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Research Article

Antipseudomonal Activity of Aqueous and Methanolic Leaf Extracts of *Lactuca serriola* Linn. (Astereceae)

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ABSTRACT

The study examined the antipseudomonal activity of aqueous and methanolic leaf extracts of *Lactuca serriola* Linn. against ten (10) *Pseudomonas aeruginosa* clinical isolates obtained from patients seen at University of Maiduguri Teaching Hospital, Nigeria. The extraction was done by maceration method using distilled water and methanol (99%) while the antipseudomonal activity was assessed by agar well diffusion assay method using varying concentrations (20, 40, 80 and 160mg/ml) of the extracts. The isolates were resistant to at least six (6) antibacterial drugs with mean (range) of 7.6 ± 1.1 (6.0 – 9.0) as against sensitivity to at least one (1) antibacterial drug with mean (range) of 2.3 ± 0.9 (1.0 – 3.0) [p = 0.0001]. The aqueous leaf extract was a dark-brown powder with percentage yield of 9.9% w/w (19.8g/200g) while the methanolic leaf extract was a greenish, sticky, oily paste with percentage yield of 6.1% w/w (12.2g/200g). Both aqueous and methanolic leaf extracts demonstrated dose-dependent antipseudomonal activity comparable to that of ciprofloxacin (p = 0.213). This finding could be an indication that the plant extract could be potential source of antipseudomonal drug(s). This may provide additional support for use of the plant in traditional medicine and could contribute to effective treatment of pseudomonal infections.

Keywords: *Lactuca serriola* Linn., *Pseudomonas aeruginosa*, Antibacterial effect, Extract.

INTRODUCTION

Traditional medicine, a vital component of healthcare system in sub-Saharan Africa especially among the rural populace, depends largely on plants and plant products for treatment of various illnesses[1]. *Lactuca serriola* Linn. (Synonym: *Lactuca scarioala* Linn.), a member of the family Astereceae, is one of such plants used in traditional medicine. It is commonly called prickly lettuce, wild lettuce or milk thistle[2] and has various local names among Nigerians. It is native to Europe, Asia, and Africa including Nigeria. The leaves are oblong or lanceolate, pinnated with fine spines along the veins and edges. They get progressively smaller as they reach the top of the plant and measure 3.5 – 25cm long by 1 – 20cm wide[3,4]. The young leaves are eaten raw as salad or cooked, although it has somewhat bitter taste[5].

L. serriola Linn. is often used traditionally to treat conditions such as cough, bronchitis, asthma, pertussis and gastrointestinal disorders[6]. Studies have shown some of its pharmacological activities to

include: sedative-hypnotic, antipyretic, antibacterial, analgesic, anti-inflammatory, antioxidant and smooth muscle activities[7-10]. These activities have been attributed to presence of certain phytoconstituents e.g. lactucin, lactucone, lactucin, triterpenoid saponin, phenols, vitamins, beta carotene, iron and sesquiterpene esters[7,9,11-13].

Pseudomonas aeruginosa, an aerobic non-fermenting Gram negative bacillus of Pseudomonadaceace family, is a major cause of nosocomial infections especially among the immuno-compromised individuals[14,15]. It has a large and complex genome[16], which probably enables it to thrive in diverse environments and infects various body tissues[17]. It is implicated as opportunistic organism in several conditions such as cancer[18], nosocomial pneumonia[19], cystic fibrosis[15], urinary tract infections[20], AIDS and severe burns[21]. In addition, it is notable for its intrinsic and acquired resistance to number of commonly used antibacterial drugs. The intrinsic resistance is attributed to the constitutive expression of β-lactamases, efflux pumps and outer membrane permeability[22]. The multi-drug resistant nature of the bacteria markedly limits the therapeutic options, hence, the need to seek for alternative means for treatment of pseudomonal infections. This study examined the antibacterial activity of aqueous and methanolic leaf extracts of *L. serriola* Linn. against multi-drug resistant (MDR) *P. aeruginosa* clinical isolates.

MATERIALS AND METHODS

Isolation of *P. aeruginosa* Clinical Isolates

Ten (10) presumptive clinical isolates of *P. aeruginosa* were obtained from the samples analyzed at Medical Microbiology Unit, University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria between February and May, 2015. The isolates were identified based on culture morphology, Gram staining and biochemical tests[14,22]. They were subjected to antibacterial sensitivity test as described by Cheesbrough[23]. All isolates were labeled, sub-cultured, stored at 4^{o}C and were used within 48 hours of collection. Multi-drug resistance was defined as resistance to at least three antibacterial drugs of different chemical class. The clinical detail of the patients from whom samples were collected was captured with a case record form. The ethical approval was obtained from Research Ethics Committee, UMTH prior to commencement of the study.

Collection and Authentication of the Plant

One whole plant of *L. serriola* Linn. was collected at University of Maiduguri, Nigeria in February, 2015 and was authenticated by Professor B. H. Kabura of Department of Crop Production, Faculty of Agriculture, University of Maiduguri, Nigeria. Following the authentication, fresh leaves were collected. A voucher specimen (Voucher number 008) of the plant was deposited at the Pharmacology Laboratory, Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri.

Preparation of the Leaf Extracts

The leaf extracts were prepared by maceration method as described by Bandar and others[24]. Briefly, the leaves were washed thoroughly with distilled water and air-dried under shade for approximately one week. The dried leaves were ground using electrical blender. For the aqueous and methanolic extracts, 200g each of the powder was soaked in 1000ml each of distilled water and absolute methanol (99%), respectively in two separate 2-liter sterile conical flasks. The solutions were allowed to stay at ambient temperature (27 ± 4^{o}C) for 24 hours with periodic shaking of the flasks to ensure proper dissolution. Then, the solutions were filtered using Whatman no 1 filter paper and the filtrates subjected to evaporation under laminar flow cabinet. The percentage yield was determined for each extract. Stock concentration (320mg/ml) was prepared for each extract using distilled water, the working concentrations (20, 40, 80 and 160mg/ml) were then prepared from the stocks.

Assessment of Antipseudomonal Activity of the Leaf Extracts

The stored isolates were sub-cultured in broth culture over-night and standardized inoculum of each isolate was prepared in normal saline to match 0.5 McFarland turbidity standard corresponding to approximately 1 x 10^{6} cells/ml[25]. Antipseudomonal activity was assessed by agar well diffusion assay[26]. Briefly, 1ml of the inoculum was aseptically mixed with 30ml of molten Mueller-Hilton agar in a sterile petri dish. The agar was allowed to dry and 5 wells were created on the agar with the aid of 6mm cork borer. Varying concentrations (20, 40, 80 and 160mg/ml) of the leaf extract were administered into the wells and distilled water was administered into the 5th well that served as negative control. The plates were left in laminar flow cabinet for 1 hour and then incubated at 37^{o}C for 24 hours. After incubation, the zone of inhibition around each well was carefully measured along two axis and the average of the two readings recorded. Each of the isolate was done in triplicate. The isolates were also tested against ciprofloxacin which served as positive control.

Data analysis

The data generated from the study were analyzed using statistical software, Statistical Package for Social Sciences version 21[27]. The mean zone of inhibition was compared using analysis of variance while proportion was compared using Chi-square. Significance was inferred at $p \leq 0.05$.

RESULTS

Antibiogram of the *P. aeruginosa* Clinical Isolates

Six (6) of the isolates were obtained from wound swab (P1, P2, P3, P6, P8 and P9); 2 isolates (P4 and P5) from urine sample, 1 isolate (P7) from ear swab and 1 isolate (P10) from catheter tip (P10). The isolates were resistant to at least six (6) antibacterial drugs with mean (range) of 7.6 ± 1.1 (6.0 – 9.0) as against sensitivity to at least one (1) antibacterial drug with mean (range) of 2.3 ± 0.9 (1.0 – 3.0) [p = 0.0001]. The isolates P3 and P7 had least sensitivity with resistance to nine (9) drugs each. Ciprofloxacin had the highest sensitivity of 70.0% (7/10) [p = 0.009]. None of the ten (10) isolates was sensitive to amoxicillin, cefuroxime and streptomycin (Table 1).

Leaf Extracts

The aqueous leaf extract was a dark-brown powder with percentage yield of 9.9% w/w (19.8g/200g) while the methanolic leaf extract was a greenish, sticky, oily paste with percentage yield of 6.1% w/w (12.2g/200g) [Table 2].

Antipseudomonal Activity of the Leaf Extracts

Tables 3 and 4 present the zones of inhibition indicating the antipseudomonal activity of the aqueous and methanolic leaf extracts of *L. serriola* Linn. respectively. Both aqueous and methanolic extracts demonstrated significant dose-dependent antipseudomonal activity against all the ten (10) isolate tested. The means zone of inhibition of aqueous extract (16.8 ± 3.1mm) and methanolic extract (19.3 ± 3.0mm) at 160mg/ml were similar to that of ciprofloxacin (30µg) with 19.7 ± 3.7mm (p = 0.213).

DISCUSSION

Infectious diseases remain major public health concern especially in developing countries[28]. *P. aeruginosa* is one of the common bacterial causes of infectious diseases. Owning to the morbidity of *P. aeruginosa* infections and the multi-drug resistance nature[14], alternative therapies are often considered[29]. In the present study, the antipseudomonal activity of the aqueous and methanolic extracts of *L. serriola* Linn. was demonstrated against ten (10) MDR *P. aeruginosa* clinical isolates. Some of the pharmacological activities of the plant have been previously reported[10,30]. However, to the best of our knowledge, this study is the first of her kind as it revealed the potential antibacterial activity of the plant extract against MDR isolates.

The antibiogram of *P. aeruginosa* clinical isolates reported in the present study indicated that most of the isolates were MDR. This is in accordance with the existing fact that the microbe is known for its resistance to wide range of commonly used antibacterial drugs[22,31]. In addition, the highest sensitivity of 70% recorded for ciprofloxacin is in line with known antibacterial activity spectrum of the fluoroquinolones[32].

Evaluation of antipseudomonal activity of the plant showed that the aqueous and methanolic leaf extracts have significant activity against all the ten (10) isolates. This finding is in contrast to the finding by Al-Marzoqi and others[30] who reported that the plant extract had antibacterial activity against only *Staphylococcus spp*. and not against *Pseudomonas spp*. This discordance could be attributed to the phytochemical constituents of the extracts. While Al-Marzoqi and others[30] used only phenolic, alkaloid and terpenoid compounds from the plant extracts, the whole extractable compounds were used in the present study. This could be an indication that phytochemicals other than the phenolic, alkaloid and terpenoid compounds may be responsible for the antipseudomonal activity. The phytochemical analysis of the plant was not done in the present study. However, previous studies have shown that *L. serriola* Linn. contains alkaloids, flavonoids, saponins, glycosides, oxalic acid, lactucin, lactucopicrin and sesquiterpene esters[2,33,34], some of which have been reported to have antibacterial activity[7]. The activity of *L.serriola* Linn. observed in the present study can be likened to that of other medicinal plants such as *Tabebuia impetigonosa, Maytenus macrocarpa* and green tea, that have exhibited inhibitory activity against MDR *P. aeruginosa*[29,35].

CONCLUSION

The present study showed that the aqueous and methanolic leaf extracts of *L. serriola* Linn. demonstrated a significant dose-dependent antipseudomonal activity against MDR *P. aeruginosa* clinical isolates. This could be an indication that the plant extract could be potential source of antipseudomonal drug(s). These findings may provide a support to use of the plant in traditional medicine and could contribute to effective treatment of pseudomonal infections.

Table 1
Antibiogram of the *P. aeruginosa* clinical isolates

Drugs	*P. aeruginosa* Clinical Isolates										Number S:R
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
Amoxicillin	R	R	-	R	R	R	-	-	-	-	**0:5**
Ceftriaxone	R	-	S	R	R	-	S	-	-	-	**2:3**
Cefuroxime	R	R	R	R	-	R	R	-	-	R	**0:6**
Chloramphenicol	-	R	R	-	R	R	R	R	R	R	**0:8**
Ciprofloxacin	S	S	R	S	S	S	R	R	S	S	**7:3**
Cotrimoxazole	S	S	R	R	R	S	R	R	R	R	**3:7**
Gentamycin	S	R	R	S	R	S	R	R	R	R	**3:7**
Levofloxacin	-	-	-	-	-	-	-	S	S	-	**2:0**
Ofloxacin	R	S	R	R	S	R	R	R	R	R	**2:8**
Pefloxacin	R	R	R	R	R	R	R	S	S	R	**2:8**
Sparfloxacin	-	R	R	S	R	R	R	R	R	R	**1:8**
Streptomycin	R	R	R	R	S	R	R	R	R	R	**1:9**
Number S:R	**3:6**	**3:7**	**1:9**	**3:7**	**2:8**	**3:7**	**1:9**	**2:7**	**3:6**	**1:8**	**-**

R Resistant
S Sensitive
P1 – P10 *P. aeruginosa* clinical isolates

Table 2
Description of the aqueous and methanolic leaf extracts of *L. serriola* Linn.

Extracts	Colour	Texture	Percentage Yield (% w/w)
Aqueous	Dark-brown	Powdery	9.9 (19.8/200g)
Methanolic	Green	Sticky, oily, and pasty	6.1 (12.2/200g)

Table 3
Antipseudomonal activity of the aqueous leaf extract of *L. serriola* Linn.

Extract Concentration (mg/ml)	Zone of Inhibition (mm)										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Mean ± SD
20	10	13	12	12	15	15	17	16	15	15	14.0 ± 2.2
40	11	13	13	14	16	16	19	17	17	16	15.2 ± 2.4
80	12	14	14	15	16	22	16	17	19	19	16.4 ± 3.0
160	12	16	14	15	17	23	17	15	20	19	16.8 ± 3.1

P1 – P10 *Pseudomonas aeruginosa* clinical isolates
SD Standard deviation
Mean zone of inhibition of ciprofloxacin (30µg) = 19.7 ± 3.7mm

Table 4
Antipseudomonal activity of the methanolic leaf extract of *L. serriola* Linn.

Extract Concentration (mg/ml)	Zone of Inhibition (mm)										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Mean ± SD
20	11	10	0	18	14	18	17	13	15	16	14.7 ± 2.9
40	13	11	0	18	18	21	18	15	15	16	16.1 ± 3.0
80	14	12	20	20	19	24	19	18	16	17	17.9 ± 3.4
160	14	19	20	20	20	26	20	19	17	18	19.3 ± 3.0

P1 – P10 *Pseudomonas aeruginosa* clinical isolates
SD Standard deviation
Mean zone of inhibition of ciprofloxacin (30µg) = 19.7 ± 3.7mm

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