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Antibacterial Activities of Nonionic and Anionic Surfactants From *Citrullus lanatus* Seed Oil

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A B S T R A C T

Background: Analysis and applications of lesser known underutilized seed oils are important, since there is little or no information on their composition and uses, most of them are discarded as waste every year.
Objectives: The present work reports the antibacterial activities of diethanolamides (nonionic surfactant) and sulphated diethanolamide (anionic surfactant) synthesized from the seed oil of *Citrullus lanatus*.
Materials and Methods: Diethanolamide biosurfactant was produced from the oil via transamidation reaction using sodium methoxide as catalyst while the diethanolamide was sulphated using chlorosulphonic acid. The conversion of the oil to the biosurfactants was monitored using FTIR spectrophotometer.
Results: The iodine and saponification values of *Citrullus lanatus* oil were 118.50 ± 0.80 g iodine/100g and 199.10 ± 2.40 mgKOH/g respectively. Linoleic acid (56.9 %) was reported to be the most abundant fatty acid in *Citrullus lanatus* oil.
Conclusions: The biosurfactants inhibited the growth of organisms such as *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli* with diethanolamide biosurfactant exhibiting better antibacterial activity than sulphated diethanolamide.

Keywords: Biosurfactant; *Citrullus lanatus*; Diethanolamide, Fatty Acids; Transamidation

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Implication for health policy/practice/research/medical education:
This study shows that biosurfactants from the seed oil of *Citrullus lanatus* have antibacterial efficacy against the growth of some pathogenic organism which presents the biosurfactants as potential antibacterial agent

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1. Background

Fats and oils are natural products which are widely distributed in both of the animal and plant kingdoms. Fats and oils are composed of carbon, hydrogen and oxygen elements in the form of the glycerides or compounds of fatty acids and glycerol (1). Oils from plant sources (seed oils) are important sources of nutritional oils, industrial and pharmaceutical oils are also important. The characteristics of oils from different sources depend mainly on their compositions and no oil from a single source can be suitable for all purposes (2, 3). Oil has different applications, including domestic and industrial applications, one of which is the production of surfactant.

Surfactants are compounds composed of both hydrophilic and hydrophobic or lipophobic groups. In view of their dual hydrophilic and hydrophobic nature, surfactants tend to concentrate at the interfaces of aqueous mixtures; the hydrophilic part of the surfactant orients itself towards the aqueous phase and the hydrophobic part orients itself away from the aqueous phase into the non polar phase. The most familiar use of surfactants includes soaps, laundry detergents, dishwashing liquids and shampoos. Other important uses are in many industrial applications such as in lubricants, emulsion polymerization, textile processing, mining flocculates, petroleum recovery, wastewater treatment, drug formulation, and many other products and processes (4).

Several known surfactants are of petroleum base and most are non biodegradable, with toxic by products and not eco-friendly. Though a few of them that are recently used as detergents and cosmetics are eco-friendly, majority of them are known to be toxic to animals, ecosystems and humans, and can increase the diffusion of other environmental contaminants into the environment (5, 6). Therefore, there is a need to develop cheap, of a renewable source, non-toxic, and eco-friendly biosurfactants; thus shifting attention to the biomass. The use of cheap and non-edible vegetable oils and animal fats as raw feed stocks to produce biosurfactant is an effective way to reduce the cost of biosurfactant. *Citrullus lanatus* is an example of underutilized plant in Nigeria which can serve as the source of a cheap seed oil for this purpose.

C. lanatus is an annual climbing or trailing herb up to 3 m high which belongs to the Cucurbitaceae family (7) of grassy savanna and bush-savanna, occurring as an introduced cultivated plant throughout the West African region (8, 9). It favors a dry climate and is mainly a dry season crop in monsoon areas, which requires only limited rainfall (10). The fruit is variable in size from about 7 cm in diameter to over 20 cm, in shape from round to long-marrow, in outside coloration, the usual pattern is a variegation of green stripes, and inside the flesh may be red or white and the seeds are black, red or pale colored (11). The flesh amounts to about 65% of the whole fruit weight, and 95% of it is water.

2. Objectives

The present study involved the synthesis of diethanolamide (non ionic) and sulphated diethanolamide (anionic) biosurfactant from *C. lanatus* oil using diethanolamine and chlorosulphonic acid in the presence of sodium methoxide catalyst. The nonionic and anionic biosurfactants produced were also analyzed for their antimicrobial activities. The formation of the diethanolamide and sulphated diethanolamide were monitored using Fourier Transform Infrared Spectroscopy (FTIR) and Proton Nuclear Magnetic Resonance Spectroscopy (HNMR).

3. Materials and Methods

3.1. Materials

The mature seeds of *C. lanatus* were purchased at the Bodija market, Bodija, Ibadan, Oyo state, Nigeria. They were identified at the herbarium unit, University of Ibadan Botany Department. All solvents and chemicals used in this study were of analytical grade and were purchased from Merck, Darmstadt, Germany.

3.2. Chemical Analysis of C. lanatus Seed Oil

The dried seeds of *C. lanatus* were extracted with n-hexane using soxhlet extractor (12). *C. lanatus* oil was analyzed for iodine value, saponification value and free fatty acid content by method described by the Association of Official Analytical Chemist (13).

3.3. Fatty Acid Analysis of C. lanatus seed oil

The determination of the fatty acids content of the oil of *C. lanatus* was reported based on the result of Oluba *et al.* (14). This was achieved using a GC coupled with a flame ionization detector.

3.4. Synthesis of Diethanolamide Surfactant From C. lanatus Oil

The diethanolamide biosurfactant was produced as follows; 0.3 moles of diethanolamine was carefully weighed into a round bottom flask fitted with a condenser, 0.02 moles of sodium methoxide was added to it and the mixture was heated while stirring to 115 °C. 0.05 moles of the oil was added intermittently over a period of 5-10 min and the reaction was allowed to run for 8 h. The reaction mixture was cooled and dissolved into hexane, washed with distilled water and dried over sodium sulphate, then it was decanted and the hexane was removed to yield the surfactant. The oil sample and its respective biosurfactants were taken for Infrared analysis using the Buck-Scientific Infrared Spectrophotometer;

Model 500.

3.5. Synthesis of Sulphated Diethanolamide Surfactant From C. lanatus

About 14.7 g of *C. lanatus* diethanolamide was dissolved in 100 mL of chloroform and 7.8 g (0.067 moles) of chlorosulphonic acid was added drop wise with stirring while maintaining the temperature below room temperature with an ice bath. Stirring at 25°C was continued for 10-15mins after the evolution of HCl had stopped. The reaction mixture was chilled and diluted with an equal volume of cold 95% ethanol and neutralized with 18N NaOH. A crude product was obtained from solution after clarification. The oil sample and its respective biosurfactants were taken for Infrared analysis using the Buck-Scientific Infrared Spectrophotometer; Model 500.

3.6. Organisms and Media

All organisms were collected from the University College Hospital (UCH), University of Ibadan, Nigeria. The bacterial strains were cultured overnight at 37°C in Muller-Hinton agar (Difco, USA).

3.7. Antibacterial Activity

Agar-well diffusion method was used. The bacteria were grown on Muller-Hinton agar medium (pH 7.3). Agar medium was poured into the plates to uniform depth of 5 mm and was allowed to solidify. The microbial suspensions at 5x106 CFU\ mL were streaked over the surface of the media using sterile cotton swab to ensure the confluent growth of the organisms. The wells (6 mm in diameter) were cut from the agar, and 60 µL of the biosurfactant solutions (100 mg\ mL) was delivered into them. The plates were incubated at 37°C for 24 h and the observed growth inhibition zones were measured. The oil and the biosurfactants were prepared and screened for the antimicrobial activities.

4. Results

4.1. Chemical Analysis and Fatty Acid Composition of C. lanatus Oil

The result of the chemical analysis of *C. lanatus* seed oil is presented in *Table 1* . The free fatty acid content was found as 1.50 ± 0.20 % while the iodine value was 118.50 ± 0.80 g iodine/100g. The fatty acid composition is shown in *Table 2* as reported by Oluba *et al.* (14). The most abundant fatty acid in the oil was reported to be linoleic (56.9 %). *C. lanatus* oil was reported to have an unsaturation value of 71.9 %. The high unsaturation value of the oil and the fairly high oil yield from the seed (47.54 %) were what led to the production of biosurfactant from *C. lanatus* oil since the unsaturated bonds were the points at which modification could be carried out on the oil by introducing different functional groups to it (4).

Table 1. Chemical Properties (%) of *C. lanatus* Seed Oil

Parameter	*C. lanatus*
Free fatty acid, %	1.50 ± 0.20
Iodine value, g iodine/100g	118.50 ± 0.80
Saponification value, mgKOH/g	199.10 ± 2.40

Table 2. Fatty Acid Composition (%) of *C. lanatus*

Fatty Acid	*C. lanatus*[a]
Lauric	0.2
Myristic	0.7
Palmitic	13.5
Stearic	13.7
Oleic	14.6
Linoleic	56.9
Linolenic	0.5
Saturated fatty acids	28.1
Monounsaturated fatty acid	14.5
Polyunsaturated fatty acids	57.4
Total unsaturated fatty acid	71.9

[a] Oluba *et al.* (Ref:14)

4.2. Synthesis of Diethanolamide and Sulphated Diethanolamide From C. lanatus Oil

The synthesis of diethanolamide and sulphated diethanolamide from *C. lanatus* was monitored using FTIR and 1HNMR. The FTIR spectrum for *C. lanatus* oil showed important peaks expressing the vibrational frequencies of the different functional groups present in the oil and biosurfactants. The absorption peak for C-H stretching of CH3 was found at 2962 cm-1 while that of CH2 appeared at 2894 cm-1 in the oil. Peaks which can be attributed the bending absorption of methylene (CH2) and methyl (CH3) groups appeared at 1460 cm-1 and 1376 cm-1 respectively.

Vibrational frequencies at 1750 cm-1 and 1160 cm-1 were due to the stretching absorption of esters; C=O and C-O respectively. The FTIR spectrum for the diethanolamide and sulphated diethanolamide biosurfactants synthesized from *C. lanatus* oil revealed the total disappearance of the vibrational frequency of C=O of esters which was at 1750 cm-1 to give an intense peak at 1640cm-1 , which signified the formation of an amide functional group. The characteristic peak at 3393 cm-1 in the biosurfactants was attributed to the vibrational frequency of OH functional group.

4.3. Antibacterial Activity of Diethanolamide and Sulphated Diethanolamide From C. lanatus Oil

The result of the antimicrobial activities of the biosurfactants from *Citrullus lanatus* oil is presented in *Table 3* . *C. lanatus* oil did not have any inhibition against the growth of the tested organisms while the biosurfactants had activity against the growth of the tested organisms (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli*). The activities of diethanolamide and sulphated diethanolamide against the growth of the microorganisms varied. The activity of the diethanolamide was found to be higher than that of the sulphated amide. The diethanolamide and sulphated diethanolamide had the same inhibition (20 mm) against *K. pneumonia*. The growth of *S. aureus* was inhibited for the most by diethanolamide (40 mm). Inhibition against the growth of *P. aeruginosa* was also exhibited higher by diethanolamide (31 mm). Diethanolamide biosurfactant from *C. lanatus* oil showed better antimicrobial activity than that of the sulphated diethanolamide from the same *C. lanatus* oil.

Table 3. Antimicrobial Activity of the Oil and Biosurfactants of *C. lanatus*

Sample	*K. pneumonia* [a]	*E. coli* [a]	*S. aureus* [a]	*P. aeruginosa* [a]
Oil	NA	NA	NA	NA
CDA [b]	20	25	40	31
CSDA [b]	20	15	15	25
Levofloxacin [c], **μg/L**	5.00	0.63	0.37	0.62

[a] Diameter of zone of inhibition including well diameter of 6mm
[b] Abbreviations: CDA, *C. lanatus* diethanolamide surfactant; CSDA, *C. lanatus* sulphated diethanolamide surfactant; NA, No activity
[c] Levofloxacin, Positive control

5. Discussion

Oil which was extracted from *C. lanatus* seeds was analyzed for free fatty acid, iodine value and saponification value. Diethanolamide (nonionic) and sulphated diethanolamide (anionic) surfactants were produced from the oil via transamidation reaction using sodium methoxide as catalyst. The biosurfactants inhibited the growth of organisms such as *P. aeruginosa*, *S. aureus*, *K.pneumonia* and *E. coli* with diethanolamide biosurfactant exhibiting better antibacterial activity than sulphated diethanolamide.

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Authors' Contribution

None declared.

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