

Original Research Articles

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

ABSTRACT

Aim: The increasing search for viable alternatives to chemotherapy in the treatment of *Salmonella* infections has doubled over the years. This informed the assessment of 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 conventional 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 for *Salmonella* species using selective medium. The isolates were identified as *Salmonella typhi* and *Salmonella typhimurium* using their colony morphology and biochemical characteristics. *Ageratum conyzoides* used was collected from Ologede Street in Oda road, Akure, Nigeria and authenticated at the Crop Science and Pest Department of the Federal University of Technology, Akure, Nigeria. Methanolic, hexane and acetone extracts of the plant leaves were obtained using standard procedures. The antibacterial effects of the extract on the *Salmonella* isolates were thereafter evaluated. Qualitative and quantitative phytochemical screenings were also carried out on the leaves extract. The antibiogram of the isolates were also determined using Antibiotics sensitivity disc.

Results: The highest zone of inhibition (4.67 ± 0.33 mm) was observed with the methanolic extract at concentration 100mg/ml for *Salmonella typhi* and the lowest (1.00 ± 0.58 mm) was observed for the acetone extract at 25mg/ml for same organism. The highest zone of inhibition (19.00 ± 0.58 mm) was however observed for the hexane extracts at concentration 100mg/ml for *Salmonella typhimurium* and the least (9.00 ± 1.00 mm) was observed for the methanol extracts at 12.5mg/ml for same organism. Phytochemical analysis revealed the presence of secondary metabolites like saponnins, tannins, flavonoids, steroids, terpenoids, alkanoids and cardiac glycosides in the plant.

Conclusion: This finding show that *Ageratum conyzoides* leaves extracts possess the potential of introducing new template into modern medicine and would require further investigation in the development of new antibacterial drugs.

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 templates 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 of microbial origin remain one of the major threats to human health today. Although a number of natural and synthetic antimicrobial agents have been developed to kill 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 costly synthetic drugs which has diverse side effects [8]. This situation has provided the needed drive to search for new antimicrobial substances from medicinal plants.

Salmonella is a gram-negative facultative rod-shaped bacterium. It is known to cause two diseases in human - salmonellosis which is an enteric fever (typhoid) resulting from bacterial invasion of the bloodstream and acute gastroenteritis resulting from a food borne infection or intoxication [9]. *Salmonella enterica* serovars are a group of Gram-negative pathogens that infect animals and humans. Systemic infections, gastroenteritis and septicaemia can occur depending on the animal species and the bacterial serovar. *Salmonella enterica* serovar *Typhimurium* (*Salmonella typhimurium*) can infect a wide range of hosts causing different symptoms, including a systemic disease in mice. In contrast, *Salmonella enterica* serovar *Typhi* (*Salmonella typhi*) infects only humans and is the aetiological agent of typhoid fever, a disease that causes significant morbidity and mortality worldwide.

Ageratum conyzoides is derived from the Greek word agera meaning non-aging referring to longevity of the flowers or the whole plant. It is commonly called goat or billy goat weed and belongs to the family Asteraceae, which is an annual herbaceous plant with a long history of traditional use in several countries of the world [10]. *Ageratum conyzoides* Linn is an annual herbaceous plant with a long history of traditional medicinal uses in the tropical and subtropical regions of the world. The weed has been known since ancient times for its curative properties and has been utilized for treatment of various ailments, such as burns, wounds, arthritis, headaches, dyspnea, and pneumonia. It has also been shown to have analgesic, anti-inflammatory, antiasthmatic, antispasmodic and haemostatic effects [11].

This study investigated the antibacterial activities of different crude extracts of the leaves of *Ageratum conyzoides* as part of the exploration for new and novel bioactive compounds.

77 **2. MATERIALS AND METHODS**

78 **2.1 Ethical Clearance**

79 Informed consent was sought in clinically suspected cases and approval for the study was
80 obtained from the Ethics Committee of the Ondo State Ministry of Health. Confidentiality
81 was maintained in accordance with standards of medical practice.

82 **2.2 Collection of blood samples**

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

86 **2.2.1 Bacteria Isolation**

87 Blood samples collected from presumptive typhoid fever patients attending the selected
88 hospitals were inoculated into Brain Heart Infusion Broth in the ratio of 2:20 that is 2ml of
89 blood into 20ml of Brain Heart Infusion Broth, and incubated at 37°C for 48hours. After
90 incubation, a loopful of the culture was streaked on *Salmonella- Shigella* agar (SSA) and
91 incubated at 37°C for 24hours.

92 **2.2.2 Sub-culturing of isolates**

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

96 **2.2.3 Identification of the bacterial isolates**

97 The bacterial isolates were identified using their colony morphological characteristics. The
98 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] (2000). 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. Their botanical identities were determined and authenticated 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 dried using the hot air oven at 40°C for three days, ground into fine powder and stored in airtight container.

2.3.1 Preparation of plant extracts

Methanolic, acetone and hexane soxhlet extraction as described by [13] Akinyemi et al, [1] and Junaide et al, [14] was adopted for this study

2.3.2 Antibacterial assay of the plant leaf extract

The antibacterial activity of the plant leaf extract was determined using the agar well diffusion method as described by Irobi *et al.*, [15] A standardized inoculum of was used for the inoculation of plates. Muller Hinton agar was prepared according to the manufacturer's instruction and allowed to cool to about 40-50°C. The freshly prepared and cooled media was poured into Petri dishes and allowed to solidify at room temperature. About 0.2ml of the standardized test inoculum was evenly spread on the surface of the solidified media using a sterile swab stick. Five equidistant wells of 5mm in diameter were then made on the seeded agar plate using a sterile cork borer and the plant extracts with concentrations ranging from 6.25-100mg/ml were introduced into the bored holes. A 5ml of tween 20 was used in re-constituting the extracts. The plates were then incubated at 37°C for 24 hrs. The resulting zones of inhibition were measured using a ruler calibrated in millimeters. The average of the

three readings was taken to be zone of inhibition of the bacteria isolate at that particular concentration.

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 [16]

2.4 Determination of the antibiotics sensitivity profile of the bacterial isolates

The antibiotics sensitivity test was carried out in order to compare the sensitivity of the organisms to the different commercially available antibiotics. The commercial antibiotics (abtek biologicals) which included Ciprofloxacin (10µg), Amoxycillin (25µg), Ofloxacin (5µg), Cotrimozazole (25µg), Gentamycin (10µg), Nitrofuranton (200µg), Ceftriazone (30µg), Pefloxacin (5µg), Tetracycline (30µg), and Augmentin (30µg) were used. The disc diffusion method described by Willey *et al.* [17] was used to determine the effect of standard antibiotics on the bacterial isolates. Test organisms were standardized as described by [18] and sterile Petri-dishes were seeded aseptically with 1ml each of standardized broth culture of the test organisms while about 20ml 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 24hrs at 37°C. 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 were measured in mm and the experiment was carried out in triplicates.

2.5 Statistical analysis

All experiments were carried out in triplicate. Data obtained were analysed using one way Analysis of Variance (ANOVA) and treatment means were compared using New Duncan's Multiple Range Test. 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 ranging from 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. Hexane and methanolic extracts inhibited *Salmonella typhi* at all concentrations, while acetone at concentration 6.25mg/ml had no inhibitory effect on 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 6.25mg/ml, methanolic extract had a zone of $(1.00 \pm 0.58 \text{ mm})$ and hexane $(21 \pm 0.58 \text{ mm})$. At concentration 25.00mg/ml however, a zone of $(5.00 \pm 0.58 \text{ mm})$ and $(24.00 \pm 0.58 \text{ mm})$ was observed for methanolic and hexane extracts respectively. When the concentration was increased to 100mg/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.

3.3 Antibiotics susceptibility profile of the isolates

Figure 1 shows the measurement of zones of inhibition using commercial gram negative antibiotics on the isolates. Highest zone of inhibition (22mm and 7mm) 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*.

193 Table 1: Zones of inhibition of the different extracts (mm) on *S. typhi*

	Zone of inhibition (mm)			
SN	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

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

195 Table 2: Zones of inhibition of the different extracts (mm) on *S. typhimurium*

	Zone of inhibition (mm)			
SN	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

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

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199 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	+	+	+

200 **Legend**

201 + present; - absent

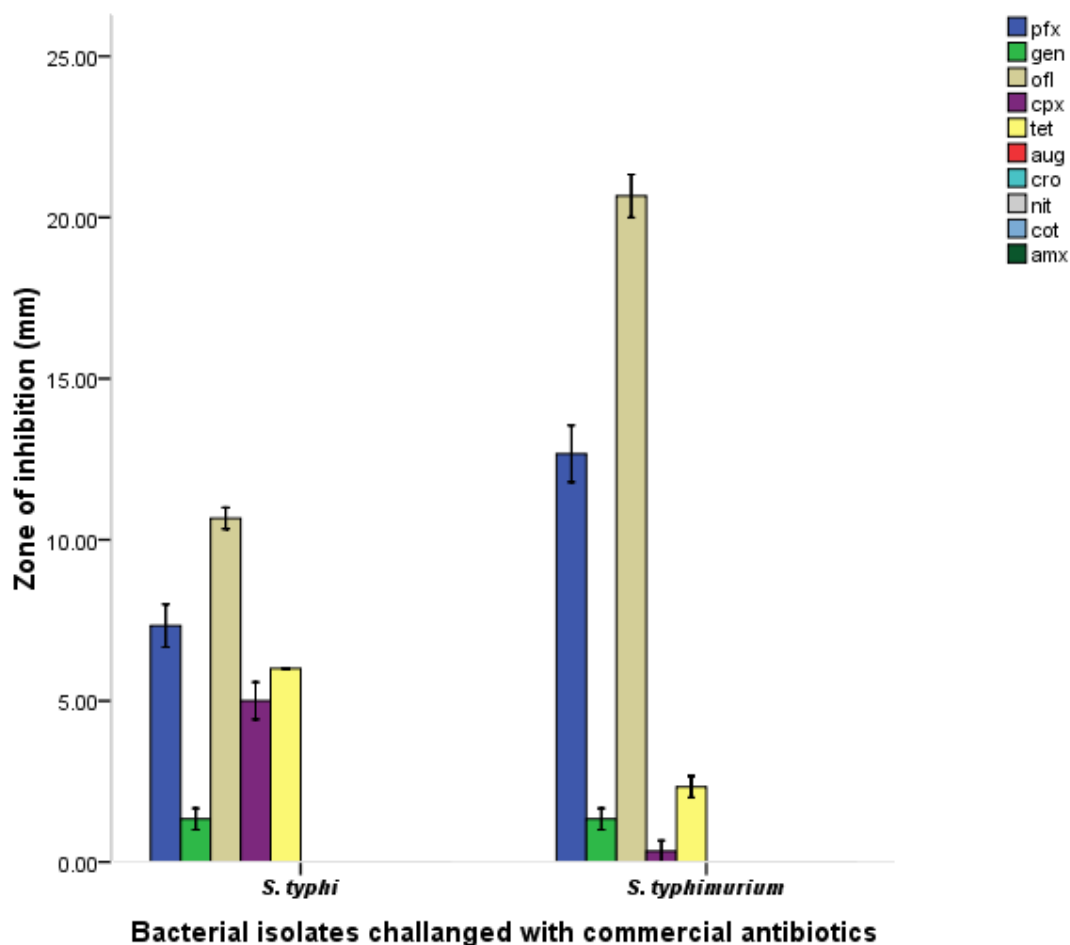
202 Table 4: Quantitative phytochemical analysis of leaf extract of *Ageratum conyzoides*

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

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

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207 Figure1: Comparative antibiotics susceptibility pattern of *S. typhi* and *S. typhimurium*

208 Legend

209 AUG- Augumentin 30µg; CRO- Ceftriazone 30µg; NIT- Nitrofuranton 200µg;

210 GEN- Gentamycin 10µg; COT- Cotrimozazole25µg; OFL- Ofloxacin 5µg

211 AMX- Amoxicillin 25µg; CPX- Ciprofloxacin 10µg; TET- Tetracycline 30µg

212 PFX- Pefloxacin 5µg

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4. DISCUSSION

Medicinal plants constitute a veritable source of both traditional and modern medicine. The World Health Organization (WHO) [19] 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 [20].

The results obtained in this study revealed the antibacterial efficacy of leaves extract of *Agerantum conyzoides*. *Salmonella typhimurium* was shown to be more susceptible to hexane extract than acetone and methanolic extracts, although the inhibitory effects of methanolic extracts of medicinal plants have been reported [21], [22], [23]. The better effectiveness of hexane extract of *Ageratum conyzoides* contradicts the findings of [24] in which case, their methanolic extract was more effective. The antibacterial activity of the hexane extracts against the *S. typhimurium* isolate suggests that the principal active ingredients of the plant must be more of lipid soluble or non-polar, since hexane is a non-polar solvent which can easily extract the lipid soluble phytochemicals. This is similar to the findings of Ijeh et al. [25], and Junaaid et al [14] but in contrast to the findings of [26], who documented alcohol as the best solvent for the extraction of plant 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 [27]. Chukwukwa et al [28] 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

242 extraction and the time of harvesting of plant materials [29]. The medicinal property of herbs
243 has been reported to be due to the presence of different complex chemical substance such as
244 secondary metabolites, which are exclusively accumulated in different parts of the plants [30]

245 Phytochemical analysis of extracts of *Ageratum conyzoides* revealed the presence of
246 alkanoids, saponins, flavonoids, tannin, steroids and cardiac glycosides. This agrees with
247 previous studies by [31], [32] and [33]. The absence of some of these phytochemicals in the
248 plant extracts could however be due to the fact that a particular type of solvent may not be
249 selective for a single compound as a result of multi-component nature of plant materials with
250 complex interaction [34]. The use of the plant extract in folkloric medicine for the treatment
251 of infectious diseases could be due to the abundant presence of secondary metabolites or
252 phytocompounds in them. Saponin for instance has been reported to have anti-inflammatory
253 and immune stimulating activity. It has also been shown to demonstrate antimicrobial
254 properties particularly against bacteria [35]. As for tannin compounds, Akiyama, 2001 [36]
255 has reported their antibacterial potential. Tannins which are polyphenolic compounds and
256 have sufficient hydroxyls and other suitable groups have been reported to possess activity
257 against many gram-negative rods [37]. Alkaloids which were also present contain basic
258 nitrogen atoms and have reported use as local anaesthetic and stimulant [38].

259 The observed antibacterial effects of *Ageratum conyzoides* leaf on selected *Salmonella*
260 isolates though *in vitro* implies that the extracts may indeed be effective *in vivo* as claimed by
261 traditional healers. In Nigeria, *A. conyzoides* has reported use in the treatment of typhoid
262 fever and diarrhoea [39]. Result of antibiotics sensitivity test revealed that the test
263 microorganisms were resistant to most of the commercially available antibiotics.

264 Resistance to antibiotics is mainly driven by the selective pressure imposed by their
265 inappropriate use especially in developing countries where people do not have minimal
266 awareness of resistance to antibiotics and infections. Often times, people want systematic

relief to which the health professionals respond by prescribing antibiotics for quick recovery. Infections with drug resistant microorganisms are associated with severity of the patient's illness, increased patient contact with health care personnel and length of stay in the hospital. [40]. Occurrence of antibiotic resistance in middle income countries like Nigeria may be due to poor access to doctors. This encourages the unacceptable practice of buying antibiotics without due prescription [41]. A relationship between the amount of antibiotic used and the frequency of resistance had been proved in many studies, even though, a direct quantitative relationship between the amount of antibiotics used and the frequency of resistance is still lacking [42]. Other factors which may be contributory to multidrug resistance are drug abuse, misuse and inappropriate prescription practices by physicians along with the intrinsic microbiological plasmid-mediated factors [43]. The increase in *Salmonella* strains resistance to antibiotics may be an indicator of the ecological impact of the use of antibiotics in animals [44]. A large amount of antibiotics have been used in veterinary medicine as therapy or feed additives which produce resistant organisms [44]. These resistant bacteria and or their resistant genes can be transferred from animals to humans through the food chain [44]. Moreover, increased population can lead to increased dissemination of resistant bacteria and resistant genes [45]. As it is typical of developing countries, increase in population density and urbanization may facilitate the spread of resistant bacteria and gene [46]. 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.

5.0

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.

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