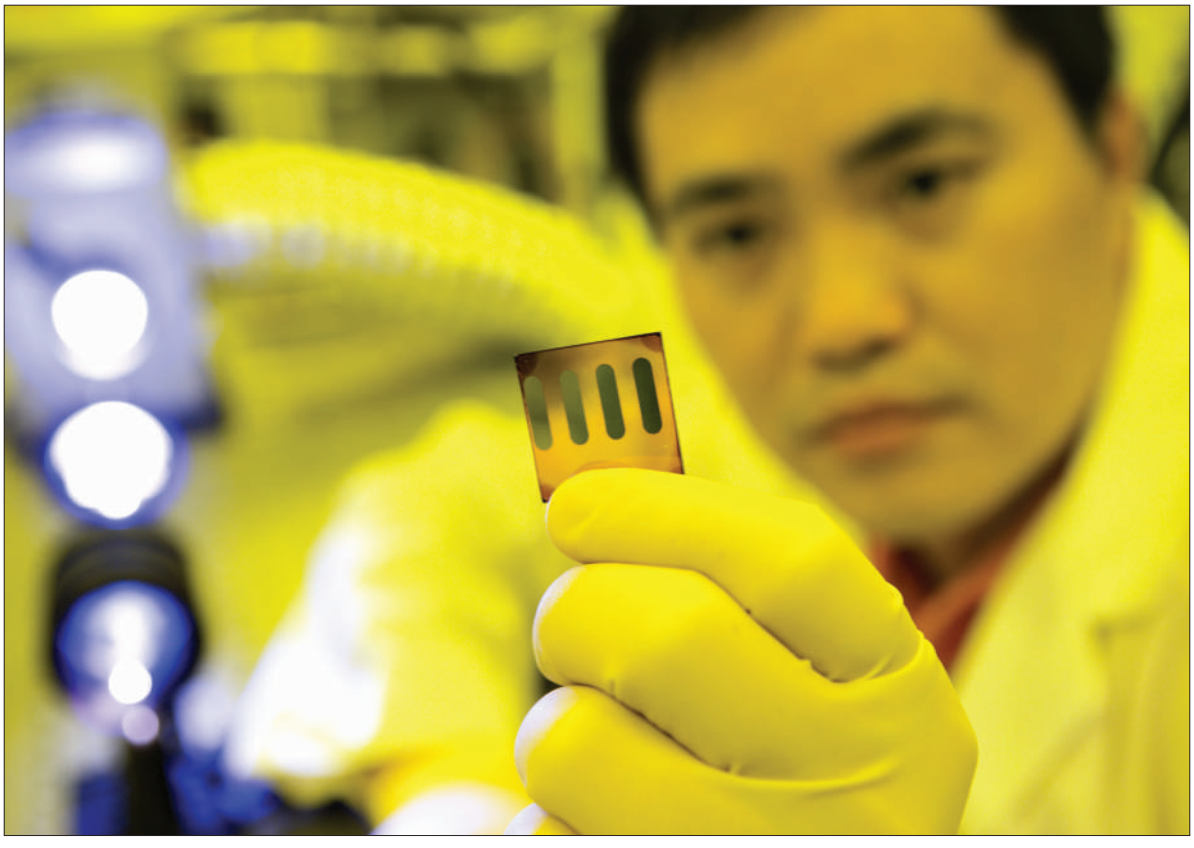

Singapore Journal of

Scientific Research

ISSN: 2010-006x

science alert

http://scialert.net/sjsr

OPEN ACCESS Singapore Journal of Scientific Research

ISSN 2010-006x
DOI: 10.3923/sjsres.2020.73.78

Research Article
Antimicrobial Efficacy of Some Herbs on Resistant Strains of *Pseudomonas* Species Isolated from West African Mud Creeper

I.O. Hakam, N.P. Akani and T. Sampson

Department of Microbiology, Rivers State University, PMB 5080, Nkpolu-Oroworukwo, Port Harcourt, Nigeria

Abstract

Background and Objective: Plant parts have been used to treat infections over the years. Some herbs were studied on resistant strains of *Pseudomonas* species isolated from *Tympanotonus fuscatus* to ascertain their antimicrobial efficacy using standard microbiological procedures of culturing, isolation and sensitivity testing with both antibiotics and methanolic herb extracts. **Materials and Methods:** Twelve *Pseudomonas* species isolates found to be most resistant to antibiotics were subjected to different concentrations of methanolic extracts of ginger (*Zingiber officinale*), garlic (*Allium sativum*), turmeric (*Curcuma longa*), bitter cola (*Garcinia kola*) seed, bitter cola(*G. kola*) seed/bark and all the herbs in a mixture. **Results:** Minimum inhibitory concentration (MIC) results showed no significant difference in the effect of the herbs at same concentrations ($p>0.05$) however, the mixed extract with mean zone of inhibition of 21.58 ± 5.20, 16.83 ± 6.71, 12.83 ± 6.83 and 10.17 ± 6.83 mm for the 10×10^4, 5×10^4, 2.5×10^4 and 1.25×10^4 µg mL^{-1} concentrations, respectively proved to be the most effective followed closely by bitter cola seed/bark, ginger, garlic, turmeric and bitter cola seed. Minimum bactericidal concentration (MBC) also showed mixed extracts proving bactericidal on 83.33, 58.33, 33.33 and 16.67% of isolates at 10×10^4, 5×10^4, 2.5×10^4 and 1.25×10^4 µg mL^{-1} concentrations, respectively. Correlation analysis of concentration of extract and efficacy revealed a strongly positive correlation as well as squared correlation coefficient (r^2) above 0.9 for all extracts. **Conclusion:** This study shows that methanol extracts the herbs used at high concentrations have some level of efficacy on antibiotic resistant strains of *Pseudomonas* species isolated from *T. fuscatus* hence, if properly standardized may prove to be a viable alternative for the treatment of food-mediated *Pseudomonas* infections.

Key words: Resistant strains, *Pseudomonas* species, medicinal herbs, antimicrobial efficacy, *Tympanotonus fuscatus*

Citation: I.O. Hakam, N.P. Akani and T. Sampson, 2020. Antimicrobial efficacy of some herbs on resistant strains of *Pseudomonas* species isolated from West African Mud Creeper. Singapore J. Sci. Res., 10: 73-78.

Corresponding Author: N.P. Akani, Department of Microbiology, Rivers State University, PMB 5080, Nkpolu-Oroworukwo, Port Harcourt, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

For thousands of years, humans have used natural products from plants either in pure forms or crude extracts for the treatment of several diseases[1]. Plants from indigenous or exotic origin have been used as an essential part of human diet as they improve taste, colour and aroma of foods[2,3]. In addition, some of these plants act as preservatives in many foods while some also have antioxidant[4] and antimicrobial properties[5].

The natural spices, garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are reported to have preservative properties[6,7] and also used as ingredients in making foods[8]. As indicated[9], natural preservatives such as spices can be appropriate alternatives to chemical preservatives used in various food industries minimizing their possible side effects and simultaneously improve the shelf life of food. Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are consumed as spices in food, while ginger is also consumed in drink form. The medicinal use of garlic has a long history, it is probably one of the earliest known medicinal plants[10]. Garlic (*Allium sativum*) is one of those plants that were seriously investigated over several years and used for centuries to fight infectious diseases[11]. Ginger (*Zingiber officinale* Roscoe) has been a part of healing strategies in Asia, India, Europe and the Middle East for centuries for treatment of such disorders as arthritis, stomach upset, asthma, diabetes and menstrual irregularities[12]. In West African countries, traditionalists and herbal medicine vendors have been observed to consistently incorporate ginger and garlic in most of their herbal preparations because these plants are believed to harbour inherent medicinal properties that cure several ailments[12]. *Garcinia kola* on the other hand is among the plants abundantly used in traditional medicine to cure diseases, this plant serves in traditional medicine for the treatment of gastroenteritis, rheumatism, asthma, menstrual cramps, malaria, throat infections, headache, colic, chest colds, cough, liver disorders, diarrhoea, bronchitis, cardiac diseases, as a poison antidote, for oral and dental hygiene[13].

Antimicrobial resistance in microorganisms has become a global public health concern in recent years[14,15]. This is chiefly because microbes in addition to the existing resistance abilities inherent within them have continued to devise new means through which they resist even a broader spectrum of agents[16]. *Pseudomonas* species happens to be one genus with great antimicrobial abilities and of very special concern is *Pseudomonas aeruginosa*, a species found to be major driver of human opportunistic infections in young children, aged adults and immunocompromised individuals[17].

This study was carried out to ascertain the efficacy of some medicinal herbs on resistant strains of *Pseudomonas* species isolated from edible parts of the West African Mud Creeper (*Tympanotonus fuscatus*).

MATERIALS AND METHODS

Study period: This study was carried out between February and August 2019.

Sample description and sample collection: Edible samples of *Tympanotonos fuscatus* (West African Mud Creeper) were collected from 3 different locations in Rivers State Nigeria, Mile 1 market in Port Harcourt City Local Government Area (4.7918°N, 6.9986°E), Rumueme Market in Obio/Akor Local Government Area (4.8273°N, 6.9820°E) and Mile 3 Market in Port Harcourt City Local Government Area (4.8042°N, 6.9924°E). Microbiological examination was carried out at the Microbiology laboratory, Rivers State University, Port Harcourt, Nigeria.

Isolation of bacteria: Stock analytical unit was prepared by weighing 10 g of edible (internal parts) of the parboiled and roasted *Tympanotonos fuscatus* samples, respectively and homogenizing in 90 mL of sterile normal saline. Ten fold serial dilution method was continued by pipetting 1 mL of the sample into 9 mL of sterile normal saline up to 6 dilutions (dilution factor from 10^{-1} to 10^{-6}). This was done for all samples collected[18].

Representative colonies were described and subcultured onto nutrient agar plates and incubated for 24 h at 37°C to obtain pure cultures. Pure cultures were stored in sterile 10% v/v glycerol for preservation and subsequently used for identification.

Identification of bacteria: This was done as described by Omokaro and Hakam[19]. The following tests were performed on each of the isolates to confirm their identity: Gram staining, sugar fermentation tests, oxidase test, catalase test, indole test, methyl red test, vogues proskauer test, citrate utilization test, haemolysis test, motility test, lecithinase test and starch hydrolysis test. Molecular identification using the 16s rRNA subunit of the DNA was also carried out to verify the identity of the isolates molecularly[20].

Collection and extraction of medicinal herbs: Selected medicinal herbs; garlic (*Allium sativum*), ginger (*Zingiber oficinale)*, turmeric (*Curcuma longa*) and bitter cola seed and bark (*Garcinia kola*) were purchased from Mile 1 market in

Port Harcourt metropolis, Rivers State, Nigeria. The samples were then transported to Rivers State University, Department of Microbiology Laboratory for preparation and subsequent extraction.

The method of extraction adopted for the study was methanol extraction as described by Jasamai *et al.*[21]. In this method, all the herbs (Garlic, ginger, turmeric and bitter kola and bark) were washed with clean water to remove sand particles and debris and subsequently sun dried. Dried herbs were then milled into coarse powder using a mechanical blender. Maceration was carried out by soaking 20 g of respective herbs in 100 mL of 99.5% methanol to give an approximate ratio of 1:5 for 3 days at room temperature with occasional shaking. Upon completion of extraction, filtration was carried out using filter paper, funnel and conical flask. The filtrate obtained was concentrated using a rotary evaporator at 40°C to yield methanol extracts of the herbs. The residue was macerated twice. The extracts were preserved in the refrigerator at 4°C throughout the period of study. These stock extracts were diluted to obtain various concentrations that were used in the study.

Statistical analysis: Statistical Package for Social Sciences (SPSS) version 22 and Microsoft Office Excel 2010 was used to analyse the data obtained from the measurement of the zones of inhibition as well as minimum bactericidal concentration (MBC) of medicinal herb extracts. Descriptive statistics was used to summarize all data obtained. Analysis of variance was carried out to test for significant difference in the minimum inhibitory concentration (MIC) obtained for different herbs at various concentrations.

RESULTS

Identity of resistant *Pseudomonas* Isolates: Twelve of the 32 isolates prior identified morphologically/biochemically and some, molecularly as shown in Table 1 were found to be resistant to 5 or more antibiotics and hence deemed resistant strains and of public health concern.

Minimum inhibitory concentration (MIC) of extracts: Table 2 shows a summary obtained from the results of minimum inhibitory concentration of the various extracts on each of the 12 isolates tested. It shows the concentrations of the herbs and their effects on the 12 isolates tested expressed by their mean zone of inhibition and standard deviation. It was observed that zones of inhibition increased with increasing concentration and vice versa with the mixed extract having the highest inhibition with 21.58±5.20, 16.83±6.71, 12.83±6.83 and 10.17±6.83 mm for the 10×10^4, 5×10^4, 2.5×10^4 and 1.25×10^4 μg mL^{-1} concentrations, respectively and bitter colas seed, the least with 16.00±9.11, 11.83±8.35, 7.00±7.79 and 4.00±6.44 for the 10×10^4 μg mL^{-1}, 5×10^4, 2.5×10^4 and 1.25×10^4 μg mL^{-1} concentrations, respectively.

Table 1: Identity of resistant *Pseudomonas* isolates

Isolate code	Morphological/biochemical Identity	Molecular identity/Accession number
1SBa	*P. aeruginosa*	
1SBb	*P. putida*	
2SAb	*P. aeruginosa*	
2SBb	*P. aeruginosa*	
2SBc	*P. aeruginosa*	
2SBd	*P. aeruginosa*	*P. aeruginosa* MK542822
3M1a	*P. aeruginosa*	
3M3a	*P. aeruginosa*	
4SCa	*P. aeruginosa*	
6SBa	*P. aeruginosa*	*P. aeruginosa* CP044006
6SAa	*P. fluorescens*	
7SAa	*P. aeruginosa*	

Table 2: Summary minimum inhibitory concentration of various herb extracts on *Pseudomonas* spp. isolates

	Concentration/zones of Inhibition (mm)			
Herb extracts	10×10^4 (μg mL^{-1})	5×10^4 (μg mL^{-1})	2.5×10^4 (μg mL^{-1})	1.25×10^4 (μg mL^{-1})
Garcinia kola seed	16.00±9.11	11.83±8.35	7.00±7.79	4.00±6.44
Garcinia kola seed and bark	18.33±7.52	15.33±6.39	11.92±6.42	8.42±6.93
Allium sativum	18.00±6.27	14.33±5.21	9.58±6.16	5.83±5.36
Zingiber officinale	18.17±7.66	13.17±6.81	8.75±5.71	4.83±5.29
Curcuma longa	17.08±11.48	12.83±10.39	8.83±8.30	7.25±6.88
Mixed	21.58±5.20	16.83±6.71	12.83±6.83	10.17±6.83

Values within represent the mean zones of inhibition in millimetres and standard deviation values summarized from raw data from minimum inhibitory concentration of each isolate

Table 3: Percentage bactericidal activity of different concentrations of extracts on *Pseudomonas* isolates

	Isolates (%)									
	10×10^4 (μg mL^{-1})		5×10^4 (μg mL^{-1})		2.5×10^4 (μg mL^{-1})		1.25×10^4 (μg mL^{-1})			
Herb extracts	NB	B	NB	B	NB	B	NB	B	Y	R^2
Zingiber officinale	41.67	58.33	66.67	33.33	100.00	0.00	100.00	0.00	0.1444x+4.4599	0.9468
Garcinia kola seed	50.00	50.00	83.33	16.67	83.33	16.67	91.67	8.33	0.1328x+3.4854	0.9423
Garcinia kola seed and bark	33.33	66.67	66.67	33.33	75.00	25.00	91.67	8.33	0.105x+8.5765	0.9001
Curcuma longa	41.67	58.33	58.33	41.67	83.33	16.67	91.67	8.33	0.1125x+6.2281	0.9775
Allium sativum	33.33	66.67	50.00	50.00	100.00	0.00	100.00	0.00	0.1315x+5.7713	0.9112
Mixed	16.67	83.33	41.67	58.33	66.67	33.33	83.33	16.67	0.1267x+9.4167	0.9706

NB: Non-bactericidal, B: Bactericidal

Bactericidal activity of extracts: Table 3 shows a unique trend for bactericidal activity of herbs. A very highly positive correlation between concentration and bactericidal effect of all the extracts used for the study was also observed. The percentage of *Pseudomonas* species isolates in which the various concentrations of medicinal herb extracts did not have bactericidal effect on as well as the percentage of *Pseudomonas* species in which the various concentrations of the medicinal herb extracts had bactericidal effect on is revealed on Table 3.

The mixed extract which had the highest bactericidal effect was bactericidal on 16.67, 33.33, 58.33 and 83.33% of the isolates at concentrations of 1.25×10^4, 2.5×10^4, 5×10^4 and 10×10^4 μg mL^{-1}, respectively. The medicinal herb extracts used in this study can be arranged in order of increasing bactericidal activity as bitter cola seed>ginger>turmeric> garlic>bitter cola seed/bark>mixed extract.

DISCUSSION

The current study reveals interesting results that proved the enormous ability of *Pseudomonas* species especially *P. aeruginosa* to resist some antimicrobial agents which were obtained after both antibiotic sensitivity testing and also upon subjection of isolates to methanolic extracts of ginger, garlic, turmeric, bitter cola seed and bark and a mixture of all herbs.

Although there was no significant difference in the zones of inhibition encountered from the various herbs at the same concentrations ($p>0.05$), some herbs proved slightly more effective on *Pseudomonas* species isolates than the others. Larger zones of inhibition were observed when the herbs were used in combination (mixed) to test *Pseudomonas* species isolates.

Results from Table 2 showed that ginger was slightly more inhibitory on *Pseudomonas* species than garlic. Similar study by Gull *et al.*[22] revealed that methanol extracts of ginger were slightly more effective than those of garlic even though differences were not statistically significant. Also, research by Karuppiah and Rajaram[4] using garlic and ginger extracts showed that ethanol extracts of both herbs were quite effective against *Pseudomonas* as well as other bacteria tested.

Studies by other researchers have suggested some phytochemicals responsible for the antimicrobial activities of some of these herbs. Guo *et al.*[23] and Fujisawa *et al.*[24] reveals allicin which is usually decomposed into more stable compounds in the presence of heat and time as the main biochemically active component in garlic and largely responsible for its antibacterial activity. Also, Lucy *et al.*[25] showed that bitter cola (*Garcinia kola*) seed extract has as active ingredients flavonoids, tannins and alkaloids but very minimal effect on *Pseudomonas aeruginosa* as also evidenced by the results from this study. Rahmani *et al.*[26] also notes gingerol and gingerol related compounds, paradol, shogaol, zingerone, zerumbone and ginger flavonoids as active ingredients of ginger that could be responsible for most of its antimicrobial activity.

CONCLUSION

Some *Pseudomonas* species isolated from the West African Mud Creeper (*Tympanotonus fuscatus*) have been found to be resistant to some conventional antibiotics previously used effectively against it and this poses a public health concern since *Pseudomonas aeruginosa* is a major driver of opportunistic infections. Resistant strains of *Pseudomonas* species from the current study have been shown to have varying sensitivity to methanol extracts of ginger, garlic, bitter cola seed, bitter cola bark, turmeric. Standardization of extracts to help manage food mediated *Pseudomonas* infections will help relieve the burden placed on antibiotic usage. However, caution must be taken to avoid

same problems of resistance currently been faced with so many antibiotics. Herbs must be used at standard and efficient concentrations to ensure that microbes do not get poorly exposed to and become resistant to the active components of these herbs.

SIGNIFICANCE STATEMENT

This study discovers the varying efficacy of methanolic extracts of herbs that can be beneficial for the management of food mediated *Pseudomonas* infections. This study will help the researcher to uncover the medicinal importance of bitter cola bark that many researchers were not able to explore.

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