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Research article

Antimicrobial and Pharmaceutical Properties of The Seed Oil of *Leucaena leucocephala* (Lam.) De Wit (Leguminosae)

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ABSTRACT: The seed oil of Leuconia *leucocephala* was investigated for its antimicrobial activity and the pharmaceutical properties of its lotion formulation determined. The oil was extracted from the pulverised dried seeds of the Leuconia leucocephala plant by cold maceration in n-hexane. The oil was tested against four bacteria (*Staphylococcus aureus, Esherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa*) and four fungi (*Aspergilus niger, Rhizopus stolon, Penicillum notatum* and *Candida albicans*) isolates. Gentamycin and tioconazole were the reference drugs respectively. The oil was later formulated as a lotion and the pharmaceutical properties of the formulation determined.The oil was found to have a concentration-dependent activity against both Gram-positive and Gram-negative bacteria, while showing no activity against the fungi tested. The lotion formulation of the oil containing oleic acid had good pharmaceutical properties and was stable over the test period. *Leucaena leucocephala* seed oil extract had a concentration-dependent activity against both Gram-positive and Gram-negative bacteria and the lotion formulation had good pharmaceutical properties.

Keywords: *Leucaena leucocephala* oil, antimicrobial activity, lotion formulation

INTRODUCTION

Leucaena leucocephala (Lam.) de Wit (Leguminosae) is one of the fastest-growing leguminous trees in drought-prone and semi-arid areas (Sethi and Kulkani, 1995). The shrub which is freely available in Ibadan, South-west Nigeria is native to Mexico and Central America (Hill, 1971), but now widely distributed throughout the tropics. It is planted as a shade tree for coffee, cacao, and other cash crops; for soil fertility improvement; erosion control; site preparation in reforestation and used for a variety of other purposes including timber and fuel wood (Rushkin, 1984; Whitesell, 1974). The protein-rich leaves and legumes are widely used as fodder for cattle, water buffalo, and goats (Sethi and Kulkani, 1995). The leaves and seeds are used as human food in Central America, Indonesia, and Thailand but are not recommended for extensive human consumption because of the mimosine toxic component (Rushkin, 1984).

Various parts of *L. leucocephala* have been reported to have medicinal properties ranging from control of stomach diseases to contraception and abortion and the seed gum has been reported to be useful as a binder in tablet formulation (Deodhar *et al*., 1998; Verma and Balkishem, 2007). Sulfated glycosylated form of polysaccharides from the seeds was reported to possess significant cancer chemo-preventive and anti-proliferative activities (Gamal-Eldeen *et al*, 2007). Also, mimosine, an amino acid from the seeds was reported to possess anticancer activity and to inhibit the growth of hair (Crounse *et al*, 1962; Chang *et al*., 1999). Other studies on the extracts of the seeds had shown varying activities including central nervous system depressant, anthelmintic and antidiabetic activities (Irene *et al*, 1997; Ademola *et al*., 2005; Syamsudin *et al*., 2010). Of recent, the seed oil was used in engineering a novel bio-device useful in

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biomembrane modeling in lipophilicity determination of drugs and xenobiotics (Idowu *et al*., 2009).

Leucaena seeds are ovoid in shape and have brown hulls and yellow kernels. The seeds are rich in protein (24.5 - 46%) and contain the following essential amino acids: cystine, arginine, methionine, glutamic acid, threonine, glycine, alanine, valine, isoleucine, leucine and lysine (Sethi and Kulkani, 1995; Mohammed *et al*., 2009). The seeds also contain a dark, green to brown oil containing approximately 26-29 % saturated acids and 71-73 % unsaturated acids. The oil is reportedly rich in linoleic acid (42.5-65 %) and contains significant quantities of arachidic (0.8-1.6 %) and lignoceric acids (0.71-7 %) (Khalid *et al*., 1989; Majumder and Chowdbury, 1987). The oil also contains sterol (mainly β-sitosterol, 55%), methylsterols, triterpenoid alcohols, tocopherols (mainly α-tocopherol), glycolipids, hydrocarbons and carotenoids (Kulkarni *et al*.,1992). The oil is the richest vegetable source of phosphatides (Jagan *et al*., 1988). However, there has been no reported study on possible antimicrobial activity of the oil seed-extract of Leucanea.

Generally, oil has been a basic ingredient for beauty treatment and body care, either on its own, or as base for the manufacture of cosmetics. It is use to preserve skin moisture and to protect the body from cold and sun. This study was therefore designed to assess the antimicrobial activity of *L. leucocephala* seed oil and its formulation as a cosmetic skin product.

MATERIALS AND METHODS

Materials

Silica gel (sorbent, thin layer chromatography grade, GF254), n-hexane, oleic acid (British Drug Houses (BDH), U.K.), polysorbate 80, gentamycin injections (80mg/2ml) (Laborate Pharmaceutical, India), tioconazole tablets 100mg (Neimeth International Pharm. Plc, Nigeria), peptone water (Acumedia, England), triethanolamine, glycerol (May and Baker Ltd, England, emulsifying wax, 96% ethanol, nutrient broth (Biotin), nutrient agar and sabouraud dextrose agar (Biotin).

Plant Material

The dried seed pods were collected from the plant *Leucaena leucocephala* (Lam.) de Wit. growing around Obafemi Awolowo Hall area, University of Ibadan and authenticated at Botany Department, University of Ibadan, Ibadan, Nigeria. The seeds were sun dried and pulverized with a milling machine.

Extraction of the *L. leucocephala* oil

The powdered seeds were weighed (3.2 kg) and packed in a cotton cloth bag and defatted by cold maceration in n-hexane. The marc was extracted continuously until the marc was exhausted. The crude oil was recovered by distillation and the combined oil residue was dried in vacuo at 40°C in a vacuum oven for 24 hours. About 80% of the total volume obtained was decanted and filtered through glass wool.

Preparation of cultures of microbial organisms

Each test organism (bacterial-*Staphylococcus aureus, Esherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa;fungi-Aspergilus niger, Rhizopus stolon, Penicillum notatum* and *Candida albicans)* was obtained from the Pharmaceutical Microbiology laboratory stock of university of Ibadan, Nigeria.

An overnight culture of each bacterial organism was prepared. Each organism in the stock was sub-cultured into freshly prepared nutrient broth aseptically using a flamed wire loop and then incubated at 37°C for 24 hours in the incubator.

For the fungal test organism, sterile wire loop was used to transfer a loopful of fungi spore to the surface of Sabouraud Dextrose agar (SDA) and streaked. They were then incubated at room temperature (28°C) until they grew and produced spores (5-7 days). The spores were harvested and used for sensitivity test.

Preparation of culture media

Nutrient broth was prepared. 5ml volumes were distributed into test tubes bottles and sterilized by autoclaving at 121°C for 15 min. The nutrient agar was similarly prepared following the manufacturer's instructions. This was distributed into 20ml universal bottles and sterilized in an autoclave at 121°C for 15 min. The SDA was also prepared following the manufacturer's instructions. It was also distributed into 20ml aliquots into universal bottles and sterilized in an autoclave at 121°C for 15 min.

Preparation of standard drug solution

A tablet of tioconazole tablet containing 100mg of the drug was powdered in a sterile porcelain mortar with pestle and dissolved with about 3ml ethanol (96%). This was then transferred into a 10ml volumetric flask and made up to volume with more ethanol to make a 10mg/ml i.e. 10,000µg/ml solution. 7ml of this was taken and diluted to 100ml with ethanol to give 0.7mg/ml (700µg/ml) solution and used as the standard drug for the fungal isolates. 0.5ml of gentamycin injection (80mg/2ml) was measured into a volumetric flask and diluted to 200µg/ml by making up the volume with sterile distilled water to 100ml. 5ml of this

solution was further diluted to 100ml with sterile distilled water to give 10µg/ml solution.

Preparation of *L. leucocephala* oil for sensitivity test

Five millilitres of the oil was used as 100% undiluted oil sample. 2.5 ml of this was taken and made up to 5 ml with methanol to give 50% concentration. From this 50% concentration, 2.5 ml was taken and made up to 5ml with methanol to give 25% concentration. Further dilutions were done to give 12.5% and 6.25% concentrations.

Antimicrobial screening

The test organism (0.1ml) was taken into 9.9ml of sterile distilled water to give 10ml at 1:100 (10^{-2}) dilution. From the 10^{-2} dilution, 0.2ml was taken into the sterile molten NA at 45-50°C. This was aseptically poured into the sterile plates, and allowed to set on the bench for about 30 minutes. The wells were made on the set plates. Inside each well, the graded concentrations of the oil samples were put inside the wells using a Pasteur pipette. 0.5% polysorbate 80 was used as control and gentamycin as drug standard. The plates were then left on the bench for about 2 hours. This is done to allow the pre-diffusion to take place.

In case of the fungi plates, the SDA was poured inside the plates, allowed the plates to set for 45-50 minutes, and then the 0.2ml of 10^{-2} organism was spread on the plates. Tioconazole was used as the drug standard, while 96% ethanol was used as the control. The wells were made and the other process followed as described above.

The bacterial plates were incubated for 18-24 hours at 37°C, while the fungi plates were incubated for 2-5days at 26-28°C. Then the zones of inhibition were measured and recorded in millimetre. Each determination was done in duplicates.

Preparation of *L. leucocephala* lotion

Seed oil lotion was prepared according to Audu-Peter *et al* (2006) from Table 1. Briefly, for each formulation, Part A was prepared by melting the emulsifying wax, adding the oil after lowering the temperature to 60°C. Oleic acid was then added for formulation I. Part B ingredients were mixed in water heated to 60°C. Part A was then added to part B at the same temperature with a glass rod until the lotion was formed.

Evaluation of lotion stability

Macroscopical evaluation: 10ml of each of the prepared lotions was poured into 10ml measuring cylinders, covered with aluminium foil to prevent evaporation, and allowed to stand at room temperature. A daily visual observation was done to note any change in general appearance, colour, odour and lotion consistency.

Determination of globule size: One drop of each of the prepared lotion formulations was placed on a slide and the globule size measured as a function of time.

Table 1.
Formulation of L. *leucocephala* lotion

Ingredient.	Formulation I	Formulation II
A		
• *Leuceana leucocephala* seed oil	32.0	32.8
• Oleic acid	2.0	-
• Emulsifying wax	8.0	8.2
B		
• Triethanolamine	1.0	1.0
• Glycerol	4.0	4.1
• Water	53.0	53.9

RESULTS AND DISCUSSION

From the investigations carried out on the anti-bacterial activity of the oil (Table 2), the oil showed activity against the Gram-positive and Gram-negative organisms used in the investigation. This activity was however concentration dependent with no activity shown by the 6.25% concentration of the oil. The highest activity was recorded against *E.* coli, while the least was against *Staph. Aureus*. In all, the anti-bacterial activity of the oil is lower compared to the standard drug used (gentamycin).

Table 2.
Diameter of zones of inhibition (mm) of bacterial isolates by different concentrations of the oil

Microorganism	Concentration of oil (%)					Polysorbate 80	Gentamycin
	100	50	25	12.5	6.25	(0.5%)	(10µg/ml)
Staphylococcus aureus	12	10	-	-	-	-	38
Esherichia coli	18	14	12	10	-	-	36
Bacillus subtilis	14	12	-	-	-	-	34
Pseudomonas aeruginosa	16	12	10	-	-	-	38

Table. 3:
Diameter of zones of inhibition (mm) of fungal isolates by different concentrations of the oil

Microorganism	Concentration of oil (%)					Ethanol	Tioconazole
	100	50	25	12.5	6.25	(96%)	(700µg/ml)
Staphylococcus aureus	-	-	-	-	-	-	24
Esherichia coli	-	-	-	-	-	-	20
Bacillus subtilis	-	-	-	-	-	-	24
Pseudomonas aeruginosa	-	-	-	-	-	-	22

Table 4.
Microscopical globule size analysis for formulation I

Globule Size (µm)	DAY 1		DAY 2		DAY 3		DAY 6		DAY 7		DAY 8	
R	C	F	FC	F	FC	F	FC	F	FC	F	FC	F	FC
0-49	24.5	27	661.50	21	514.50	17	416.50	13	318.50	9	220.50	9	220.50
50-89	69.5	26	1807.00	23	1598.50	22	1529.00	24	1668.00	27	1876.50	29	2015.50
90-129	109.5	29	3175.50	32	3504.00	27	2956.50	21	2299.50	19	2080.50	16	1752.00
130-169	149.5	18	2691.00	26	3887.00	29	4335.50	33	4933.50	31	4634.50	27	4036.50
170-209	189.5	14	2653.00	14	2653.00	17	3221.50	21	3979.50	25	4737.50	28	5306.00
210-249	229.5	4	918.00	2	459.00	6	1377.00	6	1377.00	7	1606.50	9	2065.50

R= Range, C= Class centre, F= Frequency.

Table 5.
Microscopical Globule Size Analysis for Formulation I1

Globule Size (µm)	DAY 1		DAY 2		DAY 3		DAY 6		DAY 7		DAY 8	
R	C	F	FC	F	FC	F	FC	F	FC	F	FC	F	FC
0-49	24.5	24	588.00	24	588.0	21	514.5	19	465.5	21	514.5	23	563.5
50-89	69.5	19	1320.5	18	1251.0	20	1390.0	12	834.0	16	1112.0	18	1251.0
90-129	109.5	17	1861.5	11	1204.5	13	1423.5	11	1204.5	7	766.5	7	766.5
130-169	149.5	11	1644.5	11	1644.5	9	1345.5	10	1495.0	5	747.5	7	1046.5
170-209	189.5	8	1516.0	12	2274.0	19	3600.5	21	3979.5	19	3600.5	13	2463.5
210-249	229.5	6	1377.0	9	2065.5	14	3213.0	15	3442.5	17	3901.5	17	3901.5

R= Range, C= Class centre, F= Frequency.

Table 6:
Average globule sizes for formulations I and II over eight days

	Mean globule sizes in µm					
Formulation	Day 1	Day 2	Day 3	Day 6	Day 7	Day 8
I	1984.3	2102.7	2306.0	2429.3	2526.0	2560.0
II	1384.6	1504.6	1914.5	1903.5	1773.8	1665.4

This activity was propably due to the presence of dissolved secondary metabolites in the oil as confirmed by past studies that reported varying secondary metabolites such as sterols, triterpenoid alcohols, tocopherols, glycolipids, hydrocarbons and carotenoids (Kulkarni *et al.*,1992), that have been found to be present in the oil. This is also corroborated by the work of Idowu *et al* (2009) which showed the presence UV active constituents in the oil has revealed by the tlc image of the chromatoplates. However, the oil did not show activity against the fungal organisms used in the study (Table 3).

Observation of the physical appearance of the lotions prepared using the oil shows that the lotions had brownish appearance due to the colour of the oil used. However, they had good spreadability on the skin and were easily washed off. Formulation I containing oleic acid had better consistence than formulation II.
Assessing the lotions stability, there was no visible change in colour, odour or texture of the two formulations in the first three days after formulation.

However, there was separation of the oily layer from the rest of the lotion for formulation II and the separated layer had a darker colour than the rest of the formulation.

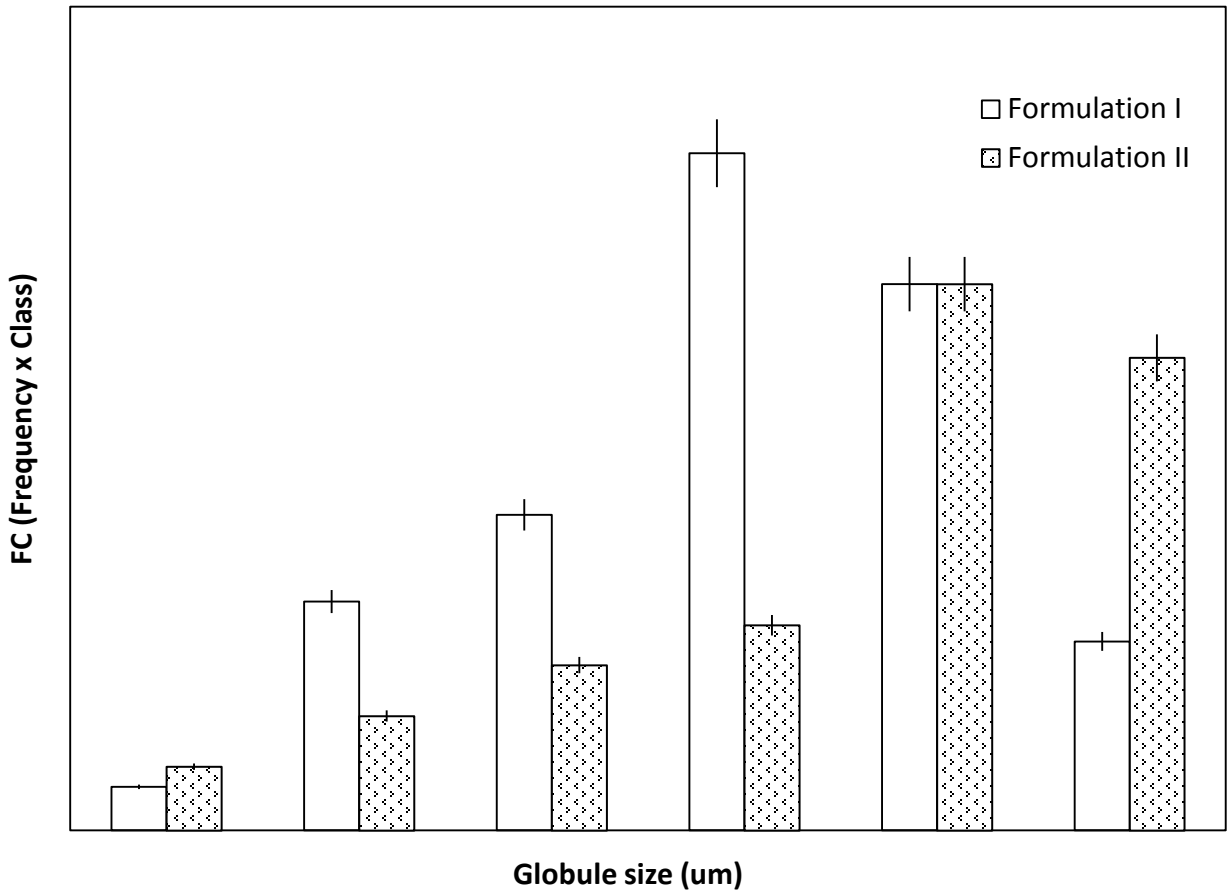

Figure 1:
Globule size distribution for the lotion formulations on day 6

The globule size analysis of the formulations over eight days are given in Tables 4, 5 and 6. Generally the there was an increase in the frequency of larger globules with a corresponding decrease in that of the smaller globules over time.

However, the increase in globule size was found to be more significant in formulation II which had no emulsifying agent (Fig 1). This confirms the need of an emulsifying agent to stabilise the lotion over the period of use. Further, presenting the oil in the lotion form will enhance its acceptability for use against skin infections caused by susceptible microorganisms.

The study showed that *Leucaena leucocephala* seed oil extract had a concentration-dependent activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Pseudomonas aeruginosa*, *Esherichia coli*) bacteria and the lotion formulation with an emulsifying agent had good pharmaceutical properties.

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