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Azadirachta Indica (Neem) Seed Oil as Adjuvant for Antimicrobial Activity

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Abstract

Neem oil, soap and a combination of neem oil neem oil soap and some already existing antimicrobial drugs, lamisil and whitfield oil (1:1 and 2:1v/v) were tested on cultured samples of bacteria and fungi. Neem oil was found to be inactive against Epidermophyton floccosum and Trychophyton tonsurans on its own but significantly increased the activity of lamisil and whitfield ointments when combined with them. Neem soap also showed some activity against the two samples of fungi. Neem oil showed considerable activity against Staphylococcus aureus and Salmonella typhi. It was observed that, the combination of neem oil and other known antimicrobial drug was more effective in the inhibition of the growth of these bacteria. Neem oil on its own may not elicit the desired activity but a mixture of neem oil an d other medicinal compounds could reduce the fatality of microbial infections.

Key words: *Neem Oil, Neem Soap, Antifungal, Antimicrobial*

Introduction

Diseases caused by harmful microbes pose a problem in our world today, especially because most of these microbes are becoming more

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resistant to existing medication. Neem extracts possess anti-diabetic, anti-bacterial and anti-viral properties (Schneider, 1986). The terpenoids are of special interest and are obtained from different parts of the neem tree Veitch et al., 2007). A number of active principles of neem oil have been reported. These include, azadirachtin, azadiradione, fraxinellone, nimbinsalannin etc.

Mammalian cells do not contain the enzymes which degrade the cell wall polysaccharides of fungi. Therefore, these pathogens are difficult to eradicate by the animal host's defence mechanism (Clayton and Midgley, 1998). Mammals and fungi are both eukaryotic. Therefore, the cellular milieu are biochemically similar. Most substances which may impair the mold fungus will usually have serious side effects on the host. Although one of the first chemotherapeutic agents, oral iodides, was an anti-mycotic used in 1903, the further development of such agents have been left far behind the development of anti-bacterial agents. The selective toxicity necessary to inhibit the invading organism with minimal damage to host has been difficult to establish within eukaryotic cells. This is why more research is being done to find natural, harmless anti-fungal medication (Clayton and Midgley, 1998).

Methodology

The *Azadirachta indica* (neem) fruits were sourced in bulk from trees located in Abubakar Tafawa Balewa University, Yelwa campus, Bauchi, Nigeria. Only ripe fruits were harvested. The seeds were removed from their shells and cleaned thoroughly after which they were air dried for 10 days to reduce the moisture content to a minimum.

The dried kernels were milled using a dry food miller to obtain the pulverised seed sample (352 g). A portion of 88 g of the pulverised kernel was extracted in a food homogenizer using n-hexane. The extraction was repeated three times using the same sample and n-hexane (250 ml) for each extraction, and by the third extraction no oil was obtained. After the extraction was done once in the food liquidizer, the mixture was left to settle until two clear layers formed, the liquid layer containing the solvent and the oil and the pasty layer. The liquid layer was removed leaving the paste for further

extraction. The solvent was recovered using the rotary evaporator at 40 °C leaving behind the concentrated neem seed oil.

Neem oil Soap

Neem oil (36 g) was weighed into a 250 ml beaker. The beaker containing the oil was placed on a hot plate and heated to boil. Then 20 % caustic soda (50 ml) was added gradually while the mixture was continuously stirred for 50 minutes till a thick cake was observed. After the saponification the soap was salted out using saturated sodium chloride (150 ml) solution. The soap was then rinsed with chilled water and left to dry (Appleton and Simmons, 2007).

Isolation of Fungi and antifungal tests

Sub-cultures of the isolated fungi were made on Sabouraud dextrose agar (SDA). Samples of *Trychophyton tonsurans* responsible for scalp infections, and *Epidermophyton floccosum* from skin infections were obtained from already cultured media. The fungus, *Trychophyton. tonsurans* was streaked randomly on two petri dishes. The same was done with *Epidermophyton floccosum*. Each petri dish was sub-divided into seven parts for all the samples to be tested. Paper discs of about 0.2 mm in diameter were punched out of a whatman filter paper No 0.1 and kept in the hot air oven at a temperature of 160 °C (Collee *et al.*, 1996). Seven samples namely lamisil ointment, whitfeild ointment, stale neem oil (15 years old, extracted in 1996), fresh neem oil, lamisil+fresh neem oil, whitfield ointment+fresh neem oil and neem soap were used. The soap was made into paste using distilled water in a petri dish. The paper discs were dipped in the soap, oil and ointment samples. One was placed on each sub-division. The plates were incubated at room temperature for 24 hours.

Antibacterial activity test.

The bacterial cultures (*Staphylococcus aureus* and *Salmonella typhi*) were grown overnight at 37 °C on nutrient agar. The antibacterial activity of neem oil was checked using the seed plate method (Collee *et al.*, 1996). In this technique the bacteria isolated previously was

seeded onto peptone water and incubated for 18 hours. 1.0 ml each of the cultures were aseptically incorporated on nutrient agar (20 ml) mixed well and allowed to solidify.

After hardening, wells were bored in the agar and each extract (0.2 ml) was placed in these wells. The plates were incubated at 37 ^{0}C and zones of inhibition read after 24 to 72 hours. The samples used for this test include fresh neem oil, neem soap and cefepime an antibacterial drug used as a control medium.

Results and Discussion

Neem oil was recovered from the neem kernels using the cold solvent extraction method. From 352 g of neem seed cake 171 g of oil (48.6 %) was obtained. The physical properties of the oil are listed in Table 1.

TABLE 1: Characteristics of neem seed oil

Properties	Observation
Colour	Greenish yellow
Quality	Non-drying oil
Taste	Acrid and bitter
Odour	Repulsive (Garlic)
Solubility	Insoluble in water
Density	0.908 g /cm^3

The neem oil recovered was used to prepare soap. The tested soap parameters are given in the Table 2.

TABLE 2: Characteristics of the laboratory prepared neem soap

Properties	Observation
Odour	Slight garlic
Lather stability	Lathers much and is stable over long periods
Hardness	Very hard
Washing efficiency	High
Moisture content	20.1%

The prepared soap, the fresh neem oil alongside other well known broad spectrum anti-fungal agents (lamisil and whitfield ointment) as control media were tested on the cultured clinical isolates of fungi. The results are represented in the Table 3. The nature of the colonies observed were as follows: Dark green colonies for *Epidermophyton floccosum* and whitish brown for *Trychophyton tonsurans*. The zone of inhibition denotes the relative susceptibility to a particular antimicrobial agent that was detected by the formation of a clean zone around the disc.

TABLE 3: Antifungal activity test results

Test Samples	Zones of inhibition (mm)	
	Trychophyton tonsurans	***Epidermophyton floccosum***
Lamisil (control)	1.1	1.5
Whitfield (control)	0.1	No activity
Fresh neem oil	No activity	No activity
Old neem oil	No activity	No activity
Neem oil soap	0.4	0.3
Neem oil + lamisil	1.8	2.1
Neem oil + whitfield	0.7	0.4

The results for the antibacterial activity test on *Salmonella typhi* and *Staphylococcus aureus* with cefepime as control agent are given in the Table 4.

TABLE 4: Antibacterial activity test results

Test sample	Zones of inhibition (mm) for	
	Salmonella typhi	***Staphylococcus aureus***
Fresh neem oil	1.7	1.8
Neem soap	No activity	0.25
Cefepime (control)	2.6	3.0

The above results indicate that the ointments (control drugs) have a significant antifungal effect on the fungi *Trychophyton tonsurans* and *Epidermophyton floccosum* while the neem oil has no significant effect on them. No zone of inhibition signifies that the fungi were resistant to the test samples. This observation is strange since neem oil has been reported to be highly effective against most human fungi. The neem soap however showed some zone of inhibition that may be as a result of the chemical combination of neem oil and caustic soda producing sodium salts of the fatty acids which are not favourable for the fungi, or due to the alkaline nature of the soap.

From the results on Table 4, neem oil showed some activity on *Salmonella typhi* although neem soap was completely inactive. Antibacterial activity was shown by both neem oil and neem soap on *Staphylococcus aureus*. The zones of inhibition for cefepime a reputable antibacterial drug against these bacteria were 2.6 mm and 3.0 mm respectively. A mixture of the fresh neem oil and lamisil (1:1 v/v) for the antifungal activity test yielded a larger zone of inhibition than the pure neem oil or soap. The same was noticed for a mixture of the fresh neem oil and whitfield ointment (1:1 v/v). This suggests that a synergy occurred between neem oil and the control drugs, a situation where the mixture of two compounds produces a greater positive result than either one on its own or even the sum of both effects.

Neem oil on its own may not produce drastic and miraculous results but a mixture of neem oil and other medicinal compounds could reduce the fatality of microbial diseases. It has been scientifically proven that two drugs may sequentially block a microbial metabolic pathway. Usually what happens is, one drug affects the cell membrane and facilitate the entry of the second drug. The combined effect may then be greater than a combination of its parts. For example, amphotericin has been synergistic with flucytosine against certain fungi, e.g., *Cryptococcus* and *Candida* species. Another way is that, one drug may prevent the inactivation of a second drug by microbial enzymes. Thus, inhibitors of such enzymes protect other drugs from inactivation. In such circumstances a form of synergism takes place (Plummer and Short, 1990).

Conclusion

Although neem oil has been used for a long time to control pathogenic fungi such as *Aspergillus niger, Penicillium expansum, Glomerella cingulata, Curvularia lunata, and bacteria such as Escherichia coli, Pseudomonas aeruginosa* and several other pathogens, this research has shown the potency of neem oil against some of the common pathogenic bacteria and fungi in our immediate environment. The effect of neem soap on some of these pathogens and the effect of the combination of neem oil and lamisil suggests that the combination of neem oil and other antimicrobial compounds may be more effective in combating microbial and fungal infections.

Recommendation

It is recommended that more attention be given to the neem plant and neem oil in particular so that neem oil can be included as synergist in medical formulations, to produce the best kind of treatment at affordable cost, especially in the third world where neem happens to be abundant. Synergy is a very important aspect of medicinal chemistry and pharmacy which should be given more attention, although synergistic drug combinations must be selected by complex laboratory procedures.

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