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Antimicrobial activities of *Oedogonium capillare* extracts on selected microorganisms

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Abstract

Antimicrobial activities of *Oedogonium capillare* extracts on selected microorganism were investigated. It was observed that the *Oedogonium capillare* extracts had antimicrobial effect on microorganisms use in the course of the research. Methanolic extracts of the *Oedogonium capillare* extracts had the highest zone of inhibition on the selected microorganisms followed by ethanolic extracts and diethyl ether extracts of the *Oedogonium capillare*. Methanolic extracts of the *Oedogonium capillare* were able to inhibit *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Psuedomonas aeruginosa, Candida albican*, *Aspergillus niger* except *Aspergillus fumigatus* at 100 mg/ml while at 200 mg/ml the Methanolic extracts of the *Oedogonium capillare* were able to inhibit all the selected microorganisms employed in the course of the research. The ethanolic extracts of the of the *Oedogonium capillare* had antimicrobial effect on *Staphylococcus aureus* and *Candida albican* at 100 mg/ml while at 200 mg/ml it was able to inhibit *Staphylococcus aureus, Escherichia coli, Psuedomonas aeruginosa, Candida albican*, *Aspergillus fumigatus, Aspergillus niger* except *Klebsiella pneumonia.* The diethyl ether extracts had antimicrobial effect on *Candida albican* at 100 mg/ml while at 200 mg/ml it was able to inhibit *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Psuedomonas aeruginosa, Candida albican*, *Apergillus niger* except *Aspergillus fumigatus.* Antimicrobial activity indicates that the presence of active constituents in the extractions of marine algae which can be exploited for the production of innovation drugs for the benefit of the humanity.
Keywords: Antimicrobial, algae, *Oedogonium capillare,* microorganisms, extracts, active

Introduction

Algae are heterogeneous complex organisms which comprise the dominant photoautotrophs in many aquatic environments, (Round, 1981; Wetzel, 1983). *Oedogonium* species belong to *Chlorophyta*, include 534 species (Mahato, 1999) and are classified as filamentous green algae. These species are cosmopolitan in freshwater ecosystems and prefer stagnant waters, such as small ponds, pools, roadside ditches, marshes, oxbows, lakes, reservoirs, rivers (Mrozińska-Weeb, 1976; Burchardt 1977; Sieminiak, 1979; Kuczyńska-Kippen, 2009; Pikosz and Messyasz, 2015). The algae *Oedogonium Capillare* belong to the *Oedogoniaceaace* (*Chlorophyta*) family (Tiffany an Britton, 1952; Hirn, 1960). This alga is very common in México during March and July. Abundance of *Oedogonium* species depends on temperature, light intensity and type of habitats (Marta and Beata, 2015).
The use of microalgae for various applications has increased in recent decades. During the past several years, there has been significant research into the use of microalgae constituent for pharmaceutical industries (Yamaguchi, 1997; Apt and Behrens, 1999; Kreitlow *et al*, 1999).

The cell extract and active constituent of various algae have been shown to have antibacterial activity in vitro against Gram-positive and Gram-negative bacteria (Borowitka and Borowitka, 1992). A wide range of result of in vitro anti-fungi activities of extracts of green algae, diatom and dinoflagellates has also be reported (Moreau *et al*., 1988)

Negrete *et al*. (2006) proved in vitro the capability of an extracts of *Oedogonium capillare*, a fresh water green algae, to be an effective antibacterial agent against 23 different bacterial species of *Enterobacteriaceae, Pseudomonadaceae, Aeromonadaceae and Vibrionaceae* families; obtained high correlation coefficient of correlation between the performance of algae extract and antibiotic like Kinamycin, tetracycline and chloramphenicol. All parts of these algae are used in the traditional medicine for the treatment of various human ailments such as dysentery, diarrhea to cure thrush on tongues of babies, wound healing and have been used as an antiseptic on various skin diseases (Perez-Gutierrez, 2006). Previous experiments carried in our laboratory revealed that the leaves possessed high antispasmodic activity produced by one novel δ-lactone named oedogonolide (Perez *et al*., 2005).

The aim of this study was to examined the antimicrobial activities of *Oedogonium capillare* on selected microorganism

MATERIALS AND METHODS

Collection of samples

Collection of Oedogonium Capillare

The algae sample was collected with a spoon type net from reservoir in Mini campus at the Olabisi Onabanjo University, Ago-Iwoye, Ogun State and was identified by Dr. Sobowale of Plant and Applied Zoology department and collaborated by Dr. (Mrs.) Adesalu of the Botany and Microbiology department, University of Lagos (UNILAG), Lagos Nigeria.

Collection of test organisms
The test organisms were collected from the culture laboratory of the Olabisi Onabanjo University Teaching Hospital (OOUTH). The microorganisms were *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Psuedomonas aeruginosa, Candida albican*, *Aspergillus fumigatus* and *Aspergillus niger*

Preparation of the extracts and percentage recovery of the extracts

The extraction method used in this study was carried out as described Jaya *et al.* (2007) with slight modification, using 3 solvents (methanol, ethanol and Diethyl ether). Ten gram of each of the dried samples powdered plant materials were extracted in a soxhlet extractor containing 40 ml of the solvent and the resulting extracts was evaporated in a rotary evaporator.

Standardization of inoculum

Standardization of test bacteria

A loopful of the bacterial culture was aseptically inoculated into freshly prepared sterile nutrient broth and incubated for 24 hours. Zero-point-two millimetre was pipette from the 24 hours broth culture of the test organism and was dispensed into 20 ml sterile nutrient broth and incubated for another 4 hours to standardise the culture to 0.5 McFarland's standard (10^6cfu/ml) before use as described by Oyeleke *et al*. (2008).

Standardization of test fungi

A loopful of the fungal culture was aseptically inoculated into freshly prepared sterile Sabouraud dextrose agar plate and incubated for 48 hours. A loopful of the fungal culture was suspended in saline solution (0.85 % NaCl) and adjusted to match a turbidity of 10^6 Cfu/ml.

Antibiotic sensitivity profile test

The antibiotic sensitivity profile was investigated in order to compare the sensitivity of the microorganisms to the different conventional antibiotics. The disc diffusion method described by Bauer *et al*. (1996) was used to determine the susceptibility and resistance of the organisms to the antimicrobial drugs.

Antimicrobial Assay of Oedogonium capilllare Extracts on Test Organisms
After standardization using 0.5 Mcfarland standard of the inoculum, sterile Petri dishes were inoculated aseptically with 0.1 ml of the 18 hours old broth cultures of the bacterial test organisms each, while 15 ml of sterilized Mueller-Hinton and Sabouraud's dextrose agar-plates for bacterial and fungal isolates respectively was poured aseptically in the inoculated plates. The plates were swirled carefully for even distribution and allowed to gel. With the aid of a sterile cork borer of 6 mm in diameter, wells were made on the solidified agar plate aseptically. A concentration of

100 and 200 mg/ml of the extracts were prepared using 30 % dimethylsulphoxide (DMSO) as the reconstituting solvent and sterilized using 0.2 µm sterile membrane pore filter paper. Using micropipette, each extract of 0.1 ml was then pipetted into the wells of appropriately labelled plates and holes. The plates were allowed to stand on the laboratory bench for 15 minutes to allow proper in flow of the solution into the medium before incubating the plates at 37ºC for 24 hours for bacteria and 27ºC for 48 hours for fungi. After incubation, the zones of inhibition (diameter) formed in the medium were measured in millimeter to determine antimicrobial effectiveness of the extracts on the test organisms.

Determination of minimum inhibitory concentrations (mic) of Oedogonium capilllare extracts on test organisms
The tube dilution susceptibility test was used to determine the MIC values of the plant extracts on the test organisms using the method of CLSI (2006). A series of Mueller-Hinton broth tubes containing varying two fold concentrations of the various plant extracts in the range of 100 mg/ml to 12.5 mg/ml were prepared and incubated with a previously standardised density of the test organisms (0.5 ml). The lowest concentration of the *Oedogonium capilllare* extracts sample resulting in no growth after 18-24 hrs of incubation for bacteria and 24-72 hrs for yeasts and moulds using spectrophotometer was recorded as the MIC.
Statistical Analysis of Data Obtained
Data obtained were subjected to one way analysis of variance, while the means were compared by Duncan's New Multiple Range Test at 95% confidence interval using Statistical Package for Social Sciences version 16.0. Differences were considered significant at p≤0.05.

RESULTS

Table 1: Antibacterial activities of *Oedogonium capillare* extracts at 100 mg/ml on selected bacteria

Bacteria	Methanol extracts	Ethanol extracts	Diethyl ether extracts	Chloramphenicol
Klebsiella pneumonia	10.33±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	11.67±0.58^{c}
Staphylococcus aureus	15.67±0.58^{c}	12.00±1.00^{b}	0.00±0.00^{a}	14.33±0.58^{d}
Pseudomonas aeruginosa	4.33±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	9.33±0.58^{c}
Escherichia coli	3.67±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	8.33±0.58^{c}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P≤0.05).

Table 1: The antibacterial activities of *Oedogonium capillare* extracts at 100 mg/ml on selected bacteria are shown in Table

Table 2: Antibacterial activities of *Oedogonium capillare* extracts at 200 mg/ml on selected bacteria

Bacteria	Methanol extracts	Ethanol extracts	Diethyl ether extracts	Chloramphenicol
Klebsiella pneumonia	14.33±0.58^{c}	0.00±0.00^{a}	3.00±1.00^{b}	14.33±0.58^{c}
Staphylococcus aureus	17.33±0.58^{c}	11.33±0.58^{b}	4.33±0.58^{a}	19.33±0.58^{d}
Pseudomonas aeruginosa	9.67±0.58^{b}	4.33±0.58^{a}	5.33±0.58^{a}	14.33±0.58^{c}
Escherichia coli	7.33±0.58^{c}	5.33±0.58^{b}	3.33±0.58^{a}	13.33±0.58^{d}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P≤0.05).

Table 2: The antibacterial activities of *Oedogonium capillare* extracts at 200 mg/ml on selected bacteria are shown in Table

Table 3: Antifungal activities of *Oedogonium capillare* extracts at 100 mg/ml on selected fungi

Fungi	Methanol extracts	Ethanol extracts	Diethyl ether extracts	Nystatin
Candida albican	16.33±0.58^{c}	9.00±0.00^{b}	4.33±0.58^{a}	16.67±0.58^{c}
Aspergillus fumigatus	0.00±0.00^{b}	0.00±0.00a	0.00±0.00^{a}	10.33±0.58^{b}
Aspergillus niger	2.33±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	11.33±0.58^{c}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P≤0.05).

Table 3: The antifungal activities of *Oedogonium capillare* extracts at 100 mg/ml on selected fungi are shown in Table

Table 4: Antifungal activities of *Oedogonium capillare* extracts at 200 mg/ml on selected fungi

Fungi	Methanol extracts	Ethanol extracts	Diethyl ether extracts	Nystatin
Candida albican	17.67±0.58^{c}	13.33±0.58^{b}	6.00±0.00^{a}	19.33±0.58^{d}
Aspergillus fumigatus	3.33±0.58^{b}	7.67±0.58^{c}	0.00±0.00^{a}	14.67±0.58^{d}
Aspergillus niger	6.67±0.58^{c}	4.67±0.58^{b}	2.00±0.00^{a}	14.33±0.58^{d}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P≤0.05).

Table 4: The antifungal activities of *Oedogonium capillare* extracts at 200 mg/ml on selected fungi are shown in Table

Table 5: Minimum inhibitory concentration (mg/ml) of *Oedogonium capillare* Extracts on Selected microorganisms

Microorganisms	Methanol extracts	Ethanol extracts	Diethyl ether extracts
Klebsiella pneumonia	25	NI	NI
Staphylococcus aureus	50	25	NI
Pseudomonas aeruginosa	100	NI	NI
Escherichia coli	100	NI	NI
Candida albican	50	25	100
Aspergillus fumigatus	NI	NI	NI
Aspergillus niger	100	NI	NI

Key: NI=No Inhibition

Table 5 is showing the Minimum inhibitory concentration (mg/ml) of the *Oedogonium capillare* extracts that inhibit some selected microorganisms. The Minimum inhibitory concentration (mg/ml) of the *Oedogonium capillare* range from 25 mg/ml to 100 mg/ml.

Discussion

The aim of this study was to examine the antimicrobial activities of *Oedogonium capillare* on selected microorganism. It was observed that the *Oedogonium capillare* had antimicrobial effect on the selected microorganisms use in the course of the research. Negrete *et al*. (2006) proved in vitro the capability of an extract of *Oedogonium capillare*, a fresh water green algae, to be an effective antibacterial agent against 23 different bacterial species of *Enterobacteriaceae, Pseudomonadaceae, Aeromonadaceae* and *Vibrionaceae* family. Previous studies by Zornitza *et al*. (2000) and Berry *et al*. (2004) on microalgae have detected antimicrobial activities in some of the species tested in their research. Rodriguez *et al.* (2010), Bhacuni and Rawat (2005) and Priyadharshini *et al.* (2011) have reported that seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids has exhibits different biological activities. On the basis of their peculiar characteristics, members of the *Oedogoniales* are important not only from academic point of view but also are of

great ecological significances especially in the field of limnology since they occupy specific niches, food for a number of aquatic organisms (Olojo *et al.,* 2003; Kone and Teugels, 2003; Awasthi *et al.,* 2006), used for the removal of heavy metals, production of antibiotics (Redondo *et al.*, 2006) and being used as indicator of water quality (Bajpai *et al.*, 2013, Srivastava *et al.*, 2014).

Methanolic extracts of the *Oedogonium capillare* extracts had the highest zone of inhibition on the selected microorganisms followed by ethanolic extracts and diethyl ether extracts of the *Oedogonium capillare*. This correlate with the report of Vijaya Parthasarathy *et al*. (2004) that methanol is a better solvent for algal extraction and separation of variety of phytochemicals that produce maximum inhibitory effect on both gram positive and gram negative bacteria.

Kausalya and Narasimha Rao (2015) reported that depending upon their solubility and polarity, different solvents shows the different antimicrobial activity. So chemical compounds should be extracted from different seaweeds in order to optimize their antibacterial activity by selecting the best solvent system (Hediat *et al.,* 2010). Methanolic extracts of the *Oedogonium capillare* were able to inhibit *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Psuedomonas aeruginosa, Candida albican, Aspergillus niger* except *Aspergillus fumigatus* at 100 mg/ml while at 200 mg/ml the Methanolic extract of the *Oedogonium capillare* was able to inhibit all the selected microorganisms employed in the course of the research. The ethanolic extracts of the of the *Oedogonium capillare* had antimicrobial effect on *Staphylococcus aureus* and *Candida albican* at 100 mg/ml while at 200 mg/ml the it was able to inhibit *Staphylococcus aureus, Escherichia coli, Psuedomonas aeruginosa, Candida albican*, *Aspergillus fumigatus, Aspergillus niger* except *Klebsiella pneumonia.* The diethyl ether extracts had antimicrobial effect on *Candida albican* at 100mg/ml while at 200 mg/ml it was able to inhibit *Staphylococcus aureus, Klebsiella pneumonia. Escherichia coli, Psuedomonas aeruginosa, Candida albican, Aspergillus niger* except *Aspergillus fumigatus*

In recent year seaweeds are wildly used in several applications such as antimicrobial (Chiheb *et al.,* 2011), antiviral (Bouhlal *et al.,* 2010, Bouhlal *et al.,* 2011; Kim and Karadeniz, 2011), antifungal (De Felicio *et al.*, 2010), anti-allergic (Na *et al.*, 2005), anti-coagulant (Dayong *et al.*, 2008), anti-cancer (Kim *et al.*, 2011), anti-fouling (Bhadury and Wright, 2004) and antioxidant activities (Devi *et al.*, 2011).

Conclusion

This study has been able prove the antimicrobial activities of *Oedogonium capillare* on some selected microorganisms and that methanolic extracts of the *Oedogonium capillare* had the highest zone of inhibition on the selected microorganisms followed by ethanolic extracts and diethyl ether extracts.

Competing Interests

Authors have declared that no competing interests exist.

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