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(RESEARCH ARTICLE)



Natural product (*Abutilon hirtum* extracts) in treating environment - related infections (Salmonellosis and Typhoid fever)

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Abstract

Salmonella is considered a serious public health burden especially in areas of low economic status and high temperature. The continuous emergence of some *Salmonella* species to even third generation antibiotics is a threat to health care delivery. Thus, this study aimed at screening multidrug resistant (MDR) *Salmonella* isolates and subjecting them to *Abutilon hirtum* extract and fraction as a means of possible discovery of new antimicrobial of plant nature in treatment of environment related infections. Ten serotyped clinical *Salmonella* isolates were obtained from Jos University Teaching Hospital (JUTH), Nigeria. The plant fractions were obtained by open column chromatography. Phytochemical screening was done using Standard Qualitative method. The bioassay of the crude and fraction extracts (AF9) against MDR *Salmonella* isolates was by Well Agar diffusion and broth micro dilution method respectively. All *Salmonella* isolates exhibited resistance against more than 3 antimicrobials (MDR). However, all the isolates (100%) were susceptible to ciprofloxacin and 60% sensitive to ceftriaxone but showed 100% resistance against amoxicillin, erythromycin, tetracycline and amoxicillin + clavulanic acid. The *A.hirtum* extract showed the presence of alkaloids, flavonoides, tannins, saponins, cardiac glycosides, terpenes and steroids, phenol and resins. 80% of the isolates were susceptible to the crude extract at concentration of 200mg/ml but susceptibility decreased as concentration decreased. The MIC and MBC of the plant fraction (AF9) against the isolates ranged from 150µg/ml–300µg/ml and 300µg/ml–600µg/ml respectively. This plant may probably be a new antimicrobial for treating MDR *Salmonella* infections.

Keywords: Multidrug Resistant Salmonellae; *Abutilon hirtum* extracts; bioactive molecules; environment; Nigeria

1. Introduction

Antibacterial agents are the most useful substances in combating the menace of infectious diseases caused by pathogenic microorganisms worldwide [1]. The burden of the infections is becoming more threatening as a result of emergence of multidrug resistant microorganisms.

Report has shown consistently that gastrointestinal infections with bacterial pathogens are positively correlated with ambient temperature. Again, Ahmad and Akil [2] have documented that socioeconomic status and climate change are said to contribute to the increased rates of *Salmonella*. The genus *Salmonella* has been responsible for both salmonellosis and typhoid fever. *Salmonella* Typhi also causes an asymptomatic and persistent (chronic) infection. These infected persons are typhoid fever carriers and are capable of shedding bacteria and sustaining transmission within the community [3, 4].

The *Abutilon hirtum* is a wild plant growing on rocks and is indigenous to Berom natives of Plateau State, Nigeria. Its leaves are used for cooking soup and have been consumed over the years without any side effect on the users and it is

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claimed to be effective in treatment of typhoid fever. Thus, this study aimed at screening multidrug resistant (MDR) *Salmonella* isolates and subjecting them to *Abutilon hirtum* extract and fraction as a means of possible discovery of new antimicrobial of plant nature in treatment of environment related infection.

Salmonella which is the causative agent of typhoid fever and salmonellosis has feature to form biofilms in abiotic surfaces outside the host, such as in farms, food processing industry, kitchen or toilets, in plant surfaces, or even in animal epithelial cells, therefore, contributing to its resistance and persistence[5]. There was an estimated disease burden of 20.6 million cases in low- and middle-income countries in 2010, presenting typhoid fever to remain an enteric disease of public health concern[6, 7]. However, marked improvement in water, sanitation and sewage removal have greatly reduced typhoid fever incidence only in most industrialized countries [3, 8, 9, 10]. *Salmonella enteric* serovar Typhi that causes typhoid fever is specific to human and the non-typhoidal *Salmonella spp* are transmitted by the fecal –oral route between humans, through the ingestion of contaminated food and water.

An epidemic of invasive non-typhoidal *Salmonella* (iNTS) infections had occurred in sub-Saharan Africa and genomic analysis and clinical observation revealed these organisms are evolving to become more typhoid like in regard to patterns of transmission and virulence. It is worrisome that a prototypical African NTS strain has lost traits required for environmental stress resistance, consistent with an adaptation to a human-model of transmission [11].

Over time *Salmonella* species have demonstrated resistance to many antibiotics as a result of mutation, horizontal and vertical gene transfer. These factors are promoted by miss-used of antibiotics, use of antibiotics as growth promoters in agriculture, poverty, over crowdedness, lack of portable water, poor hygiene practices of handling, raw animal product and food in general and biofilm barriers. Particularly improper cooking methods by food handlers in restaurants and canteens could be responsible for spread of multidrug resistant *Salmonella* since most of developing countries populations live below the average level of \$1 per day meal, hence, they made up their minds to patronizing restaurants and canteens of questionable cooking standards [12, 13]. Also flooding and dumping of untreated waste /sewage have been reported to contribute to the transmission of these agents [14, 15].

Right from the time memorial, man had learned to use plants around his environment by trial and error for treatment of ailments. This is in line with what is documented in Ezekiel 47:12 that plants are for healing and food. Today, use of plants is the basis of sophisticated traditional medicine system that has existed for thousands of years and has continued as new remedies for man and animal [16]. Majority of the rurals in developing countries like Nigeria largely depend on traditional use of medicinal plants handed over to them from generation to generation. Traditional medicines have continued to be more relevant due to some features that are unique to this medicine in terms of efficacy and safety [17]. Medicinal plants are sources of novel chemical entities that have beneficial pharmacological and therapeutics potentials. They can either be administered directly or their extracts used as starting material in formulation of conventional drugs [18].

2. Material and methods

2.1. Bacterial isolates

Ten clinical *Salmonella* isolates serotyped by polyvalent antiserum Poly O, 1-67 and Poly H-1+2(SIFINGERMANY) Subgroup and Monovalent sera (Carper Laboratories, London) were collected from Medical Micro biology Department of the University of Jos Teaching Hospital.

2.2. Antibigram

Kirby-Bauer's method was adopted using ceftriaxone, cotrimoxazole, ciprofloxacin, erythromycin, chloramphenicol, tetracycline, gentamicin, cloxacillin, amoxicillin and amoxicillin + clavulanic acid to obtain the antibiogram against the isolates.

2.3. Collection and preparation of the plant sample

Abutilon hirtum was collected from BarkinLadi L.G.A of Plateau State, Nigeria and identified by a plant taxonomist; Late Dr.FidelisTiseer of A.B.U Zaria. The procedure of Ndip et al. [19] was used for the extraction of the plant material with slight modification by using thermostatic water Cabinet (model HH-W420, XMTD-204 and TT42D Multipurpose use. Techmel and Techmel, USA) at 1000C instead of rotor vapor to concentrate the plant extracts. Analytical grade 86% methanol was used for the cold extraction.

2.4. Column chromatography

The extract of *A.hirtum* was fractionated by column chromatography process as reported by Dawang et al. [20] using the following mobile phase; n-hexane: Methanol(MeOH) as (10:0, 15:1,9:4,8:2,7: 3,6:4,5:5,4:6,3:7,2:8) and MeOH:Ethanol (EtoAC) (10:0,9:1,8:2, 7:3,6:4,5:5) and the effluent was collected in a small fraction (150cm³) in a beaker. Fractions from crude plant extract were pooled together based on similar profile on Thin Layer Chromatography (TLC, AlugramXtra SIL G/uv 254, MAC HERY-NAGEL GmbH and Co. Kg, Germany) to yield 11 *A.hirtum* leaf fractions of AF1-AF11 due to variance in polarity and types of constituents extracted [21]. However only 1 most effective fraction obtained after preliminary bioassay of the 11 fractions was tested against the isolates.

2.5. Phytochemical screening

The phytochemical screening of ethyl acetate extract of *A.hirtum* extract was carried out using standard qualitative procedure [22, 23].

2.6. Sensitivity test of the crude extract

Agar Well Diffusion Method was used to determine the sensitivity of the isolates to the plant crude extract. A Mueller-Hinton agar plate was inoculated with 0.7ml of suspended isolate of inoculum size equivalent to 1X10⁸cfu/ml and the excess fluid at the edge of the petri dish was removed with sterile cotton wool to obtain confluent growth. The plates were then kept for few minutes to dry. Wells of 6ml in diameter were aseptically punched with a sterile cork borer. A stock concentration of the crude plant extract was obtained by dissolving 2g of crude plant extract in 10ml of DMSO and 100mg/ml of the crude extract was prepared. Two wells punched in a plate were filled with 200mg/ml and 100mg/ml of separately and one well in the same plate was separately filled with DMSO (negative control). The plates were left again for some time for the extracts to diffuse into the agar, after which they were incubated at 37°C for over 18 hours. The zone of inhibition was measured to the nearest millimeter and mean zone of inhibition was calculated for each extract concentration [24].

2.7. Determination of minimum inhibition concentration (MIC) of fraction of *A.hirtum*

The MIC of the leaf fraction (Methanolic AF9) against the various isolates was done by using the 96-well micro dilution method described by Nvau et al. [25] (2011), but with slight modification of using 8 wells instead of 12 wells. After about 24 hours sub-culturing of the isolates on nutrient agar and Xylose Lysine Deoxycholate (XLD), 4-5 colonies of the same appearance of each isolate were emulsified in sterile normal saline according to Ndip et al. [19] documented by Nyenje and Ndip [26] and adjusted to 0.5 McFarland Scale (1x10⁸cfu/ml). Fifty microlitres (50µl) of Brain Heart Infusion (BHI) broth was introduced into wells 2 to 8. One hundred microlitre of 0.006g of the extract dissolved in 10ml of DMSO was dispensed into well 1 and 50µl was then transferred from well 1 and delivered into well 2. After thorough mixing, 50µl was again transferred from well 2 to 3 and the same procedure was repeated through to well 8 and from well 8, 50µl was discarded. Thereafter, 50µl of inoculum was introduced to all the wells. The wells were then covered with plastic tape, incubated for about 24 hours and observed for turbidity. The well before the one that showed turbidity (growth) was noted as Minimum Inhibitory Concentration (MIC).

2.8. Determination of the minimum bactericidal concentration (MBC) of fraction of *A.hirtum*

A sterile wire loop was dipped into the wells of minimum inhibitory concentration that showed no turbidity (no bacterial growth) and streaked on nutrient agar and incubated overnight. The MBC was obtained as the lowest concentration preventing the growth of bacteria [21].

3. Results and discussion

The clinical salmonellae isolates showed varied multidrug resistant (MDR) patterns with AmoxEryTetCxcCotChlAuGen appearing the most (Table 1). The methanolic leaf extract of *Abutilon hirtum* showed the presence of alkaloids, flavonoids, saponins, cardiac glycoside, terpenes and steroids, phenols and resins whereas balsam was absent (Table 2). Table 3 showed that at concentration of 200mg/ml, 80% of the isolates were susceptible to the extract. The AF9 Methanolic fraction showed MIC ranging from 150µg/ml to 300µg/ml while MBC varied from 300µg/ml to 600µg/ml against the clinical *Salmonella* isolates (Table 4). Figure 1 showed percentage susceptible and resistant of the isolates to the various antimicrobials used with 100% resistant to amoxicillin, erythromycin, tetracycline, cloxacillin and amoxicillin + clavulanic acid but 100% and 60% susceptible to ciprofloxacin and ceftriaxone respectively.

Table 1 Distribution of Susceptibility/ Resistance to Antimicrobials among *Salmonella* Isolates from Patients of University of Jos teaching hospital, Jos, Nigeria.

Isolate id/antimicrobials											
No	Tx	Cl	Amox	Ery	Tet	Cxc	Cot	Chl	Au	Gen	MDR Pattern
1	S	S	R	R	R	R	R	S	R	R	AmoxEryTetCxcCotAuGen.
2	S	S	R	R	R	R	R	R	R	S	AmoxEryTetCxcCotChlAu
3	R	S	R	R	R	R	R	S	R	S	TxAmoxEryTetCxcCotAu
4	R	S	R	R	R	R	R	S	R	R	TxAmoxEryTetCxcCotAuGen
5	S	S	R	R	R	R	S	R	R	S	AmoxEryTetCxcChlAu
6	R	S	R	R	R	R	R	R	R	R	TxAmoxEryTetCxcCotChlAuGen
7	S	S	R	R	R	R	R	R	R	R	AmoxEryTetCxcCotChlAuGen
8	R	S	R	R	R	R	R	S	R	R	TxAmoxEryTetCxcCotAuGen
9	S	S	R	R	R	R	R	R	R	R	AmoxEryTetCxcCotChlAuGen
10	S	S	R	R	R	R	R	S	R	S	AmoxEryTetCxcCotAu

Tx (Ceftriaxone), Cl(Ciprofloxacin), Amox(Amoxicillin), Ery (Erythromycin) Tet (Tetracycline), Cxc (Cloxacillin), Cot (Cortimoxazole), Chl (Chloramphenicol), Au (Amoxicilin + Clavalanic acid), Gen (Gentamicin), S(Susceptible) R(Resistant).

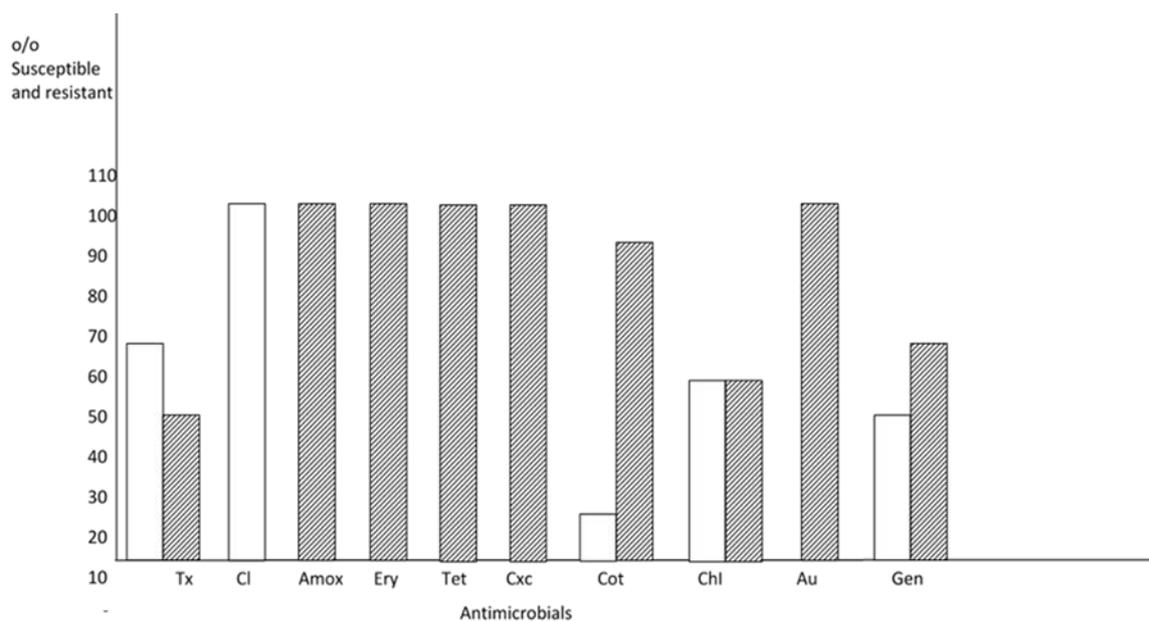


Figure 1. Susceptibility and Resistance of *Salmonella* isolates to Antimicrobials

Tx (Ceftriaxone), Cl(Ciprofloxacin), Amox(Amoxicillin), Ery (Erythromycin) Tet (Tetracycline), Cxc (Cloxacillin), Cot (Cortimoxazole), Chl (Chloramphenicol), Au (Amoxicilin + Clavalanic acid), Gen (Gentamicin), (Susceptible)

(Resistant).

Table 2. Phytochemical constituents of methanolic leaf extract of *Abutilon hirtum*.

Chemical Components	<i>Abutilon hirtum</i>
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Cardiac glycosides	+
Terpenes & steroids	+
Balsam	-
Phenols	+
Resins	+

+ (positive/present) , - (Negative/Absent)

Table 3. Efficacy of *Abutilon hirtum* methanolic crude leaf extract against clinical *Salmonella* isolates.

Isolate i.d no	Serogroup	200mg/ml	100mg/ml
1	B	S	R
2	C	S	R
3	C	S	S
4	A	S	S
5	C	R	R
6	C	S	R
7	D	S	R
8	C	R	R
9	C	S	R
10	B	S	S

i.d no. (Identification Number), Zone to inhibition <14mm (resistant), >14mm (susceptible).

Table 4. MIC and MBC of AF9 methanolic *Abutilon hirtum* fraction against the clinical *Salmonella* isolates.

Isolate i.d no	MIC ($\mu\text{g/ml}$)	MBC ($\mu\text{g/ml}$)
1	300	600
2	150	600
3	150	600
4	300	600
5	300	>600
6	300	600
7	300	300
8	300	300
9	300	300
10	300	600

i.d No (identification number), MIC (minimum inhibition concentration, MBC (Minimum bactericidal concentration, AF9 (*Abutilon* fraction 9).

The salmonellae multidrug resistant patterns observed in this study are in conformity to several reports indicating varied antibiotic profiles of salmonellae in sub-Saharan African countries. This could be due to the fact that infectious diseases are much more serious in the tropical regions such as Southeast Asian, South Asian, and Africa (Nigeria, where

this recent research was carried out) because the warm and humid environment favour the growth and propagation of microorganisms [27]. The swapping between plasmid(s) and the bacterial chromosome and integration of resistance genes into genetic elements like integrons probably contributed in acquisition and dissemination of resistance genes partly as a result of environmental factors [28]. Marks *et al.* [29] have reported ciprofloxacin resistant *S. paratyphi* A and iNTS in Senegal and Ghana respectively although it is not similar to this finding which could be as a result of geographical location. Also, reported was iNTS isolate from Kenya found to be resistant to ceftriaxone as the case in this present study.

The presence of bioactive compounds in *A. hirtum* confirms its antisalmonellae potentials. This is in line with the World organization [30] which reported that medicinal plants would be the best source to obtain a variety of drugs. At 200mg/ml of *A. hirtum* methanolic leaf extract, all the salmonellae isolates were susceptible unlike at 100mg/ml. This shows that as the concentration of the extract increased, its potency also increased. Thus, effective treatment of this multidrug resistant menace entails the development of new pharmaceuticals or some potential source of novel drugs. Some commonly used medicinal plants in our environment might be an excellent source of drugs to fight off this problem [31].

The effectiveness of the antibacterial activity of *A. hirtum* was demonstrated by low MIC of 150µg/ml to 300µg/ml against the tested Salmonellae. At 300µg/ml to 600 µg/ml range of the MBC, the plant showed bactericidal effect against all the organisms except one. This finding is similar to that of Dawang *et al.* [20] who reported sub fraction, AF9, and AF2 of *A. hirtum* to exhibiting antisalmonellae potency at 75µg/ml and 150 µg/ml respectively. The medicinal properties of the plant extract are due to the synergetic effects of the bioactive substances present. Some researchers have suggested that the main active molecules in plants that are responsible for anti-diarrhoeal activity are tannins and tannic acid, flavonoids, alkaloids, sesquiterpenes, terpenes and terpenoids [32].

There are several confirmations on the effectiveness of plants extracts on salmonellae. For instance, *Urtica aurens* aqueous extract was demonstrated to have a significant effect on mice mortality when administered in *S. Typhimurium* infected rats at considerably low dosages (3 mg/kg) [33]. Also, *Terminalia bellerica* fruits aqueous extract administered to mice infected with lethal doses of *S. Typhimurium* showed a dose dependent effect, with 83.3% of the mice surviving after 15 days, while all of the controls have died within seven days [34]. Additionally, Kengni *et al.* [35] experimented that the faeces of infected rats treated with *Harungana madagascariensis* aqueous leaf extract (25mg/kg) were free of *Salmonella* after 16 days in both male and female animals, even at low extract dose concentrations.

4. Conclusion

In conclusion, tropical countries are more prone to microbial infections due to the conducive environment where the causative agents can easily thrive beside the socioeconomic status of the developing countries. *Abutilon hirtum* as one of the wild plants found within the environment with further processing could be used in treating microbial infections beside sustained clean environment.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

I sincerely declare that there is no conflict of interest in the course of this work.

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