Antimicrobial activity of diethanolamide: a nonionic surfactant from *Calophyllum inophyllum* and *Cyperus esculentus*

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The present work reports the antimicrobial activities of diethanolamides synthesized from the seed oils of *Calophyllum inophyllum* and *Cyperus esculentus*. Oil was extracted from the seeds of *Calophyllum inophyllum* and *Cyperus esculentus* using hexane. The iodine value of the oil of *Calophyllum inophyllum* was 106.22±0.50 g iodine/100 g while that of *Cyperus esculentus* was 115.20±0.80 g iodine/100 g. The saponification value of *Cyperus esculentus* (201.30±1.20 mgKOH/g) was found higher than that of *Calophyllum inophyllum* (198.25±0.40 mgKOH/g). Diethanolamine biosurfactant was produced from the oils via transamination reaction using sodium methoxide as catalyst. The conversion of the oils to the biosurfactant was monitored and confirmed using the Perkin-Elmer FTIR spectrophotometer. The biosurfactants inhibited the growth of organisms such as *Klebsiella Pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The diethanolamide biosurfactant from the oil of *Calophyllum inophyllum* exhibited better antimicrobial activity than that of *Cyperus esculentus*.
Keywords: *Calophyllum inophyllum*, *Cyperus esculentus*, diethanolamide, fatty acids, biosurfactant

Attività antimicrobica di dietanolammine da oli di semi di *Calophyllum inophyllum* e *Cyperus esculentus*

Il presente lavoro relaziona sull'attività antimicrobica di dietanolammine sintetizzate da oli di semi di *Calophyllum inophyllum* e *Cyperus esculentus*

L'olio è stato estratto dai semi di *Calophyllum inophyllum* e *Cyperus esculentus* utilizzando esano. Il numero di iodio dell'olio di *Calophyllum inophyllum* era 106,22±0,50 g iodine/100 g mentre quello di *Cyperus esculentus* era 115,20±0.80 g iodine/100 g. Il valore di saponificazione di *Cyperus esculentus* (201,30±1,20 mg KOH/g) è stato trovato superiore a quello di *Calophyllum inophyllum* (198,25±0,40 mgKOH/g).

La dietanolammina dei biotensioattivi è stata prodotta dagli oli tramite reazione di transaminazione con metossido di sodio come catalizzatore.

La conversione degli oli per ottenere il biotensioattivo è stata monitorata e confermata con uno spettrofotometro Perkin-Elmer FTIR. I biotensioattivi inibiscono la crescita di organismi come la *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus subtilis* e *Pseudomonas aeruginosa*.

La dietanolammide dall'olio di *Calophyllum inophyllum* ha mostrato miglior attività antimicrobica di quello di *Cyperus esculentus.*
Parole chiave: *Calophyllum inophyllum*, *Cyperus esculentus*, dietanolammide, acidi grassi, biotensioattivi

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INTRODUCTION

Triglycerides obtained from oil seed crops represent a renewable source of fatty acids that are suitable for the preparation of surfactants and related products [1]. Surfactants are applicable to both edible and non edible industrial products. Edible applications include use as emulsifiers or thickening agents. Monostearate and oleate glycerides are used mainly in the food industry where they act as viscosity modifiers and stabilizers that provide texture to processed foods [2]. Surfactants are important components in formulations of agrochemicals, corrosion inhibitors, cosmetics, detergents, lubricants, drugs, polymers and textile finishes [3].

Several known surfactants are of a petroleum base which are non biodegradable, with toxic by-products and are not ecofriendly, though a few of them that have been recently used as detergents and cosmetics are ecofriendly. Therefore, there is need to develop biosurfactants that are cheap, of a renewable source, non-toxic, and ecofriendly; thus shifting attention to the biomass. So, the use of cheap and non-edible vegetable oils and animal fats as raw feed stocks for biosurfactant production is an effective way to reduce the cost of biosurfactant.

Cyperus esculentus and *Calophyllum inophyllum* are underutilized plants in Nigeria which could serve as sources of seed oils for cheap production of biosurfactants. *Calophyllum inophyllum*, commonly known as "Indian laurel" or "Alexandrian laurel" is a broad leaved evergreen tree occurring as a littoral species along the beach crests, although sometimes occurring inland [4]. It is known to have cancer chemopreventive agents [5], coumarins and xanthones with antimicrobial activity [6]. It is a large evergreen plant. It is widely cultivated in the tropical regions of the world, including several Pacific Islands. The leaf is decorative with fragrant flowers and a spreading crown, it is best known as an ornamental plant. It is a low-branching and slow-growing tree with a broad and irregular crown. The oil has various medicinal uses in rheumatism, skin diseases, joint pains and haemorrhage [7-9]. The fruit is a round, green drupe reaching 2 to 4 cm in diameter and having a single large seed. The fruit being a drupe, has a hard endocarp with long dormancy period and low rate of germination. Being a littoral species, the seeds are taken away in the tidal water thereby limiting the propagation rate. Conventional propagation via vegetative cuttings is not practiced due to difficulty in rooting [10] in almost all species of *Calophyllum* and immediate protective measures are essential for the continued existence of the genus [11].

Tigernut (*Cyperus esculentus*) is an underutilized crop of the family *Cyperaceae* which produces rhizomes from the base and tubers that are somewhat spherical. It is commonly known as "earth almond", "chufa", "chew-fa" and "Zulu nuts". There are three varieties (black, brown and yellow) which are cultivated [12]. Among these only two varieties yellow and brown are readily available in the market. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size, attractive color and fleshier body [13]. Tiger nut can be eaten raw, roasted, dried, baked or be made into a refreshing beverage (called Hochata De Chufas) or tiger nut milk [14]. Also, it can be used as a flavoring agent for ice cream and biscuits [15]. It is an annual or perennial plant, growing to 90 cm in height, with solitary stems growing from a tuber. The stems are triangular in section, and bear slender leaves 3-10 mm wide. The flowers of the plant are distinctive, with a cluster of flat oval seeds surrounded by four hanging leaf-like bracts positioned 90 degrees from each other. The plant foliage is very tough and fibrous, and is often mistaken for a grass.

Surfactants based on seed oil offer significant advantages in handling, processing and formulation. They are ideal candidates for a broad range of applications. They provide excellent surface tension reduction, excellent wetting, low foam and excellent detergency. They are readily biodegradable and are not a source of VOCs (Volatile Organic Compounds). These Surfactants are excellent materials for use in "green" products such as environmentally friendly cleaners but little is known about their antimicrobial activities. Assays for plants with anti-microbial activity are particular important in developing countries due to the prevalence of infectious disease of bacterial or fungal origin. For instance, there is a great need of cheaper and more effective anti-microbial agents for tropical or systemic use.

Fatty acids diethanolamides find various uses, particularly in detergents and cosmetic formulations, as foam boosters, viscosity builders and emulsifying, wetting, superfattening and conditioning agents [16]. The reaction conditions, processing equipments, product composition and end use depend on whether fatty acids, methyl esters or oils are used [17].

In this present work we have produced diethanolamide based biosurfactant from the oils of *Calophyllum inophyllum* and *Cyperus esculentus* using diethanolamine in the presence of sodium methoxide catalyst.

The biosurfactants produced were also analyzed for their antimicrobial activities. The formation of the diethanolamides was monitored and confirmed using Fourier Transform Infrared Spectroscopy (FTIR).

MATERIALS AND METHODS

MATERIALS

The mature seeds of *Calophyllum inophyllum* were collected from the trees grown at the garden of University of Ibadan while the seeds of *Cyperus esculentus* were purchased at the Bodija market, Bodija, Ibadan, Oyo state, Nigeria. They were identified at the herbarium unit, Botany Department University of Ibadan. All solvents and chemicals used in this study were of analytical grade and were purchased from Merck, Darmstadt, Germany.

CHEMICAL ANALYSIS OF THE SEED OILS OF *CALOPHYLLUM INOPHYLLUM* AND *CYPERUS ESCULENTUS*

The dried seeds of *Calophyllum inophyllum* and *Cyperus esculentus* were extracted with *n*-hexane for 10 h using soxhlet extractor [18]. The oils of *Calophyllum inophyllum* and *Cyperus esculentus* were analyzed for their iodine value, saponification value and free fatty acid content by method described by the Association of Official Analytical Chemist (AOAC) [19].

FATTY ACID ANALYSIS OF THE SEED OILS OF *CALOPHYLLUM INOPHYLLUM* AND *CYPERUS ESCULENTUS*

The determination of the fatty acids content of the oil of *Calophyllum inophyllum* was reported based on the result of Ajayi *et al.*, [20] while that of *Cyperus esculentus* was the report of Shaker *et al.*, [21]. This was achieved using a GC coupled with a flame ionization detector was used.

SYNTHESIS OF DIETHANOLAMIDE FROM THE OILS OF *C. INOPHYLLUM* AND *C. ESCULENTUS*

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 (*Calophyllum inophyllum* or *Cyperus esculentus*) was added intermittently over a period of 5-10 min and the reaction was allowed to run for 8 h. After this, the reaction mixture was cooled and dissolved into hexane, washed with distilled water and dried over sodium sulphate after which it was decanted and the hexane removed to yield the surfactant.

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).

ANTIBACTERIAL ACTIVITY

Agar-well diffusion method was used. Briefly, the bacterial were grown on Muller-Hinton agar medium (pH 7.3). Agar medium were poured into the plates to a uniform depth of 5 mm and allowed to solidify. The microbial suspensions at $5x10^6$ 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 observed growth inhibition zones were measured. Both the oils and the surfactants were prepared and screened for the antimicrobial activities.

RESULTS AND DISCUSSION

ANALYSIS AND FATTY ACID COMPOSITION OF THE OIL OF *CALOPHYLLUM INOPHYLLUM* AND *CYPERUS ESCULENTUS*

The chemical properties of the oils of *Calophyllum inophyllum* and *Cyperus esculentus* is presented in Table I. The free fatty acid was found to be 1.10±0.20% in *Calophyllum inophyllum* and 1.20±0.20% in *Cyperus esculentus*. The saponification value of *Cyperus esculentus* (201.30±1.20 mgKOH/g) was found higher than that of *Calophyllum inophyllum* (198.25±0.40 mgKOH/g). The iodine value of *Cyperus esculentus* was 115.20±0.80 g iodine/100g while that of *Calophyllum inophyllum* was found to be 106.22±0.50 g iodine/100g. The result of the fatty acid composition of *Calophyllum inophyllum* and *Cyperus esculentus* is shown in Table II. The fatty acid composition of *Calophyllum inophyllum* was presented as reported by Ajayi *et al.*, [20] while that of *Cyperus esculentus* was as presented according to the report of Shaker *et al.*, [21]. C18:1 is the major fatty acid present in the oil of *Calophyllum inophyllum* (39.82%) and *Cyperus esculentus* (69.50%). C18:2 was found higher in the oil of *Calophyllum inophyllum* (27.60%) than that of *Cyperus esculentus* (8.80%). C18:3 was only detected in *Cyperus esculentus* as 0.40%. C18:0 was reported higher in *Calophyllum inophyllum* (16.48%) than in *Cyperus esculentus* (3.40%) while C16:0 was found in almost equal amount in both oils. C20:1 and C22:1 were not detected in *Cyperus esculentus* but in *Calophyllum inophyllum* as 0.86 and 0.24% respectively.

SYNTHESIS OF DIETHANOLAMIDE FROM THE OILS OF *C. INOPHYLLUM* AND *C. ESCULENTUS*

The FTIR spectrum for the oils of *Calophyllum inophyllum* and *Cyperus esculentus* showed important peaks expressing the vibrational frequencies of the different functional groups present in the oils and biosurfactants. We observed the absorption

Table I - Fatty acid composition (%) of *Calophyllum inophyllum* and *Cyperus esculentus*

Parameter	*Calophyllum inophyllum*	*Cyperus esculentus*
Free fatty acid (%)	1.10±0.20	1.20±0.20
Iodine value (g iodine/100g)	106.22±0.50	115.20±0.80
Saponification value (mgKOH/g)	198.25±0.40	201.30±1.20

peak for C-H stretching of CH_3 at 2924 cm^{-1} and 2918 cm^{-1} in the oils of *Calophyllum inophyllum* and *Cyperus esculentus* respectively; another C-H stretching absorption was also noticed for CH_2 which occurred at 2847 cm^{-1} in the oil of *Calophyllum inophyllum* and at 2853 cm-1 in the oil of *Cyperus esculentus*. Two peaks which can be attributed the bending absorption of methylene (CH_2) and methyl (CH_3) groups appeared at 1458 cm^{-1} and 1374 cm^{-1} respectively. The vibrational frequencies at 1745 cm^{-1} and 1163 cm^{-1} were due to stretching absorption of esters; C=O and C-O respectively.

The FTIR spectrum for the diethanolamide surfactants produced from the oils of *Calophyllum inophyllum* and *Cyperus esculentus* revealed the total disappearance of the vibrational frequency of C=O of esters which was at 1745 cm^{-1} to give an intense peak at 1625 cm^{-1} thus, signifying the formation of an amide functional group. We also observed that the stretching, and bending absorptions due to CH_3 and CH_2 were still retained. Peaks were also noticed at 3391 cm^{-1} in *Calophyllum inophyllum* and at 3399 cm^{-1} in *Cyperus esculentus* which were attributed to the vibrational frequencies of OH functional groups. The characteristic strong peaks of OH stretching at 3391 cm^{-1} and 3399 cm^{-1} as well as the peaks at 1625 cm^{-1} and 1622 cm^{-1}; which was attributed to the amide functional groups are strong indicators of fatty acid diethanolamides.

The result of the antimicrobial activities of the oils and surfactants of *Calophyllum inophyllum* and *Cyperus esculentus* is presented in Table III. The oil of *Calophyllum inophyllum* showed inhibition against the tested organisms except *Klebsiella pneumonia* while the oil of *Cyperus esculentus* did not show any activity against the organisms. The diethanolamide biosurfactants from the oil of *Calophyllum inophyllum* demonstrated activity against all the microorganisms screened with inhibition zone above 17 mm but in the case of diethanolamide from the oil of *Cyperus esculentus*, the least inhibition zone was 7 mm which was in *Bacillus subtilis*. The highest inhibition zone was found against *Staphylococcus aureus* (25 mm) and *Pseudomonas aeruginosa* (25 mm) for diethanolamide of *Calophyllum inophyllum* while the highest inhibition zone was recorded against *Klebsiella Pneumoniae* (19 mm) and *Proteu mirabilis* (19 mm) for the diethanolamide of *Cyperus esculentus*. Diethanolamide biosurfactant from the oil of *Calophyllum inophyllum* exhibited better antimicrobial activity than that of *Cyperus esculentus*.

Table II - Fatty acid composition (%) of *Calophyllum inophyllum*

Fatty acid	*Calophyllum inophyllum*[a]	*Cyperus esculentus*[b]
C14:0	ND	0.80
C16:0	14.62	14.50
C16:1	ND	1.50
C18:0	16.48	3.40
C18:1	39.82	69.50
C18:2	27.60	8.80
C18:3	ND	0.40
C20:0	0.38	0.20
C20:1	0.86	ND
C22:1	0.24	ND

a) Ajayi et al. (2002); b) Shaker et al. (2009), ND = Not detected

CONCLUSION

Oil was extracted from the seeds of *Calophyllum inophyllum* and *Cyperus esculentus*. The extracted oils were analyzed for free fatty acid, iodine value and saponification value. C18:1 was reported to be the most abundant fatty acid in the oils of *Calophyllum inophyllum* and *Cyperus esculentus*. Diethanolamine surfactant was produced from the oils via transamidation reaction using sodium methoxide as catalyst. The biosurfactants inhibited the growth of organisms such as *Klebsiella Pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* with biosurfactant from *Calophyllum inophyllum*

Table III- Antimicrobial activity of the oils and biosurfactants of *Calophyllum inophyllum* and *Cyperus esculentus*

Sample	*Klebsiella Pneumoniae*[a]	*Proteus mirabilis*[a]	*Staphylococcus aureus*[a]	*Bacillus subtilis*[a]	*Pseudomonas aeruginosa*[a]
Calophyllum inophyllum	NA	14	13	12.5	8
CIS	18	19	25	22	25
Cyperus esculentus	NA	NA	NA	NA	NA
CES	19	19	15	7	15

CIS = *Calophyllum inophyllum* surfactant, CES = *Cyperus esculentus* surfactant, NA = No activity,
a) Diameter of zone of inhibition including well diameter of 6 mm

exhibiting better antimicrobial activity than that of *Cyperus esculentus*. The conversion of the oils to the biosurfactant was monitored and confirmed using the Perkin-Elmer FTIR spectrophotometer.

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