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Research Article

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Anti-microbial activity of solvent extracts of leaves of *Azadirachta indica* (dogonyaro) and production of antiseptic soap and antifungal cream

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Abstract Aqueous extract of *Azadirachta indica* is drunk by the Igbos as medicine for curing malaria fever. The dried leaves are kept at strategic places to drive away insects. In this research the effect of two solvent extracts of *A. indicia* were investigated on five micro-organisms that attack the skin. These micro-organisms include; *Malassezia globosim, Atopic dermatitis, Propionibacterium acnes, Tricophyton microsporum* and *Staphylococcus aeurus*. Disc agar diffusion method was used to assay for antimicrobial activity on the test isolates. The antimicrobial activity shows some inhibitory effects against the test organisms. The result shows that the ethanol and methanol extracts inhibited the growth of all the test organisms. The minimum inhibitory concentration (MIC) of both ethanolic and methanolic extracts for the five test organisms were found graphically. *A. indica* extracts were used to produce effective anticeptic soap and antifungal cream for the treatment of skin infection.

Keywords Micro-organisms, *Azadirachta indica*, solvent extracts, antiseptic soap, antifungal cream

Introduction

Neem plant (*Azadirachta indica*) grows in tropical and semitropical regions. All parts of the neem are used for curing of leprosy, eye defect, skin problems, ulcer, infections and many more and that is why it is called the "nature drug store" [1], The aim and objectives of this work is to determine the microbial activity of *A. indica* leaves extracts on some of those micro-organisms that attack the skin, and then use the extracts to produce antiseptic soap and antifungal cream. The English name is neem borrowed from Hindi, the Urdu, Arabic and Nepali. Other vernacular names are Niim in Sindhi and Punjabi, Niim in Bengali, Dogonyaro in Northern Nigeria, Magosanim tree in Vepu, Minba in Indonesia, Muanbiani in East Africa (Swahili), Kusaal in some Ghanaian language [2]. A neem tree (*A. indica*) is a fast growing tree that can reach a height of 45-131ft. It is evergreen but in drought, it may shed most or nearly all of its leaves [3]. The root system of neem is comprised of a strong tap root and excellently developed lateral roots [4].

The pinnate leaves of the neem are alternating and they can be about 20-40cm long with about 20-30 dark green to medium green leaflets about 3 to 8cm in length [5]. The inflorescence of the flowers bears about 150-250 flowers. The neem is a polygamous tree with protandous bisexual as well as male flowers growing in the same individual plant [6]. Many parts of the neem tree have antimicrobial properties; they provide effective ingredients for traditional tooth paste, medicines, cosmetics and insect repellant in South Asia. Neem insecticides are active against more than 200 different types of insects, including head lie, fleas, locust and mosquitoes. The tender young leaves of neem are astringent, good for the eye, skin diseases and leprosy while the old leaves are anthelmintic, alexeteric, insecticidal, good in ophthalmic biliousness and cures ulcer quickly. The leaves are carminative and expectorant, anti-inflammatory and anti-rheumatic [1], it is also useful in syphillic sores, ear ache boils and all blood impurities.

There are several reports on the biological and pharmacological action of neem leaf such as antiviral, anti-bacteria;, anti-fungal, anti-inflammatory, anti-pyretic and anti-paralytic uses [7].

Experimental Work

Sample Collection and Preparation

The leaves of *A. indica* were collected on the tree growing in Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State of Nigeria. The leaves were washed, air dried for three weeks and ground or blended into powder. It was then stored in polyethylene bottle until needed for analysis. Extraction of the active component was done using the method described by Adebayo [8]. 500g of the sample was measured into a beaker. About 1000cm^3 of ethanol was poured into it. The mixture was allowed to stay for four hours with occasional shaking of the mixture. The mixture was filtered. The filtrate was evaporated to dryness in a water bath. Then 1g of the residue was dissolved in 10ml of ethanol to make an ethanolic solution of the extract. Then the ethanolic extract was taken in these concentration for the inhibition of the test micro-organisms. 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml, respectively. The antimicrobial activity of leaves of *A. indica* was determined by agar well diffusion method [9-10]. The MIC was found graphically.

Results

The results of the analysis carried out on leaves of *A. indica* are given in table 1-3.

Table 1: Physio-chemical characteristics of leaves of *A. indica* in two solvents: Ethanol and Methanol

Parameters	Ethanolic Extract	Methanolic Extract
Appearance and texture	Dried and powdery	Dried and powdery
Colour	Dark green	Dark green
Odour	Characteristics smell of ethanol and slightly chocking	Characteristic smell of methanol and slightly chocking
Solubility	Soluble	Soluble

Table 2: Antimicrobial activity of two solvent extracts of *A. indica* on some micro-organisms

Test organism	Concentration mg/ml	Average diameter of zone of inhibition in mm on test organisms			
		Ethanolic extract	Methanolic extract	-ve control ethanol 50%	-ve control methanol 50%
Molassezia globosin	0.1	2.5	3.0	NA	NA
	0.2	5.0	4.5	NA	NA
	0.3	7.6	9.0	NA	NA
	0.4	10.0	11.5	NA	NA
	0.5	12.5	12.8	NA	NA
Atopic dermatitis	0.1	2.0	3	NA	NA
	0.2	6.5	7.0	NA	NA
	0.3	8.5	9.1	NA	NA
	0.4	10.0	10.2	NA	NA
	0.5	11.5	12.0	NA	NA
Propionibacterium	0.1	3.0	1.5	NA	NA
acnes	0.2	6.0	3.5	NA	NA
	0.3	8.2	5.0	NA	NA
	0.4	9.5	8.5	NA	NA
	0.5	11.3	10.0	NA	NA
Trichophyton	0.1	2.5	2.0	NA	NA
microsporum	0.2	5.3	5.0	NA	NA
	0.3	7.6	8.0	NA	NA

	0.4	9.0	11.2	NA	NA
	0.5	12.0	13.0	NA	NA
Staphylococcus	0.1	1.8	1.5	NA	NA
aeurus	0.2	2.7	3.0	NA	NA
	0.3	5.5	6.0	NA	NA
	0.4	7.5	8.5	NA	NA
	0.5	11.8	10.9	NA	NA

Table 3: Result of Minimum Inhibitory Concentration (MIC) of two solvent extracts of leaves of *A. indica*

Test organisms	Ethanolic extract	Methanolic extract
M.globosin	0.05	0.06
A. dermatitis	0.06	0.07
P. bacterium	0.07	0.08
T. microsporum	0.08	0.07
S. aeurus	0.08	0.08

Discussion

Table 1 shows physio-chemical characteristics of leaves of *A. indica*. It is dry and powdery in texture. It possesses dark green colour in both ethanolic and methanolic solution with characteristic odour of the solvent. The dried sample is soluble in both ethanol and methanol. Table 2 exposes the zones of inhibition of ethanolic and methanolic leaf extracts of *A. indica* on some pathogenic micro-organisms. At 0.1 – 0.5mg/ml concentration, ethanolic and methanolic extracts inhibited the five test micro-organisms. These organisms are *M. globosin, A. dermatitis, P. bacterium, T. microsporum* and *S. aeurus.*At 0.5mg/ml concentration, ethanolic extract can inhibit all the five test micro-organisms effectively because the zone diameter is up to or approximately 12mm. At 0.5mg/ml concentration, methanolic extract inhibited effectively three out of the five test microorganisms. These are *M. globosin,* (12.8mg/ml) *A. dermatitis,* (12.0mg/ml) and *T. microsporum* (13.0mg/ml). 50% ethanol and 50% methanol (negative controls) show no action against five test organisms. Table 3 is the result of the minimum inhibitory concentration (MIC) of ethanolic and methanolic extracts of *A. indica* on five test micro-organisms. The MIC of both ethanolic and methanolic extracts ranges from 0.06 – 0.08mg/ml.

Ethanolic and methanolic leaf extracts of *A. indica* were used to produce antiseptic soap and anti-fungal cream. These two products were subjected to quality control tests and were found to be of high quality. Antiseptic soap was tested for its pH, foaming capacity, solubility and degree of hardness, while anti-fungal cream was tested for its pH, sun screening effectiveness and path test.

Production of Antiseptic soap using the *Azadirachta indica* leaf extracts

Ingredients: Caustic soda, soda ash, palm kernel oil, disinfectant, sodium benzoate, *A. indica* leaf extract, sulphuric acid.

Procedure

- Measure out palm kernel oil, caustic soda and soda ash in the ratio of 2:1:1 respectively (500ml, 25ml, 250ml respectively).
- Put the caustic soda, soda ash, and palm kernel oil in a basin.
- Add a pinch of sodium chloride
- A continues stirring in clockwise direction to avoid separation.
- Put 50ml of ***A. indica*** extract e.g. (ethanol or methanol extract in two different basins).
- Add 70ml of foaming agent (sulphonic acid).
- Continue to stir to get a homogenous mixture, pour it out in soap mould and allow to cool and solidify.

Production of antifungal cream using the *Azadirachta indica* leaf extracts

Ingredients: Water, crod wax, stearic acid, paraffin oil, glycerine, *A. indica* leaf extracts, perfume, sodium benzoate.

Procedure

Aqueous phase

- Boil 250 ml of water for about ten minutes
- Put little quantity of preservative (sodium benzoate)
- Allow it to cool.
- Oil phase
- Measure out 330grams of stearic acid
- Measure out 460 grams of crod wax and put them together. Then heat then to melt into one homogenous mixture
- Measure out glycerine and paraffin oil in the ratio of 2:1 (440ml, 220ml) respectively
- Check the boiling point with the thermometer to a temperature of 100°C
- Then add the boiled and cooled 250ml of water (aqueous phase)
- Add 50ml of *A. indica* leaf extracts into the mixture and continue to stir.
- Add little quantity of hot water if the cream is sticky
- Add perfume to the cream

Stir to get a desired result.

Conclusion

Solvent extracts of *A. indica* leaves are medicinal. They can be used in curing various skin infections caused by the above mentioned micro-organisms. The extracts can be employed industrially in the manufacture of drugs, antiseptic soap and anti-fungal cream.

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