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Antibacterial Efficacy of *Ageratum conyzoides* on *Salmonella* Species Isolated from Suspected Typhoid Fever Patients in Akure Metropolis, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OEA and SIA designed the study. Author AGO performed the statistical analysis. Authors OEA and SIA wrote the protocol and first draft of the manuscript. Authors FNO and BFO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The study aimed at assessing the antibacterial efficacy of *Ageratum conyzoides* on *Salmonella* species isolated from suspected typhoid fever patients in Akure metropolis, Nigeria.
Study Design: The study evaluated the prospective use of *Ageratum conyzoides* as an alternative to commonly used drugs in the treatment of salmonellosis and gastroenteritis.
Place and Duration of Study: Five selected hospitals within Akure metropolis in Ondo State, Nigeria were used for the study which was conducted between June and September, 2015.
Methodology: Two hundred (200) blood samples collected from presumptive typhoid fever patients attending selected Hospitals in Akure metropolis were screened, and *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Typhimurium were isolated from them. Plant used (*Ageratum conyzoides* Linn) was collected from Ologede Street in Oda road, Akure, Nigeria. Authentication of the plant was done at the Crop Science and Pest Department of the Federal

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University of Technology, Akure, Nigeria. Extract (Methanol, hexane and acetone) of the plant leaf were gotten using standard procedures. Antibacterial effects of the extract on the *Salmonella* isolates were thereafter evaluated followed by qualitative and quantitative phytochemical screenings on the leaf extract. The antibiogram of the isolates were also determined using Antibiotics sensitivity disc.

Results: The highest zone of inhibition was observed with the methanol extract at a concentration of 100 mg/ml for *Salmonella* Typhi and the lowest with the acetone extract at 25 mg/ml. The highest zone of inhibition was however observed for the hexane extract at a concentration 100 mg/ml for *Salmonella* Typhimurium and the least with methanol extract at 12.5 mg/ml. Phytochemical analysis revealed the presence of various secondary metabolites in the plant.

Conclusion: *Ageratum conyzoides* leaves extracts if further investigated for its antibacterial properties, possess the potential of creating a roadmap for drug formulation especially against salmonellosis.

Keywords: In-vitro; anti-bacterial; phytochemical; inhibition; Ageratum conyzoides; salmonellosis.

1. INTRODUCTION

The under-exploration of herbal medicine which abounds in many readily accessible vegetation in Nigeria has greatly limited the potentials of introducing new drugs with herbal origin into modern medicine. Medicinal plants have been used globally before the advent of commercial antibiotics [1]. Herbal medicine has documented use especially in countries such as China [2] Ethiopia [3], Argentina [4] and Papau New Guinea [5]. Several modern day pharmaceutical products are designed using medicinal plants [6] and [7].

Diseases caused by microorganisms remain one of the major challenges to human health today. In spite of the development of natural and synthetic antimicrobial agents against many pathogenic microorganisms, antimicrobial resistance seems to be a major bane of their effectiveness. There is thus a renewed interest in traditional medicine and an upsurge in the quest for drugs of plant origin. This renewed interest in drugs of plant origin is borne out of the belief in the safety and dependability of plants than the synthetic drugs which are costly and have deleterious effects on man [8]. This situation has provided the needed drive to search for new antimicrobial substances from medicinal plants.

Salmonella is a rod-shaped Gram negative enteric bacterium. It is the causative agent of two human diseases viz; salmonellosis otherwise called enteric fever (typhoid) which usually arise from bacterial invasion of the bloodstream, and acute gastroenteritis which is due to either a food borne infection or intoxication [9]. *Salmonella enterica* serovars infect mainly animals and humans. The type of resulting salmonella infection however depends on the animal species and the bacterial serovar. *Salmonella enterica* serovar Typhimurium (*Salmonella typhimurium*) has a wide host range and produces diverse symptoms in them. *Salmonella enterica* serovar Typhi (*Salmonella typhi*) however infects only humans where it causes typhoid fever- a disease that causes significant morbidity and mortality worldwide.

Ageratum conyzoides took its origin from the Greek word ‘agera’ meaning non-aging. More commonly it is called goat or billy goat weed and belongs to the Astreracae family. *Ageratum conyzoides* is an herbaceous plant that grows annually and has been used in several countries of the world for various medicinal purposes [10]. The curative properties of the leaves especially in the treatment of ailments such as burns, wounds, arthritis, headaches, dyspnea, and pneumonia have been documented. There are also reports of its analgesic, anti-inflammatory, antiasthmatic, antispasmodic and haemostatic effects [11].

This study thus investigated the antibacterial potentials of different leaf extracts of *Ageratum conyzoides* with a view to sourcing for alternative treatment for gastroenteritis and salmonellosis.

2. MATERIALS AND METHODS

2.1 Ethical Clearance

The consent of the clinically suspected patients was sort, while the Ethics Committee of the Ondo State Ministry of Health gave approval for the study. Confidentiality of the subjects’ identities was duly maintained.

2.2 Collection of Blood Samples

Blood samples used were collected from selected hospitals in Akure metropolis. The blood samples were kept in Ethylene Diamine Tetra Acetic (EDTA) bottles and transferred to the laboratory for analysis.

2.2.1 Bacteria Isolation

Blood samples collected from presumptive typhoid fever patients attending the selected hospitals were inoculated into Brain Heart Infusion Broth in the ratio of 2:20. Incubation was done for 48 hours 37℃. After incubation, a loopful of the culture was streaked on *Salmonella- Shigella* agar (SSA) and incubated at 37℃ for 24 hours.

2.2.2 Sub-culturing of isolates

A colony of the grown organism was sub-cultured on a fresh *Salmonella- Shigella* agar plate and incubated for 24 hours at 37℃. This was done to get a pure and distinct colony strain on plate.

2.2.3 Identification of the bacterial isolates

The bacterial isolates were identified using their colony morphological characteristics. The appearance of each colony on the agar media and characteristics such as shape, edge, colour, elevation and texture were observed as described by Olutiola et al. [12]. The isolates were thereafter subjected to relevant biochemical tests and identified using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology.

2.3 Collection and Authentication of Plant Materials

Plant materials used were collected from Ologede Street, Oda road, Akure. The plants were identified at the Crop Science and Pest Department (CSP) of the Federal University of Technology, Akure (FUTA) using the handbook of West African weeds. Leaves of the collected plant material were oven dried for 3 days at 40℃, ground into fine powder and stored in airtight container.

2.3.1 Preparation of plant extracts

Methanol, acetone and hexane extract of the plant leaf was obtained using soxhlet extraction method as described by Akinyemi et al. [1] and Junaid et al. [13].

2.3.2 Antibacterial assay of the plant leaf extract

Agar well diffusion as described by Irobi *et al.,* [14] was used to determine the antibacterial activity of the plant leaf extract. A standardized inoculum of was used for the inoculation of plates. Muller Hinton agar was prepared according to the specifications and allowed to cool to about 40-50℃. The freshly prepared and cooled media was poured into Petri dishes and allowed to solidify at room temperature. A sterile swab stick was used to spread about 0.2 ml of the standardized test inoculum evenly on the surface of the solidified media. Five equidistant wells of 5 mm in diameter were then made on the seeded agar plate using a sterile cork borer and the plant extracts with concentrations ranging from 6.25-100 mg/ml were introduced into the bored holes. A 5 ml of tween 20 was used in re-constituting the extracts. The plates were then incubated at 37℃ for 24 hrs. Zones of inhibition around the isolates were measured using a calibrated ruler. The experiment was carried out in triplicate.

2.3.3 Phytochemical screening of plant extract

The extract was subjected to phytochemical tests of quantitative and qualitative screening for plant secondary metabolites in accordance with [15].

2.4 Determination of the Antibiotics Sensitivity Profile of the Bacterial Isolates

The antibiotics sensitivity test was carried out determine the antibiogram of the isolates to conventional antibiotics. The commercial antibiotics (abtek biologicals) which included Ciprofloxacin (10 µg), Amoxycillin (25 µg), Ofloxacin (5 µg), Cotrimozazole (25 µg), Gentamycin (10 µg), Nitrofuranton (200 µg), Ceftriazone (30 µg), Pefloxacin (5 µg), Tetracycline (30 µg), and Augmentin (30 µg) were used. The disc diffusion method described by Willey et al. [16] was used to determine the antibiogram of the bacterial isolates. Test organisms were standardized as described by [17] and sterile Petri-dishes were seeded aseptically with 1 ml each of standardized broth culture of the test organisms while about 20 ml of sterilized Muller-Hinton agar was poured aseptically on the plates. The plates were swirled carefully for even distribution of the agar and later allowed to gel. With the aid of sterile forceps, the antibiotics disc were firmly placed on solidified plates and incubated for 24 hrs at 37℃. After incubation, clear zones around the disc (which represent the zones of inhibition) were measured. Seeded agar plates without antibiotics served as the control experiment. The zones of inhibition around the bacterial isolates were measured accordingly and the experiment was carried out in triplicate.

2.5 Statistical Analysis

The experiments were carried out in triplicate. Numerical data obtained were analysed using one way Analysis of Variance (ANOVA). New Duncan's Multiple Range Test was used to compare the treatment means. Differences were considered significant at P<0.05.

3. RESULTS

3.1 Antibacterial Effects of *Ageratum conyzoides* on the Isolates

Table 1 shows the susceptibility pattern of the *S.* Typhi isolate to the leaf extract at different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml respectively. Hexane and methanol extracts inhibited *Salmonella* Typhi at all concentrations, while acetone at concentration 6.25 mg/ml had no inhibitory effect on the same organism*.*

The antibacterial effect of the leaves extract on *S.* Typhimurium is shown on Table 2. At all the tested concentrations, no zones of inhibition was observed for the acetone extract while at 6.25 mg/ml, methanolic extract had a zone of (1.00±0.58 mm) and hexane (21±0.58 mm). At concentration 25.00 mg/ml however, a zone of (5.00±0.58 mm) and (24.00±0.58 mm) was observed for methanolic and hexane extracts respectively. When the concentration was increased to 100 mg/ml, the zones of inhibition increased accordingly for the two extracts.

3.2 Phytochemical Screening Results

The qualitative and quantitative phytochemical screening results of the plant extract are represented on Tables 3 and 4 respectively. Table 3 showed that all the extracts were not rich in phlobatannin, and anthraquinone but they all possess saponin, tannin, flavonoid, terpenoid, alkanoid, and cardiac glycosides. The result for the quantitative analysis which revealed the various quantities of the secondary metabolites present in the plant is depicted by Table 4. Hexane extract had the highest quantity for cardiac glycosides and flavonoid, while methanolic extract had the highest quantity for terpenoid, alkaloid, tannin, and saponin. The phytochemical screening showed that cardiac glycoside was highest in acetone while the least was recorded in the methanolic extract. For terpenoids, methanolic extract gave the highest yield followed by hexane and acetone extracts respectively. Tannin was highest in the acetone extract, and least in the hexane extracts. Saponin however yielded more in methanolic extract than the acetone and hexane extracts respectively. The percentage of flavonoid was best with acetone and reduced significantly in the hexane and methanolic extracts respectively. As for alkaloid, higher yield was obtained in the methanolic extract than was in the hexane and acetone extracts.

Table 1. Zones of inhibition of the different extracts (mm) on *S.* Typhi

SN	**Zone of inhibition (mm)**			
	Concentration (mg/ml)	**Methanol**	**Acetone**	**Hexane**
1	100	4.67^{c}±0.33	2.00^{b}±0.58	0.33^{a}±0.33
2	50	4.00^{c}±0.33	1.67^{ab}±0.67	0.33^{a}±0.33
3	25	3.00^{ab}±0.58	1.00^{ab}±0.58	0.00^{a}±0.00
4	12.5	2.67^{a}±0.88	0.33^{a}±0.33	0.00^{a}±0.00
5	6.25	2.67^{a}±0.33	0.00^{a}±0.00	0.00^{a}±0.00

Values with the same alphabet along the column are not significantly (P<0.05) different

3.3 Antibiotic Susceptibility Profile of the Isolates

Fig. 1 shows the measurement of zones of inhibition using commercial gram negative antibiotics on the isolates. Highest zone of inhibition (22 mm and 7 mm) was observed with ofloxacin for *Salmonella* Typhimurium and *Salmonella* Typhi respectively. However, other antibiotics like augumentin, ceftriazone, nitrofuraton, cotrimozazole and amoxycillin had no inhibitory effect on the isolate. Ciprofloxacin had no inhibitory effect on *Salmonella* Typhimurium but inhibited *Salmonella* Typhi.

4. DISCUSSION

Plants with medicinal value constitute a veritable source of both conventional and modern medicine. The World Health Organization (WHO) [18] has reported that herbal medicine has genuine utility, and about 81% of rural population world round depend on its efficacy for their primary health care. Various studies conducted by researchers in different parts of the world have established the significance of *Salmonella* Typhi, and *Salmonella* Typhimurium as the causative agent of Salmonellosis [19].

Table 2. Zones of inhibition of the different extracts (mm) on *S.* Typhimurium

SN	Zone of inhibition (mm)			
	Concentration (mg/ml)	**Methanol**	**Acetone**	**Hexane**
1	100	10.67^{a}±0.88	12.67^{a}±0.33	19.00^{c}±0.58
2	50	10.00^{a}±0.00	12.33^{a}±0.67	15.33^{b}±0.88
3	25	9.33^{a}±0.33	11.00^{a}±1.15	14.67^{b}±0.67
4	12.5	9.00^{a}±1.00	10.67^{a}±0.33	11.67^{a}±0.33
5	6.25	8.33^{a}±0.88	10.00^{a}±1.15	11.00^{a}±1.15

Values with the same alphabet along the column are not significantly (P<0.05) different

Table 3. Phytochemicals present in *Ageratum conyzoides* leaf extract (qualitative screening)

Phytochemicals	Methanol	Acetone	N-Hexane
Saponin	+	+	+
Tannin	+	+	+
Flavonoid	+	+	+
-Steroid	+	+	+
Terpenoid	+	+	+
Alkaloid	+	+	+
Phlobatannin	-	-	-
Anthraquinone	-	-	-
Cardiac glycosides			
Legal test	+	+	+
Keller kiliani	+	+	+
Salkowski	+	+	+

Legend: + present; - absent

Table 4. Quantitative phytochemical analysis of leaf extract of *Ageratum conyzoides*

SN	Phytochemicals	Methanol (mg/g)	Hexane (mg/g)	Acetone (mg/g)
1	Glycoside	0.32^{a}±0.00	6.21^{c}±0.00	8.81^{c}±0.00
2	Terpenoid	11.80^{d}±0.04	8.27^{d}±0.00	5.45^{ab}±0.00
3	Tannin	3.48^{c}±0.00	2.24^{a}±0.02	4.95^{a}±0.01
4	Saponnin	20.73^{e}±0.55	8.18^{d}±0.55	12.18^{d}±0.36
5	Flavonoid	1.68^{b}±0.00	4.53^{b}±0.01	5.78^{b}±0.00
6	Alkaloid	25.23^{f}±0.00	18.58^{e}±0.00	13.08^{e}±0.00

Values with the same alphabet along the column are not significantly (P<0.05) different

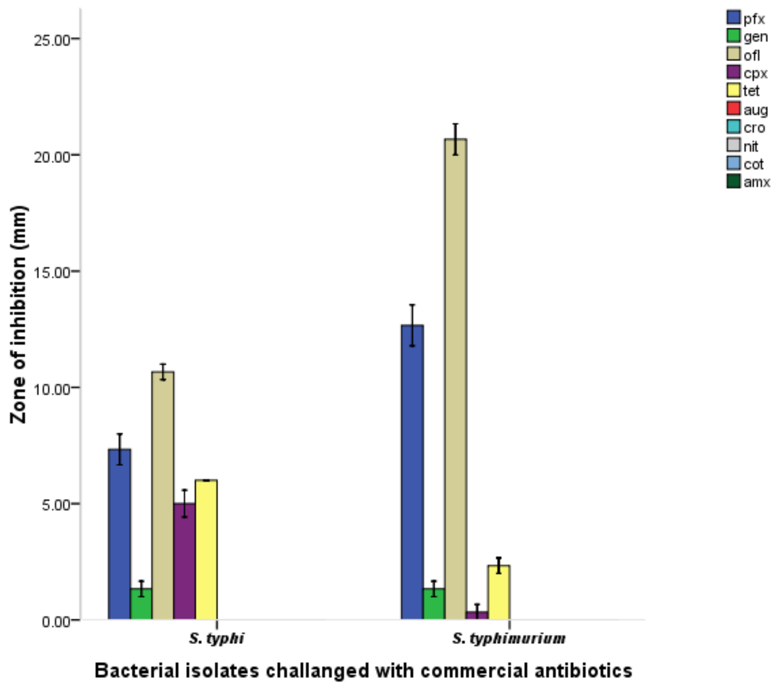

Fig. 1. Comparative antibiotics susceptibility pattern of *S.* Typhi and *S.* Typhimurium

Legend: AUG- Augumentin 30 µg; CRO- Ceftriazone 30 µg; NIT- Nitrofuranton 200 µg; GEN- Gentamycin 10 µg; COT- Cotrimozazole 25 µg; OFL- Ofloxacin 5 µg; AMX- Amoxicillin 25 µg; CPX- Ciprofloxacin 10 µg; TET- Tetracycline 30 µg; PFX- Pefloxacin 5 µg

In this study, the antibacterial efficacy of leaves extract of *Agerantum conyzoides* on some *Salmonella* strains was assessed*. Salmonella* Typhimurium was shown to be more susceptible to hexane extract than acetone and methanol extracts. Reports by [20-22] have however proved the inhibitory effects of methanol extract of medicinal plants. The better effectiveness of hexane extract of *Ageratum conyzoides* contradicts the findings of [23] in which case, their methanol extract was more effective. The antibacterial activity of the hexane extracts against the *S.* Typhimurium isolate is indicative of the non-polarity of the principal active ingredients of the plant. The non-polar nature of hexane makes it extract the lipid soluble phytochemicals easily. Ijeh et al. [24] and Junaid et al. [13] reported a similar result, but [25] contradicted it with his documentation of alcohol as the best solvent for the extraction of plants' active substances of medical importance. As for the *S.* Typhi isolate, slight susceptibility was observed for the methanol extract while the hexane and acetone extracts showed resistance. The differences observed in the antimicrobial properties of the plant extracts can be linked with factors such as age of the plant material, physical influence such as temperature, light and water, as well as incorrect preparation and dosage [26]. Chukwukwa et al. [27] noted that medicinal plants with no antibacterial activity may be due to astringent properties possessed by extract of the plants. Inactivity of plant extracts could also be traced to the extraction solvent, method of extraction and the time of harvesting of plant materials [28]. The medicinal property of herbs has been traced to the presence of various complex chemical substance such as the secondary metabolites, which abound adequately in different parts of plants [29].

Phytochemical screening of extracts of *Ageratum conyzoides* showed the presence of alkaloids, saponins, flavonoids, tannin, steroids and cardiac glycosides. This agrees with previous studies by [30-32]. The absence of some of these phytochemicals in the plant extracts could however be due to the fact that a particular type of solvent may not be selective for a single compound as a result of multi-component nature of plant materials with complex interaction [33]. The use of the extracts from plant in folkloric medicine for the treatment of infectious diseases could be ascribed to the abundant presence of phytocompounds in them. Take for instance, the anti-inflammatory and immune stimulating activity of saponin which was found to be a principal constituent of the plant has been reported. It has also been shown to demonstrate antimicrobial properties particularly against bacteria [34]. As for tannin compounds, Akiyama, 2001 [35] has reported their antibacterial potential. Tannins which are polyphenolic compounds and have sufficient hydroxyls and other suitable groups have been shown to be active against many gram-negative rods [36]. Alkaloids which were also present contain basic nitrogen atoms and have reported use as local anaesthetic and stimulant [37].

The observed *in-vitro* antibacterial effects of *Ageratum conyzoides* leaf on selected *Salmonella* gives credence to its possible use *in-vivo* as opined by some traditional healers. In Nigeria, [38] has reported the use of *A. conyzoides* in the treatment of typhoid fever and diarrhoea. Result of antibiotics sensitivity test revealed that the test microorganisms were resistant to most of the commercially available antibiotics.

The selective pressure imposed by the inappropriate use of antibiotics is the major drive for resistance. This is prominent in developing countries where the level of awareness of resistance to antibiotics and infections is relatively low. People most times prefer a systematic relief, and health professionals respond to such needs by prescribing antibiotics. Factors such as severity of the patient's illness, increased patient contact with health care personnel and length of stay in the hospital also play a major role in patients' resistance to antibiotics. [39]. Poor access to doctors seems to be another reason for the high rate of antibiotic resistance observed in middle income countries like Nigeria. This development make individuals engage in the purchase of antibiotics without due prescription [40]. Studies by [41] has established a relationship between the amount of antibiotic used and the frequency of resistance, although, information on the quantitative relationship between the antibiotics used and the frequency of resistance is still lacking. Drug abuse, misuse and inappropriate prescription practices by physicians as well as the intrinsic microbiological plasmid-mediated factors are also contributory to the problem of antibiotic resistance [42]. The increase in *Salmonella* strains resistance to antibiotics has been reported to be directly linked with the effect of antibiotics use in animal feeds. Nandi et al. [43] opined that the production of resistant organisms may have arisen from the use of antibiotics either as therapy or feed additives in veterinary medicine. He stated further that that the resistant bacteria and their genes can be transferred from animals to humans through the food chain. Increase in population has over time led to the increased dissemination of these resistant bacteria and genes [44]. Urbanization especially in developing countries may also facilitate the spread of resistant bacteria and genes [45]. Antibiotic resistance is both an observable occurrence in the young and adult population, misuse of antibiotics could be the brain behind the resistance in children while resistance in adults can be traced to continuous antibiotics use.

5. CONCLUSION

It was evident from the study that *Ageratum conyzoides* leaves extracts possess antibacterial properties against *salmonella* species and this can be traced to their rich phytochemical content. The antibacterial activity of the extracts could however be enhanced if the components are purified. *Ageratum conyzoides* leaves extract possess the potential of introducing new template into modern medicine especially in the treatment of *salmonella* infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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