapy [25, 26].

Similarly, new treatment methods such as laser, iontophoresis, photodynamic and ultrasound treatment or therapy are not effective or efficient. The reason been that the fungus infection is very hard to treat with a high percentage of relapse once treatment is stopped. The residual fungal spores existing in the infected person nails and foot wears couple with some favourable environmental conditions may probably be responsible for the relapse of the infection. Treatments including creams, gels, nail lacquers, or oral medications provide little help and lots of side effects to the ailing patients [25,27].

Application of amorolfine 5% nail lacquer once or twice weekly for up to 6 months produced mycological and clinical cure in approximately 40-55% of patients with mild onychomycosis Application of amorolfine 5% nail lacquer once or twice weekly for up to 6 months produced mycological and clinical cure in approximately 40-55% of patients with mild onychomycosis.

In their study, Shenoy and Shenoy, [17], concluded that there was mycological and clinical success of 40 to 50% with patients having mildonychomycosis, when 5% of nail lacquer Amorolfine was applied one to two times weekly for 6 months. In the same vein, Monod and Méhul, [28] in their review concluded that

there was likely failure of Terbinafine treatment against dermatophytes and this could be as a result of a missense alteration by the drug and recommended a switch to an azole-based therapy for difficult cases of *Onychomycosis*.

The use of natural substances (e.g. salt) to inhibit microbial growth and development is an ancient procedure that remains significant in today's world. It should be noted that all natural treatments are low-cost, non-toxic with no noticeable side effects and more importantly, they completely destroy the toe and nail fungus. However, the general conception is that natural therapies takes time and are more often than not disregarded [16]. Although not much documented researches have been evaluated using sea salt, however, some e-books have reported the use of salt and these include Chang, [29] and Shazia and Siddiqui, [30].

Salt can limit the growth of microorganisms, since it has the capability to cause detrimental effect on the organism it comes in contact with. At lower concentrations of the test chemical, the growth of fungi was promoted, however at higher concentration growth is inhibited by creating low water activity in the fungi cell and this could likely result in the decrease of flow of transportation in and out of the cell of the fungi. It is most probable that the mechanism of action for the sea salt inhibition of microbial growth could likely be by osmosis or dehydration of cells. The salt in its aqueous form in contact with the organism attempts to reach an equilibrium with the cells of the fungi it is in contact with. This have the tendency to draw available fluid from within the host to the surrounding and introducing the salt into the host thus killing the cells which in turn inhibit their growth. In this way, the salt is able to destroy the disease-carrying fungi at specific concentrations. Other antimicrobial mechanisms of salt include interfering with the functions of enzyme, thereby weakening the molecular structure of its deoxyribonucleic acid (DNA) [31, 32].

The minimum inhibitory concentration (MIC) is considered the gold standard for determining the proneness of organisms to antimicrobial agents or chemicals [33]. In this assessment, the efficacy of the sea salt was more pronounced at higher concentrations than at lower levels, although the MIC is commonly used for chemical and/or drugs to determine the dose concentration to administer to an infected person [24].

CONCLUSION

Onychomycosis have been regarded as one of the most common nail condition in adults. In this study, the application of sea salt (non-oral) inhibited the growth of the nail fungi. Since inhibition was possible, it could be used for the treatment of the nail fungi condition (*Onychomycosis*) possibly since topical treatment do have side effect on most patients and also to reduce the intake of oral drug for treatment. The cost of treatment of the nail condition (*Onychomycosis*) with sea salt could be economical when compared to the ingestion of drugs that could damage internal organs on prolonged intake with no meaningful success story. Similarly sea salts can be readily obtained all the year round and are cheap to purchase as compared to anti-fungi drugs, which are expensive and are usually imported into developing countries.

Although, it is important to prevent the infection from occurring by keeping one nails dry and healthy since prevention, is widely said to be better than cure. In that vein, excessively tight hose or shoes will give raise to a moist environment favourable for onychomycotic infections. Some important steps are required to prevent *Onychomycosis*: Individuals should be encouraged to wear smacks made of fibers, which can absorb moisture readily than those made of cotton or wools, and also tools used for manicure and pedicure should be sterilized before and after each use. In addition, since water and moisture that gather underneath the surface of a nail do not evaporate and can be trapped, nail vanish should not be applied to infected nails. Hands and feet should be kept short and washed regularly and kept clean and dry, other preventive measures include, giving up polishing nail, applying artificial nails and washing hands after contact with an infected nail.

The results from this evaluation revealed that sea salt was effective in the treatment of the nail condition *Onychomycosis*. The method in this appraisal is simple, natural, non-oral with short response time for inhibiting the development of the fungi pathogens.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

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CITATION OF THIS ARTICLE

D F Ogeleka and L Esivweneta Tudararo-Aherobo An appraisal of the effectiveness of native salt (sea salt) in the treatment of fungal nail infection (*Onychomycosis*). Bull. Env. Pharmacol. Life Sci., Vol 9[5] April 2020 : 23-32