Advances in Bioscience and Bioengineering
2019; 7(2): 22-26
http://www.sciencepublishinggroup.com/j/abb
doi: 10.11648/j.abb.20190702.12
ISSN: 2330-4154 (Print); ISSN: 2330-4162 (Online)

Anti-bacterial Activity of *Tithonia diversifolia*, *Secamore afzelli* and *Jaundea pinnata* Against Plasmid-bearing Multiple Antibiotics Resistant Bacteria from Different Water Sources in Akure, Nigeria

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To cite this article:
Alaofin Sefunmi, Onifade Anthony Kayode. **A**nti-bacterial Activity of *Tithonia diversifolia*, *Secamore afzelli* and *Jaundea pinnata* Against Plasmid-bearing Multiple Antibiotics Resistant Bacteria from Different Water Sources in Akure, Nigeria. *Advances in Bioscience and Bioengineering*. Vol. 7, No. 2, 2019, pp. 22-26. doi: 10.11648/j.abb.20190702.12

Received: June 6, 2019; **Accepted**: July 10, 2019; **Published**: July 26, 2019

Abstract: This study aims at assessing the antibacterial efficacies of some plant leaves. In this study, the antibacterial efficacies of *Tithonia diversifolia* Harm, *Secamore afzelli* Linn, and *Jaundea pinnata* Linn were assayed against plasmid-bearing multiple antibiotics resistant Gram-negative bacteria isolated from different water sources in Akure local government, Nigeria. The extracts of the plant leaves were prepared using cold water, hot water, Petroleum ether and Ethanolic solvents. The plant leaves of *Tithonia diversifolia*, *Secamore afzelli* and *Jaundea pinnata* were air-dried and pulverized using an electric blender which were soaked in 1 liter of solvent each for 72 hours after which it was sieved using muslin cloth and filtered using Whatman No 1 filter paper. Filtrates collected in beaker were concentrated in vacuousing rotary evaporator. The extracts were then reconstituted in tween 20 and sterilized with the aid of Millipore membrane filter. Agar well diffusion technique was done by using 1 ml aliquot of 18 hours broth culture that had been adjusted to the 0.5 McFarland standards which was dispensed into sterile Petri dishes and molten sterile Muller-Hinton agar was aseptically poured into the plates and incubated at 37°C for 24 hours. Clear zones around the wells were measured in millilitres. The minimum inhibitory concentration of extracts that showed antimicrobial activity were reconstituted by diluting 0.5 g of each in 10 ml of Tween 20 and then sterilized by passing through sterile Millipore membrane filter (0.45 µl). Different concentration of the extracts (50, 25, 12.5, 6.25, 3.125 mg/ml) was used. From this research work, it was observed that the highest plant extract yield was seen in the ethanolic extracts of *Tithonia diversifolia*, *Secamore afzelli* and cold water extracts of *Jaundea pinnata* having 17.8%, 8.6% and 15.7% respectively while the lowest yield was observed in petroleum ether extracts of *Secamore afzelli*. From the antibacterial analysis, it was observed that Hot water extract of *J. pinnata* inhibited *Acinetobacter baumanni*, *Salmonella typhimurum* while the cold water extracts of *T. diversifolia* inhibited *Enterobacter aerogenes* and *Shigella dysenteriae*.

Keywords: Antibacterial Efficacies, Plasmid-bearing, Multiple Antibiotics Resistant Bacteria

1. Introduction

Scientists have carried out many studies on the determination of antimicrobial properties of many natural products; however, continued research on this area is still promising because of phenomena like spread of multiple drug resistance and emergence of new diseases [1]. Before the early 20th century, treatments for infections were based primarily on medicinal folklore and some studies described herbal mixtures with antimicrobial properties that were used in treatments of infections over 2000 years ago [2]. Medicinal plants constitute an effective source of natural products antimicrobials. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into Africa continent [3]. Plants have been used in traditional medicine for many centuries as arbotifacients, contraceptives, for menstrual regulation, fertility control, as well for the treatment of ailments of both microbial and non-microbial origins [3]. The Nigeria flora is

rich in medicinal plants that are exploited by herbal doctors; precisely, the indigenous population of Southwestern, Nigeria for example has developed a vast knowledge on the use of plants as traditional remedies [3]. Some of the plants collections are used against a variety of diseases such as typhoid fever, gastroenteritis, dysentery, malaria and others which are typical diseases of tropical countries [3]. The medicinal power of these plants lies in their phytochemical constituents that cause definite pharmacological actions on the human body [4]. Phytochemical, natural compound occur in plants such as medicinal plants, vegetables and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases [4]. This work aims to investigate the antibacterial efficacy of *Tithonia diversifolia*, *Secamore afzelli* and *Jaundea pinnata* against plasmid-bearing multiple resistant bacteria.

2. Materials and Methods

2.1. Collection and Identification of Plant Samples

Fresh leaves of *Tithonia diversifolia Harms* (Mexican Sunflower), *Secamore afzelli Linn* (American plane tree), and *Jaundea pinnata Linn* ("Erebe") were collected from Akure, Nigeria.

2.2. Preparation of Plant Extracts

The leaves of *T. diversifolia*, *S. afzelli* and *J. pinnata* were prepared into extracts as described by [1] with slight modifications. The leaves were air-dried for three weeks and pulverized using an electric blender. The solvents used for the extraction were ethanol, petroleum ether, cold water and hot water. A 100 g portion of the powdered sample was soaked in 1 litre of each solvent. Each solution was allowed to stand for 72 hours, after which it was sieved with a muslin cloth and filtered using Whatman No 1 filter paper. The filtrate was collected in a beaker and concentrated in vacuousing rotary evaporator. The extracts were then reconstituted in tween 20 (10% v/v) prior to use and sterilized with the aid of Millipore membrane filter (0.22 µm). The dry weights of the extracts were measured using weighing balance.

2.3. Antibacterial Susceptibility Testing of Plant Extracts

The agar well diffusion technique was done as described by [5]. A 1 ml aliquot of 18 hours broth culture that had been adjusted to the 0.5 McFarland standards was dispensed into sterile Petri dishes. Molten sterile Muller-Hinton agar was aseptically poured into the plates and the plates were gently rotated for the organisms to be homogenously distributed in the medium. The agar was allowed to solidify, after which a sterile cork borer of 6 mm in diameter was used to cut uniform wells in the agar plates. The wells were later filled with 0.5 ml of each extract. In addition, 20% Tween 20 was used as the negative control while Chloramphenicol served as the positive control. The experiment was conducted in triplicate. All the plates were incubated at 37^{o} C for 24 hours. Clear zones around the wells were measured in millilitres.

2.4. Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extracts

The minimum inhibitory concentration of extracts that showed antimicrobial activity were reconstituted by diluting 0.5 g of each in 10 ml of Tween 20 and then sterilized by passing through sterile Millipore membrane filter (0.45 µl). Different concentration of the extracts (50, 25, 12.5, 6.25, 3.125 mg/ml) was used. The reconstituted extracts were serially diluted in sterile broth culture and 0.1 ml of the 18 hours broth culture of each of the test organisms that have been adjusted to turbidity equivalent to 0.5 McFarland standard was introduced to each test tube containing the serial diluted extract and incubated for 24 hours at 37^{o}C. The tube with the least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration [1, 6, 7].

3. Result

The percentage recovery of the different leaf extracted using Organic solvent (Petroleum ether and Ethanol) and Aqueous solvent is as shown in Table 1. The ethanolic extract had the highest yield of 17.8%, 8.6% from *T. diversifolia, S. afzelli* respectively while the least recovery was obtained from petroleum ether extracts with 2.6%, 2.5% and 4.5% in *T. diversifolia, S. afzelli* and *J. pinnata*. The result of the antibacterial activities of the three plant extracts is as shown in Table 2, 3 and 4. Table 2 shows the antibacterial activities of the aqueous, petroleum ether, and ethanolic extract against plasmid-bearing multiple antibiotics bacteria. From this table, it was observed that the ethanolic extract of *Jaundea pinnata* was capable of inhibiting *Shigella dysenteri*, *K. pneumoniae* and *Salmonella typhi* while the petroleum extract of the leaf was capable of inhibiting *Salmonella*. *Typhimurum, V. cholerae* and the cold and hot water extracts was capable of inhibiting *Vibrio cholerae* and *A. baumanni* respectively. Table 3 show the antibacterial activity of *Tithonia diversifolia* against some plasmid-bearing bacteria. From this table, it was observed that the ethanolic extracts of the leaf was capable of inhibiting *Shigella dysenteri*, *Salmonella paratyphi*, *K. pneumoniae* while the petroleum ether extract of the leaf was capable of inhibiting *Vibrio cholerae* and the cold water was capable of inhibiting *Salmonella typhimurum*, *E. aerogenes* and *Shigella dysenteri*. Table 4 shows the Antibacterial activity of *Secamore afzelli* against the plasmid-bearing multiple antibiotics bacteria. From this table, it was observed that the ethanolic extract of *Secamore afzelli* was capable of inhibiting *Shigella dysenteri*, *Klebsiella pneumoniae* and *S. typhi* while the petroleum ether extract of the leaf was capable of inhibiting *S. typhimurum* and *Vibrio cholerae* and the cold water extract was capable of inhibiting *V. cholerae* while the hot water extract of the leaf was capable of inhibiting *A. baumanni* and *Salmonella typhimurum*. The minimum inhibitory concentration is as shown in Table 5. The inhibitory property of *T. diversifolia*

ranges from 6.25 to 25mg/ml for the hot water extract while it ranges from 3.125 to 12.5mg/ml for the cold water and petroleum ether extracts and it ranges from 3.125 to 25mg/ml for the ethanol extracts on the test bacteria. The inhibitory property of *S. afzelli* ranges from 3.125 to 25mg/ml of the hot water extract while it ranges from 12.5 to 25mg/ml for the cold water extracts and it ranges from 6.25 to 25mg/ml for the petroleum ether extracts and ranges from 3.125 to 12.5mg/ml for the ethanolic extracts. The inhibitory property of *J. pinnata* ranges from 3.125 to 25mg/ml for the hot water extracts while it was 6.25 to 12.5mg/ml for thecold water extracts and it is 3.125 to 12.5mg/ml for the petroleum ether extract and it ranges from 3.125 to 12.5mg/ml on the test isolates.

Table 1. *Percentage yield of T. diversifolia, S. afzelli and J. pinnata leaf extracts.*

Extracts	OW(g)	AE(g)	PR(%)
Tithonia diversifolia			
Hot water	100	10.5	10.5
Cold Water	100	13.6	13.6
Ethanol	100	17.8	17.8
Pet ether	100	2.6	2.6
Secamore afzelli			
Hot water	100	3.8	3.8
Cold Water	100	4.5	4.5
Ethanol	100	8.6	8.6
Pet ether	100	2.5	2.5
Jaundea pinnata			
Hot water	100	13.6	13.6
Cold water	100	15.7	15.7
Ethanol	100	11.2	11.2
Pet ether	100	4.5	4.5

Keys: OW-Original weight, AE- Amount extracted, PR-Percentage recovery, g-gram.

Table 2. *Antibacterial activities of Jaundea pinnata against some bacterial isolates.*

Ex	AV	CV	BI	BV	FV	FI
Et	0.0±0.00a	7.67±0.577^{b}	0.0±0.000a	0.0±0.000^{a}	0.0±0.000a	13.67±0.577^{d}
P.E	5.67±0.577b	15.33±1.155	0.0±0.00a	0.0±0.000^{a}	0.0±0.000^{a}	0.0±0.000^{a}
CW	0.0±0.000^{a}	0.0±0.000^{a}	4.67±0.577b	0.0±0.000^{a}	5.67±0.577^{b}	0.0±0.000^{a}
HW	19.67±0.577e	19.67±0.577^{c}	0.0±0.000^{a}	0.0±0.000^{a}	0.0±0.000^{a}	9.67±0.577^{c}
Co	24.67±0.577^{f}	27.67±0.577^{g}	29.67±0577^{g}	19.67±0.577^{e}	19.67±0.577^{e}	29.67±0.577^{g}

Table 2. *Continued.*

Ex	FS	FE	AS	ST	VC	PR
Et	9.67±0.577^{c}	12.67±0.577^{d}	0.0±0.00a	12.67±0.577^{c}	10.67±0.577^{c}	0.0±0.000^{a}
P.E	0.0±0.000^{a}	0.0±0.000^{a}	0.0±0.000^{a}	0.0±0.000^{a}	12.67±0.577^{c}	0.0±0.000^{a}
CW	10.67±0.577^{c}	5.67±0.577^{b}	0.0±0.000^{a}	0.0±0.000^{a}	14.67±0.577^{d}	0.0±0.000^{a}
HW	7.67±0.577^{b}	4.67±0.577^{b}	5.67±0.577^{b}	4.67±0.577^{b}	0.0±0.000^{a}	0.0±0.000^{a}
Co	25.67±0.577^{f}	27.67±0.577^{g}	14.67±0.577^{d}	21.67±0.577^{e}	21.67±0.577^{e}	14.67±0.577^{d}

Values are means±SD for 3 samples. Mean followed by the same letter(s) within the group along the same column are not significantly different at P≤0.05 using Duncan's New Multiple range test. KEYS: BV = *E. coli*, FV = *E. aerogenes*, FE= *K. pneumoniae*, FS= *S. paratyphi*, ST=*S typhi,* AS =*P. aeruginosa,*, CV=*S. typhimurum,* AV= *A. baumanni,* PR= *P. vulgaris,* FI =*S. dysenteriae*, BI= *S. marcescens*, VC= *V. cholerae,* PE=Petroleum ether, CW= Cold water, HW=Hot water, Co= Control, Et=Ethanol, Ex=Extracts.

Table 3. *Antibacterial activity of Tithonia diversifolia against some isolated bacteria.*

Ex	AV	CV	BI	BV	FV	FI
Et	9.67±0.577^{c}	9.67±0.577^{c}	0.0±0.000a	0.0±0.000^{a}	9.67±0.577^{c}	16.67±0.577^{d}
PE	0.0±0.000^{a}	10.67±0.577^{c}	10.67±0.577^{d}	0.0±0.000^{a}	10.67±0.577^{c}	0.0±0.000^{a}
CW	7.67±0.577^{b}	13.67±0.577^{d}	0.0±0.000^{a}	0.0±0.000^{a}	14.67±0.577^{d}	15.67±0.577^{d}
HW	0.0±0.000^{a}	9.67±0.577^{c}	0.0±0.000^{a}	1.67±0.577^{a}	0.0±0.000^{a}	0.0±0.000^{a}
Co	24.67±0.577^{f}	27.67±0.577^{g}	29.67±0577^{g}	19.67±0.577^{e}	19.67±0.577^{e}	29.67±0.577^{g}

Table 3. *Continued.*

Ex	FS	FE	AS	ST	VC	PR
Et	13.67±0.577^{d}	15.67±0.577^{d}	11.67±0.577^{c}	11.67±0.577^{c}	10.67±05777	0.0±0.000^{a}
PE	0.0±0.000^{a}	0.0±0.000^{a}	0.0±0.000^{a}	0.0±0.000^{a}	12.00±1.732	0.0±0.000^{a}
CW	11.67±0.577^{c}	11.67±0.577^{c}	9.67±0.577^{c}	7.67±0.577^{b}	7.67±0.577	0.0±0.000^{a}

Ex	FS	FE	AS	ST	VC	PR
HW	0.0±0.000[a]	0.0±0.000[a]	0.0±0.000[a]	9.67±0.577[c]	0.0±0.000[a]	0.0±0.000[a]
Co	25.67±0.577[f]	27.67±0.577[g]	14.67±0.577[d]	21.67±0.577[e]	1.67±0.577[e]	14.67±0.577[d]

Values are means±SD for 3 samples. Mean followed by the same letter(s) within the group along the same column are not significantly different at P≤0.05 using Duncan's New Multiple range test. LEGENDS: BV = *E. coli*, FV = *E. aerogenes*, FE= *K. pneumoniae*, FS= S. paratyphi, ST=*S typhi,* AS =*P. aeruginosa,*, CV=*S. typhimurum,* AV= *A. baumanni,* PR= *P. vulgaris,* FI =*S. dysenteriae*, BI= *S. marcescens*, VC= *V. cholerae,* PE=Petroleum ether, CW= Cold water, HW=Hot water, Co= Control, Et=Ethanol, Ex=Extracts.

Table 4. *Antibacterial activity of Secamore afzelli against some isolated bacteria.*

Ex	AV	CV	BI	BV	FV	FI
Et	0.0±0.00[a]	7.67±0.577[b]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	13.67±0.577[d]
PE	5.67±0.577[b]	15.33±1.155[d]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]
CW	0.0±0.00[a]	0.0±0.00[a]	4.67±0.577[a]	0.0±0.00[a]	5.67±0.577[b]	0.0±0.00a
HW	19.67±0.577[e]	19.67±0.577[e]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	9.67±0.577[c]
Co	24.67±0.577[f]	27.67±0.577[g]	29.67±0.577[g]	19.67±0.577[e]	19.67±0.577[e]	29.67±0.577[g]

Table 4. *Continued.*

Ex	FS	FE	AS	ST	VC	PR
Et	9.67±0.577[c]	12.67±0.577[c]	0.0±0.00[a]	12.67±0.577[c]	10.67±0.577[c]	0.0±0.00[a]
PE	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	12.00±1.732[d]	0.0±0.00[a]
CW	10.67±0.577[c]	5.67±0.577[b]	0.0±0.00[a]	0.0±0.00[a]	14.67±0.577[d]	0.0±0.00[a]
HW	7.67±0.577[b]	4.67±0.577[b]	5.67±0.577[b]	5.67±0.577[b]	0.0±0.00[a]	0.0±0.00[a]
Co	25.67±0.577[f]	27.67±0.577[g]	14.67±0.577[d]	21.67±0.577[e]	21.67±0.577[e]	14.67±0.577[d]

Values are means±SD for 3 samples. Mean followed by the same letter(s) within the group along the same column are not significantly different at P≤0.05 using Duncan's New Multiple range test. LEGENDS: BV = *E. coli*, FV = *E. aerogenes*, FE= *K. pneumoniae*, FS= S. paratyphi, ST=*S typhi,* AS =*P. aeruginosa,* C= *C. freundii*, CV=*S. typhimurum,* AV= *A. baumanni,* PR= *P. vulgaris,* FI =*S. dysenteriae*, BI= *S. marcescens*, VC= *V. cholera,* PE=Petroleum ether, CW= Cold water, HW=Hot water, Co= Control, Et= Ethanol, Ex=Extracts.

Table 5. *Minimum inhibitory concentration (mg/ml) of Jaundea pinnata, Secamore afzelli, Tithonia diversifolia.*

Isolates	*J. pinnata*				*S. afzelli*				*T. diversifolia*			
	Hot water	Cold Water	Pet. Ether	Ethanol	Hot water	Cold water	Pet. Ether	Ethanol	Hot water	Cold water	Pet. ether	Ethanol
A.baumanni	3.125	6.26	12.5	3.125	3.125	NI	6.25	NI	NI	12.5	NI	3.125
S. typhimurum	NI	6.25	12.5	6.25	6.25	NI	25	3.125	6.25	3.125	6.25	6.25
S. marcescens	NI	NI	NI	12.5	NI	12.5	NI	NI	NI	NI	6.25	NI
E.coli	NI	NI	NI	NI	NI	NI	NI	NI	25	NI	NI	NI
E. aerogenes	NI	NI	NI	6.25	NI	12.5	NI	6.25	NI	6.25	12.5	6.25
S. dysenteriaeae	12.5	6.25	NI	NI	6.25	NI	NI	6.25	NI	6.25	NI	25
V.cholerae	NI	6.25	6.25	12.5	NI	12.5	25	6.25	NI	6.25	3.125	12.5
P. vulgaris	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
S. paratyphi	25	6.25	NI	6.25	12.5	12.5	NI	12.5	NI	6.25	NI	6.25
K. pneumoniae	NI	12.5	NI	12.5	25.0	25.0	NI	6.25	NI	6.25	NI	12.5
P. aeruginosa	NI	NI	3.125	NI	3.125	NI	NI	NI	NI	3.125	NI	3.125
S. typhi	NI	12.5	NI	3.125	12.5	NI	NI	3.125	6.25	6.25	NI	6.25

4. Discussion

The different recovery percentage of plants extracts in the study may have resulted from various solvents used in extraction as reported by [8, 9] that different solvents have different extraction capacities. The highest percentage recovery observed in ethanolic extract of *T. diversifolia, S. afzelli* leaves might be due to the polar bonds present in ethanol which is more active in extracting plants' metabolites. This observation is in agreement with [10] who reported that polar solvents have been shown to be more effective in extracting organic and inorganic materials from plants. The ethanolic extract of *J. pinnata* was capable of inhibiting *Salmonella typhi, K. pneumoniae* and *S. dysenteriae* while the petroleum ether extract of the plant was capable of inhibiting S. *typhimurum* and *V. cholera.* In the same vein, the cold water extract of the same plant was capable of inhibiting *V. cholerae* while the hot water extract of the plant was capable of inhibiting *S. typhimurum* and *A. baumanni.* The ethanolic of extract of *T. diversifolia* was capable of inhibiting *S. dysenteriae*, *S. paratyphi*, *K. pneumoniae* while the petroleum ether extract was capable of inhibiting *V. cholerae* and the cold water extract inhibited*S. typhimurum*, *E. aerogenes* and *S. dysenteri*. The hot water extract of *T. diversifolia* showed mild inhibition against *S. typhi* and *S. typhimurum* which might be because the leaf extract was denatured by the high temperature of the water. The ethanolic extracts of *S. afzelli* was capable of inhibiting*S. dysenteriae*, *K. pneumoniae* and *S. typhi* while the petroleum ether inhibited *S. typhimurum* and *V. cholerae* and the cold water extract inhibited *V. cholerae* and hot water extract of *S. afzelli* inhibited *A. baumanii* and *S. typhimurum*.

Some of the extract recorded zones of inhibition ranging between 5mm and 31mm on the various species of *Acinetobacter sp*., *Enterobacter aerogenes, Pseudomonas*, *Shigella sp*, *Salmonella spp.*, *Escherichia coli*, *Klebsiella spp.* isolated from water at 50 mg/ml. The Antibiotic used as control was more effective in inhibiting test organisms than the plant extracts which may be due to purity level of commercial antibiotic. This is in line with the findings of [11] who reported that antibiotic are in a refined state while the plant extracts are still in a crude state. This is also in line with [12] who reported that antibiotic have high degree of purity; conventional antibiotic and other pharmaceutical products are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures expressing purity and high fractionation which certainly will enhance antimicrobial effect than crude extracts. Also, the small molecular size possessed by antibiotic as reported by [13] aids the solubility of antibiotic in diluents as this could enhance their penetration through the cell wall into the cytoplasm of the organism. Varying degrees of susceptibility of organisms to Ethanol, Petroleum ether and aqueous extracts of *T. diversifolia, S afzelli and J. pinnata are* compatible with other results published by other workers such as [14, 15]. The result from the MIC indicates that some the plant extract can be effective at concentration as low as 3.125mg/ml.

5. Conclusion

Conclusively, antibiotics resistance in human pathogens is a great concern to public health, thus the search for alternative to antibiotics is of utmost importance. From this research work, ethanolic extracts of *T. diversifolia* has been shown to produce rate percentage recovery. Hot water extracts of *S. afzelli* has been shown to inhibit *A. baumanni* and *S. afzelli* while cold water extracts of *T. diversifolia* has been able to inhibit *E. aerogenes* and *S. dysenteriae* and petroleum ether extracts of J. pinnata has been able to inhibit *S. typhimurum* and *V. cholerae*. The finding of this study has been able to reveal the bactericidal action of *T. diversifolia*, *S. afzelli* and *J. pinnata* against some plasmid-bearing multiple antibiotics bacteria even at concentration as low as 3.125mg/ml.

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