Microbiology Research Journal International

23(3): 1-6, 2018; Article no.MRJI.39911
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Antimicrobial Studies on Leaf and Stem Extracts of *Solanum erianthum*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/MRJI/2018/39911
Editor(s):
(1) Marcin Lukaszewicz, University of Wroclaw, Poland.
Reviewers:
(1) Muhammad Imran, Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan.
(2) Milena Kalegari, Universidade Federal do Paraná, Brazil.
(3) Akpi, Uchenna Kalu, University of Nigeria, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/24017

Original Research Article

Received 17th January 2018
Accepted 24th March 2018
Published 6th April 2018

ABSTRACT

Aims: The current study examines the leaves and stem extracts of *Solanum erianthum* for antimicrobial activities.
Place and Duration of Study: Department of Chemistry, Federal University of Technology Akure between September 2014 and July 2015.
Methodology: The extracts were screened for activity against bacterial (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebisidlae pneumonae*) and fungal (*Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer*) organisms at concentrations between 6.25 and 200 mg/ml. Antimicrobial assays were carried out using agar diffusion method. The Minimum Inhibitory Concentration (MIC) of the extracts were determined.
Results: Of all the extracts tested, the hexane extracts of the leaves and stem of *S. erianthum* had the highest activities against all the test organisms. The MIC values of both hexane extracts ranged between 1.25 mg/ml and 5.00 mg/ml.
Conclusion: This result corroborates the traditional usage of the plant for the treatment of microbial infections.

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Keywords: Antimicrobial; bacterial; fungal; Solanum erianthum.

1. INTRODUCTION

The emergence of resistant pathogens as a result of misuse of the existing antibiotics has necessitated the search for cheaper and more effective compounds to combat various diseases. Plants have been employed for ages in the treatment of various diseases. Medicinal plants are known to produce a variety of compounds to protect them against many pathogens. Researchers are increasingly turning their attention to herbal products as sources of lead compounds in search for better drugs against resistant microbe strains.

Solanum *erianthum* belongs to the family 'Solanaceae'. It is called '*ewuro-igbo*' in South-western Nigeria. Herbalists in this part of the country use the plant for the treatment of veneral diseases, leprosy, dermatological problems, wound healing, headache, leucorrhoea, and piles. Solanum *erianthum* leaf volatile oil demonstrated potent inhibitory activity against Hs 578T and PC-3 human breast and prostate tumor cells respectively. In addition, the Solanum essential oils exhibited significant antimicrobial activity [1]. The compounds (−)-Solavetivone, Solanerianones A and B, (+)-anhydro-β-rotunol, solafuranone, lycifuranone A, N-trans-feruloyltyramine, palmitic acid, acetovanillone, β-sitosterol and stigmasterol have been isolated from the hexane fraction of the root of the plant. (−)-Solavetivone possesses fungitoxic, antimicrobial and weak cytotoxic activities [2].

In a previous study [3] the in vitro antioxidant and anti-inflammatory activities of S. *erianthum* leaf extracts were determined. In the current study, the antibacterial and antifungal activities of the leaf and stem extracts of the plant are reported.

2. METHODOLOGY

2.1 Plant Sample Collection

Solanum *erianthum* leaves were collected from an uncompleted building in Osogbo, Osun State Nigeria. Identification and deposition of voucher specimens were carried out at the Herbarium of the University of Ibadan, Nigeria.

2.2 Sample Preparation and Extraction

The samples were ground after drying under mild sunlight for several days. A 1kg portion of ground sample was subjected to successive extraction using hexane, ethylacetate and methanol. The extracts were thereafter concentrated.

2.3 Inoculum Preparation

A loop full of each organism was taken from the stock and inoculated into a sterile nutrient broth of 5mls. This was followed by incubation for 18-24hrs at 37°C. From overnight culture, 0.1 ml of each organism was taken and put into 9.9 mls of sterile distilled water to get 1:100 (10^{-2}) of the dilution of the organism.

2.4 Antimicrobial Tests

This was carried out as described by [4]. Bacterial (*Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi* and *Klebisidlae pneumonae*) and fungal (*Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer*) pathogens were employed in the assay. The pour plate and surface plate methods were employed for the antibacterial and antifungal assays respectively. The extracts were assayed against the test organisms at concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. Serial dilutions of the extracts were carried out in order to arrive at these concentrations.

2.4.1 Antibacterial assay (agar diffusion-pour plate method)

From the diluted organism (10^{-2}), 0.2 ml was taken into the prepared sterile nutrient agar which was at 45°C. This was then poured asceptically into sterile petri dishes and allowed to solidify for about 45-60 minutes. Using a sterile cork borer of 8 mm diameter, wells were made according to the number of graded concentration (6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) of the sample. In each well, the different concentrations of the sample were introduced. This was done in duplicates. The plates were allowed to stay on the bench for 2hrs to allow pre-diffusion. The plates were incubated uprightly in the incubator for 18-24 hrs at 37°C. The standard drug, Gentamicin was used as the control.

2.4.2 Antifungal assay (agar diffusion-surface plate method)

A sterile Sabouraud Dextrose Agar (62 g/l) was prepared and asceptically poured into sterile plates in duplicates and allowed to set properly. The diluted organism (0.2 ml of the 10^{-2}) was used to cover all the surface of the agar using sterile spreader. Wells were made on the plates using a sterile cork borer of the 8mm diameter. In each well, the graded concentrations of the extract and control were introduced. The plates were left on the bench for 20 minutes so as to allow extracts to diffuse properly into the agar. The plates were incubated uprightly in the incubator for 48 hrs at 26-28°C. The standard drug, Tioconazole was used as the control.

2.4.3 Determination of minimum inhibitory concentration (MIC)

Graded concentrations (0.625 mg/ml, 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml, 10 mg/ml and 20 mg/ml) of the samples were prepared. Two ml of each concentration was added to 18 mls of nutrient agar at 45-50°C. These were mixed together and poured asceptically into the sterile plates. The plates were allowed to set. After this, the organisms were streaked on the plates at different concentrations in order to determine the minimum concentration that would inhibit/hinder the growth of the organisms. All the plates were incubated appropriately (bacterial plates at 37°C for 24 hours and fungal plates at 26°C-28°C for 48 hours). The plates were observed for the growth of the microorganisms after the incubation period [5-7].

3. RESULTS AND DISCUSSION

Multiple drug resistance in human pathogenic microorganism, coupled with the high costs and side effects of synthetic drugs and inefficient public access to medical and pharmaceutical care are some of the factors contributing to the central role of medicinal plants in health care systems [8]. In developed countries, about 80% of the populace use traditional medicines derived from plants. There is therefore a need for further studies on plants in order to have a better understanding of their pharmacological properties.

Furthermore, a significant revival of interest in natural products as a potential source for new medicines has been observed among academia as well as pharmaceutical companies. Several modern drugs in use (approximately 40%) have been developed from natural products. In addition, traditional medicine programs are being incorporated into the primary health care systems of Mexico, the People's Republic of China, Nigeria, and other developing countries [9-10]. The World Health Organization has advocated that countries should interact with traditional medicines with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origin [11]. Tables 1 and 2 show the results of the antimicrobial screening of the S. *erianthum* leaf and stem extracts. The hexane extracts (of the leaves and stem), showed the highest activity against all the organisms at all the test concentrations. All the extracts showed a dose-dependent activity (with antimicrobial activity increasing with the concentration of the extracts). The ethylacetate extract of the leaves of S. *erianthum*, showed no activity against Rhizopus stolonifer at all the test concentrations. The ethylacetate and methanol extracts of the stem and leaf extracts generally showed poor activity against all the test organisms at low concentrations. None of the extracts showed activity as high as the standard drugs at all the concentrations tested.

The MIC values for the organisms are shown on Table 3. In determining the MIC of the extracts, their concentrations were scaled down ten times and the least concentration at which the extract inhibits the growth of the microorganisms determined. The hexane extracts of the leaves and stem demonstrated a broad range of antibacterial and antifungal properties against the test organisms. The hexane extracts of the leaves and stem of S. *erianthum* showed the highest activity against S. aureus (with MIC values as low as 1.25 mg/ml). S. aureus can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections. Furthermore, a MIC value of 2.50 mg/ml was observed against all the other bacterial and fungal organisms. This implies that the hexane extracts possess broad-based antimicrobial activities. For example, the leaves and stem extracts of this plant could also show some effectiveness in the treatment of typhoid fever, a disease spread through contaminated food and water. *P. notatum* is widely distributed in the environment can be an allergen, produce skin reactivity, and colonize the airways of patients with respiratory allergies. It is a thermotolerant fungus and has been described as a human pathogen despite its low

pathogenicity. There are reported cases of skin infections, esophagitis, keratitis, endophthalmitis, pneumonia, endocarditis, central nervous system infections, and even rare cases of disseminated infection in immunocompromised patients [12,13,14]. The ethylacetate and methanol extracts of the stem and leaves of S. *erianthum* generally showed far weaker antimicrobial activities with MIC values between 10 mg/ml and 20 mg/ml.

Interestingly, the hexane extracts showed high activity against gram-negative bacteria. Gram-negative organisms have been reported to be less susceptible to the action of antibacterials since they possess an outer membrane surrounding the cell wall which restricts diffusion of hydrophobic compounds through their lipopolysaccharide covering [15]. Phytochemical investigations indicated that the hexane extracts contain saponins, flavonoids, alkaloids, glycosides, steroids and terpenoids [3]. Saponins, naturally occurring plant compounds are known for their biological and pharmacological activity. This activity is strongly related to the amphiphilic character of saponins that allows them to aggregate in aqueous solution and interact with membrane components [16].The presence of saponins in the hexane extract could be partly responsible for their activities against gram-negative bacteria. Phytoconstituents are responsible for a broad range of pharmacological activities displayed by plants. The presence of a broad range of phytochemicals in the plant extracts under study could be responsible for the antimicrobial activities displayed by this plant. Further studies aimed at isolating and characterizing the compounds responsible for the high antimicrobial properties exhibited by the hexane extract should be carried out.

Table 1. *In vitro* antimicrobial activity of the S. *erianthum* leaves against the test organisms

Extracts	Microorganism/zone of inhibition (mm)										
	Conc. (mg/ml)	SA	EC	BS	PSA	ST	KP	CA	AN	PN	RS
SELHE	200	28	26	26	24	20	24	22	20	20	18
	100	24	22	22	22	18	20	20	18	18	16
	50	20	18	20	20	16	18	18	14	16	14
	25	18	14	16	18	14	14	14	12	14	12
	12.5	14	12	14	12	12	12	12	10	12	10
	6.25	10	10	12	10	10	10	10	-	10	-
SELEE	200	16	16	16	14	16	14	16	14	14	-
	100	14	14	14	12	14	12	14	12	12	-
	50	12	12	12	10	10	10	12	10	10	-
	25	10	10	10	-	-	-	10	-	-	-
	12.5	-	-	-	-	-	-	-	-	-	-
	6.25	-	-	-	-	-	-	-	-	-	-
SELME	200	20	18	18	18	18	16	16	16	16	16
	100	18	16	14	16	16	14	14	14	14	14
	50	14	14	12	14	14	12	12	12	12	12
	25	12	12	10	12	12	10	10	10	10	10
	12.5	10	10	-	10	10	-	-	-	-	-
	6.25	-	-	-	-	-	-	-	-	-	-
Control	-ve	-	-	-	-	-	-	-	-	-	-
	GENT	40	40	40	38	40	40	NT	NT	NT	NT
	TIOC	NT	NT	NT	NT	NT	NT	26	28	28	28

SELHE-Hexane Extract of S. erianthum leaves; SELEE-Ethylacetate Extract of S. erianthum leaves; SELME-Methanol Extract S. erianthum leaves; GENT- Gentamicin (10mg/ml); TIOC- Tioconazole (70%); Microorganisms: SA- Staphylococcus aureus; EC- Escherichia coli; BS-Bacillus subtilis; PA- Pseudomonas aeruginosa; ST- Salmonella typhi; KP-Klebsiella pneumonia; CA-Candida albicans; AS-Aspergillusniger; PN-Penicilliumnotatum and RH-Rhizopusstolonifer; -=No zone of inhibition; NT= Not tested; - - No activity

Table 2. *In vitro* antimicrobial activity of *S. erianthum* stem extracts against test organisms

Extracts	Microorganism/zone of inhibition (mm)										
	Conc. (mg/ml)	SA	EC	BS	PSA	ST	KP	CA	AN	PN	RS
SESHE	200	28	26	24	26	26	26	20	22	20	20
	100	24	24	20	24	20	20	18	20	18	18
	50	20	20	18	20	18	18	14	18	16	14
	25	18	18	16	18	14	14	12	14	14	12
	12.5	14	14	12	14	12	12	10	12	12	10
	6.25	10	10	10	10	10	10	-	10	10	-
SESEE	200	16	16	16	14	14	14	14	14	16	12
	100	14	14	14	12	12	12	12	12	14	10
	50	12	12	12	10	10	10	10	10	12	-
	25	10	10	10	-	-	-	-	-	10	-
	12.5	-	-	-	-	-	-	-	-	-	-
	6.25	-	-	-	-	-	-	-	-	-	-
SESME	200	20	18	18	16	16	16	16	16	18	14
	100	18	14	16	14	14	14	14	14	14	12
	50	14	12	14	12	12	12	12	12	12	10
	25	12	10	12	10	10	10	10	10	10	-
	12.5	10	-	10	-	-	-	-	-	-	-
	6.25	-	-	-	-	-	-	-	-	-	-
CONTROL	-ve	-	-	-	-	-	-	-	-	-	-
	GENT	40	38	40	38	40	38				
	TIOC	NT	NT	NT	NT	NT	NT	28	26	26	26

SESHE-Hexane Extract of S. erianthum stem; SESEE-Ethylacetate Extract of S. erianthum stem; SESME-Methanol Extract S. erianthum stem; GENT- Gentamicin(10mg/ml); TIOC- Tioconazole (70%); Microorganisms: SA- Staphylococcus aureus; EC- Escherichia coli; BS-Bacillus subtilis; PA- Pseudomonas aeruginosa; ST-Salmonella typhi; KP-Klebsiella pneumonia; CA-Candida albicans; AS-Aspergillus niger; PN- Penicillium notatum and RH-Rhizopus stolonifer; -=No activity; NT= Not tested

Table 3. Minimum inhibitory concentration of *S. erianthum* extracts

Extracts	Minimum inhibitory concentration (mg/ml)									
	SA	EC	BS	PSA	ST	KP	CA	AN	PN	RS
SELHE	1.25^{a}	2.50^{a}	2.50^{a}	2.50^{a}	2.50^{a}	2.50^{a}	2.50^{a}	5.00^{a}	2.50^{a}	5.00^{a}
SELEE	10.00^{b}	10.00^{b}	10.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}
SELME	5.00^{c}	5.00^{c}	10.00^{b}	10.00^{c}	10.00^{c}	10.00^{c}	20.00^{b}	10.00^{c}	10.00^{c}	10.00^{c}
SESHE	1.25^{a}	1.25^{d}	2.50^{a}	2.50^{a}	2.50^{a}	2.50^{a}	5.00^{c}	2.50^{d}	2.50^{a}	5.00^{a}
SESEE	10.00^{b}	10.00^{b}	10.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}	20.00^{b}
SESME	10.00^{b}	10.00^{b}	10.00^{b}	10.00^{c}	10.00^{c}	10.00^{c}	10.00^{d}	10.00^{c}	10.00^{c}	20.00^{b}

SELHE-Hexane Extract of S.erianthum leaves; SELEE-Ethylacetate Extract of S. erianthum leaves; SELME-Methanol Extract S. erianthum leaves; SESHE-Hexane Extract of S.erianthum stem; SESEE-Ethylacetate Extract of S.erianthum stem; SESME-Methanol Extract S. erianthum stem Microorganisms: SA- Staphylococcus aureus; EC- Escherichia coli; BS-Bacillus subtilis; PA- Pseudomonas aeruginosa; ST- Salmonella typhi; KP-Klebsiella pneumonia; CA-Candida albicans; AS-Aspergillus niger; PN-Penicillium notatum and RH-Rhizopus stolonifer; R-Resistant; Columns with different superscripts are significantly different at $P < 0.05$

4. CONCLUSION

The current study has justified the use of the plant in the treatment of microbial diseases by traditional medical practitioners.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24017