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ORIGINAL RESEARCH

Evaluation of the Antimicrobial Activity of Leaf Extracts of *Acokanthera schimperi* against Various Disease-Causing Bacteria

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Background: In traditional medicine of Ethiopia, *Acokanthera schimperi* is the one used to treat different infectious diseases. Hence, this study was conducted with the main aim of determining active compounds in the leaves of *A. schimperi*.

Methods: The antimicrobial activities of using disk diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and acute oral toxicity of the fraction.

Results: The average bacterial zone of inhibition of the dichloromethane (DCM), chloroform (TCM), petroleum ether (PE) and ethyl acetate (EA) fractions ranged from 7.67 mm to 18.12 mm. The average values of MIC of the DCM, TCM, PE and EA fractions ranged from 4.17 mg/mL to 33.34 mg/mL. The most susceptible bacterium at 200 mg/mL was *S. typhi*, (18.12mm), while the less susceptible bacterium was *C. freundii* (14.33mm).

Conclusion: The solvent fractions demonstrated significant antibacterial activities with varying spectrum and safe up to 2000mg/kg.

Keywords: *Acokanthera schipheri*, acute toxicity, antibacterial activity, phytochemical profile and solvent fractions

Introduction

In Ethiopia, plant remedies are still the most important and sometimes the only source of therapeutics for nearly 80% of human and more than 90% of the livestock population.[1] The country endowed with rich flora and fauna, due to its rugged topography and climatic diversity. For instance, Ethiopian flora is estimated to consist of about 7000 species. Out of these, it is believed that 10% are endemic to the country and about 14% are used as medicinal plants.[2]

One of the plants applicable in this study is *Acokanthera schimperi* (A.DC.) Schweinf, which grows in Ethiopia, and where it is known locally as Merz inchet (Amharic). It is a tree of dry woodland, thickets and grasslands 1100–2300 m, often at the margin of dry forest or forest remnants. It prefers rich, well-drained forest soil but also grows on black cotton and poor soils in the arid mid-lands. *Acokanthera schimperi*, has been reported to possess a varied range of therapeutical and pharmacological applications; traditionally it is used for wound,[3–5] hepatitis,[5,6] tonsillitis,[7] malaria,[8] scabies[9] and insect repellent.[10]

Phytochemical studies reveal that *A. schimperi* has a number of secondary metabolites; flavonoids, terpenes, alkaloids, coumarin, cardiac glycosides,

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anthraquinone, tannin and phenolic compounds.[11,12] To the best of our search, there were no studies conducted beyond screening of the aqueous and hydroalcoholic extracts of the plant.[13–15]

Expertise in ethno-veterinary is important given that veterinary pharmaceuticals substantially reduce livestock mortality rates and by promoting prudent antibiotic use may preserve the long-term efficacy of drugs by limiting the development of antimicrobial resistance plant.[16] Non-prudent drug usage is particularly concerning in low-income countries where pharmaceutical use often occurs outside of a professional veterinary context plant.[17] Nevertheless, it is believed that many more animals die due to a lack of access to antimicrobials compared to infections caused by resistance bacteria plant.[17]

Therefore, the present study was conducted to evaluate the antibacterial activity of different solvent fractions of *Acokanthera schimperi* leave on selected pathogenic bacterial species, to determine the phytochemical constituents of solvent fractions qualitatively and to determine the acute toxicity of solvent fractions of *A. schimperi*.

Materials and Methods

Study Area

Leaves of *A. schimperi* were collected from their natural habitat around Arema natural forest, which is found in the Arema-biderkun peasant association, Takusa district (12° 09′01″N and 39°12′01″E), North Gondar, Ethiopia. The in-vitro experimental study was conducted in the microbiology laboratory of the College of Veterinary Medicine and Animal Sciences (CVMAS), University of Gondar, Ethiopia.

The experimental study involving an in-vitro antimicrobial activity of *A. schimperi* leave fractions on disease causing bacterial species and phytochemical screening were conducted in microbiology laboratory of College of Veterinary Medicine and Animal Sciences (CVMAS), University of Gondar from October 2018 to April 2019.

Collection, Identification, and Preparation of Plant Materials

The leave was identified at Addis Ababa University by an experienced botanist and the voucher specimen (AK0012) was deposited at the National Herbarium, College of Natural Science of Addis Ababa University, Ethiopia. The leaves were transported to the University of Gondar, College of Veterinary Medicine and Animal Science, Veterinary Pharmacology Laboratory. Then, washed thoroughly with tap water and dried under shade. The dried leave materials were grinded by an electric mill.

Drugs, Reagents and Bacteriological Media

The solvents used for extracting the plant material were distilled water, methanol, dichloromethane dimethyl sulfoxide (DMSO), phosphate buffered saline, iodine, ferric chloride and Resazurin and all solvents (40–60°C bp) (LobaChemie, India). The standard antibiotic discs that were used in the antibacterial activity tests were purchased from Oxoid, UK. The bacteriological media that were used in the study include Mueller Hinton agar (MHA), 0.5% McFarland standard, Resazurin, Mueller Hinton broth, Plate count agar (Tryptone glucose yeast extract agar) (HiMedia, India). All chemicals and reagents used were of laboratory grade.

Plant Extraction and Fractionation

Two kilo gram of *A. schimperi* leave powder were soaked in 10L of 80% methanol and socked for 48hr in a flask, three times, and agitated thoroughly to mix the slurry. The extracts were filtered using Whatman filter paper No. 1 and the aqueous crude extracts were kept at 4°C and all the three extracts were mixed and subjected to evaporation by a Rota-vaporizer (Buchi, England) which was set at 50°C and then after it were dried by using a freeze drier (Ningbo, China).

Fractionation was done according to,[18] with slight modification of the steps. Briefly, 200g of crude methanol extract was suspended in 100 mL distilled water and transferred into a separation funnel. Thirty milliliters of n-hexane, dichloromethane and petroleum ether were added separately to it and shaken by hand for some minutes. Then the mixture was left to have a separate mixture and the upper layer or the lower layers were separated depending on the solvent. The solvent fractions were taken (dichloromethane, chloroform, petroleum ether and ethyl acetate) and evaporated using a Rota-vaporizer to obtain the fraction.

Phytochemical Screening

The dichloromethane, chloroform, ethyl acetate and petroleum ether fractions of *A. schimperi* leave were screened for the presence phenolic compounds, flavonoids, terpenoids, cardiac glycosides, alkaloids, saponins and tannins

were performed using standard phytochemical procedures as stated elsewhere[19] and.[20]

Antimicrobial Assay

To screen the antibacterial effect of the fractions using the disk diffusion method according to,[21] with slight modification. Briefly, 6mm (diameter) discs were prepared from Whatman No. 1 filter paper. The discs were sterilized by autoclaving. Then, disks were soaked in 50µL fractions with a concentration of 200mg/mL, 100mg/mL, 50mg/mL and 25mg/mL of the solvent fractions.[22,23]

The microbial suspensions were prepared by making a saline suspension of isolated colonies selected from nutrient agar, and the agar plates were grown for 18–24 h. The suspension was adjusted to match the tube of 0.5 McFarland turbidity standard, which equals to 1×10^8CFU/mL.

A 6mm diameter sterilized Whatman filter paper discs were impregnated with 100µL of 200 mg/mL, 100 mg/mL, 50 mg/mL and 25mg/mL of each of the solvent fractions. Standard antimicrobial discs (vancomycin and chloramphenicol) were used as a positive control and 100µL of 5% dimethyl sulfoxide (DMSO) was used as a negative control. Then incubation of the media at 37°C for 24 hrs in an inverted position. Then, it was incubated at 37°C for 24 hours. The zone of inhibition was measured using digital caliper. The experiment was performed in three independent tests for each bacterial strain and the mean of zones of inhibition was calculated for each fraction.

The minimum inhibitory concentration (MIC) was conducted according to CLSI.[24] Briefly 100µL Muller Hinton Broth (MHB) was transferred aseptically to the well of a 96-well micro-titer plate. The stock solutions of the fractions were diluted in 5% DMSO, and then 100µL of stock solution containing approximately 200mg/mL of each fraction was transferred aseptically to the first six rows and serial two-fold dilution to the 10th column by transferring 100µL sequentially. The bacterial strains were prepared using MHB then 0.1mL was diluted with 1mL of distilled water in the ratio of 1:1000 to give a final dilution of 10^5 CFU/mL of the standard inoculums following the dilution susceptibility technique. Fifty microliter of the adjusted inoculum (turbidity adjusted equivalent to 0.5 McFarland) was transferred aseptically to the 96-well micro-titer plate up to 10th of the wells containing the test dilutions, negative control (11th column) and sterility control (12th column). Then, 30µL resazurin was added as an indicator for bacterial growth; bacteria metabolize it and change its color to pink.[25,26] The micro-titer plates were incubated at 37°C for 24 hrs for all the organisms. The wells which had no change in color after the 24 hrs incubation indicated no growth of the microorganisms and they were taken as MIC values. The tests were run in triplicate.

The minimum bactericidal concentration (MBC) is defined as the lowest concentration where no bacterial growth is observed. It was determined by first selecting tubes that showed no growth during MIC determination; 3µL samples were taken and streaked on the surface of plate count agar. The plates were allowed to dry and then kept in an inverted position in an incubator at 37°C for 24 hrs. After the incubation period, the colonies were counted and compared with the negative control. The concentration of the plant extract that completely inhibited the growth of the test organism was considered as MBC.[25,26]

Acute Toxicity

Acute toxicity test (LD_{50}) was conducted by using female white albino mice which were brought from the Ethiopian Public Health Institute (EPHI); Addis Ababa, Ethiopia. These experiment animals weigh 25–30g (average weight) and are 9 weeks of age. Prior to using the experiments, it starved for 3hrs. It was kept in plastic cages at 22°C and on a 12 hr light dark cycle with free access to pellet food and water.[27] This study was done using the limit test dose of 2000mg/kg according to the Organization for Economic Cooperation and Development (OECD) guideline for testing of chemicals using white albino mice.[28] They were randomly distributed into five groups. The first group (control group) received orally distilled water (10m1/kg) as negative control. The white albino mice groups (n=3) with the same sex and age were individually given orally 2000 mg/kg of DCM, TCM, PE and EA fraction of *A. schimperi*.

Ethical Approval

All animals were maintained on a standard mouse diet and water was provided ad-libitum. All animal experimentation was done in accordance with the University of Gondar, Research and community service vice president office. Ethical clearance with approval number O/V/P/RC/05/813 was obtained from the Research and community service vice president office, University of Gondar before the commencement of the study. All animal experiments were conducted in accordance with the National Institutes

of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Statistical Analysis

The experimental data is expressed as mean ± Standard Error of the Mean (SEM). Data are analyzed using the Statistical Package for the Social Sciences (SPSS), version 24.0 software. The statistical differences in the mean zone of inhibition of solvent fractions for individual bacterium were carried out by ANOVA followed by a Tukey Post Hoc Multiple Comparison test at a significance level of $P<0.05$. The MIC and MBC values of fractions were carried out by one way analysis of variance. Moreover, the concentration dependent antibacterial activities of the dichloromethane and petroleum fractions for each bacterium were determined by independent *t*-test analysis using the SPSS software.

Results

Phytochemical Screening

The result of the Phytochemical screening test is shown in Table 1. According to the qualitative Phytochemical screening of the DCM, TCM, EA, and PE fractionates of the leaf of *A. schimperi* were found to be positive for the presence of all of the tested secondary metabolites.

Antibacterial Activity

According to the agar well diffusion test, the growth of all test bacterial strains was inhibited by the tested concentrations of DCM, TCM, PE, and EA fraction of *A. schimperi*. However, there were a statistically significant difference as compared to that of their respective positive control ($p<0.05$) against all test bacteria (Tables 2–5).

E. coli, S. aureus, K. pneumoniae by PE fraction were among the most susceptible than those of the rest of the tested bacterial species. As indicated in (Table 2), the most susceptible bacteria at 200 mg/mL were *E. coli, S. typhi* and *K. pneumonia* on DCM fraction with the same mean zone of inhibition of 16.67 mm while *E. coli, S. aureus* and *P. aeruginosa* with the mean zone of inhibition of 17.67 mm, 17.33 mm and 17.33 mm by PE fractionate, respectively. *S. typhi* showed a higher zone of inhibition in the EA and in the TCM fractions, 18.25mm and 18.00mm, respectively.

With the exception of *C. freundii* which appears relatively resistant to four fractionates. The zone of inhibition of the PE fraction was greater than that of the DCM fraction at equal concentrations against the growth of each test bacterium except *S. typhi*, with a significant difference ($p<0.05$). The TCM fraction (Figure 1A) and EA fractions (Figure 1B) exhibits a low inhibition zone against *S. typhi*; while the PE fraction (Figure 2) exhibit low inhibition zone against *K. pneumonia*.

Minimum Inhibitory Concentration of Solvent Fractions

The MIC value of the DCM fraction ranges from 4.17 mg/mL to 22.24 mg/mL in all bacterial species for which it was active, with the lowest MIC (against *K. pneumoniae*) and maximum MIC (against *C. freundii*) respectively. The highest MIC value of PE fraction was 33.34 mg/mL (against *C. freundii*) and the lowest MIC value was 11.13 mg/mL (against *K. pneumoniae, P. aeruginosa*, and *S. typhi*) with equal values. When compared the MIC value of solvent fractions; DCM fraction was more effective against all tested bacteria (Table 6).

The MIC value of the TCM fraction ranged from 8.35 mg/mL to 55.56 mg/mL in all bacterial species for which it was active with lowest MIC (against *S. typhi*) and maximum MIC (against *C. freundii*) respectively. The highest MIC value of EA fraction was 44.45 mg/mL (against *C. freundii*) and the lowest MIC value was 4.17 mg/mL (against *K. pneumoniae, P. aeruginosa*, and *S. typhi*) with equal values (Table 7).

Table 1 Phytochemical Investigation of Solvent Fractions of *A. schimperi* Leave

Phytochemical Tests	DCM Fractionate	TCM Fraction	PE Fractionate	EA Fraction
Terpenoids	+	+	+	+
Tannin	+	+	+	+
Alkaloid	+	+	+	+
Cardiac glycoside	–	–	+	–
Phenolic compounds	+	+	+	+
Saponin	+	+	+	+
Flavonoids	+	+	+	+

Abbreviations: +, present; –, absent.

Table 2 Zone of Inhibition (in mm) of the Different Concentrations of DCM Fraction of *A. schimperi* Leave Against the Tested Bacteria

	DCM					
	200mg/mL	**100mg/mL**	**50mg/mL**	**25mg/mL**	**Vanc**	**CAF**
C. freundii(ATCC 43864)	13.33±0.66^{a***b***c*}	11.67±0.88^{a***b**}	9.67±0.88^{a***e*}	7.67±0.33^{a***d**e***}	22.33 ±0.33	22.67 ±0.33
K. pneumonia (ATCC 700603)	16.67±0.66^{a***b***c**}	14.33±0.88^{a***b***c*}	11.00±0.57^{a***d*e***}	8.67±0.33^{a***d***e***}	24.00 ±0.57	21.67 ±0.66
S. typhi (ATCC 6539)	16.67 ±0.33^{a***b***c***d*}	14.00 ±0.33^{a***b***c*e*}	11.67 ±0.33^{a***b**d*e***}	9.67 ±0.33^{a***c**d***e***}	22.67 ±0.66	22.33 ±0.33
E. coli (ATCC 25922))	16.67 ±0.33^{a***b***c***d*}	15.00 ±0.00^{a***b***c**e*}	13.00 ±0.00^{a***b*d**e***}	11.33 ±0.33^{a***c*d***e***}	24.00 ±0.57	23.33 ±0.33
P. aeruginosa(ATCC)	15.67 ±0.33^{a***b***c***}	14.00±0.57^{a***b***c*}	11.67±0.33^{a***d*e***}	9.67±0.33^{a***d***e***}	22.33 ±0.66	22.33 ±0.66
S. aureus(ATCC 29213)	15.00 ±0.57^{a***b***c***}	13.33 ±0.33^{a***b***c**}	10.67 ±0.33^{a***b*d*e***}	8.67 ±0.33^{a***c*d***e***}	19.33 ±0.33	22.33 ±0.33

Notes: Values are expressed as Mean ± S.E.M (n=3), analysis was performed with One-Way ANOVA followed by Tukey's test; [a]Compared to vancomycin, [b]Compared to 25 mg/mL, [c]To 50 mg/mL, [d]To 100 mg/mL, [e]To 200 mg/mL; *P<0.05, **P<0.01, ***P<0.001. The negative control has shown no antibacterial activity.
Abbreviations: CAF, chloramphenicol; Vacho, vancomycin.

Minimum Bactericidal Concentration of Solvent Fractions

The maximum mean MBC (least dilution) was 33.34mg/mL (against *C. freundii*) by PE fractionate and the minimum mean MBC (highest dilution) of the study plant was 11.13 mg/mL (against *K. pneumoniae, S. typhi* and *P. aeruginosa*). The corresponding values of the DCM fraction range from 6.95 mg/mL to 22.24 mg/mL for *K. pneumoniae* and C. *freundii*, respectively. Generally, the DCM fractionate was more potent in stopping bacterial growth against all tested bacteria's with a lower concentration compared to that of the PE fractionate (Tables 6 and 7).

Table 3 Zone of Inhibition (in mm) of the Different Concentrations of TCM Fraction of *A. schimperi* Leave Against the Tested Bacteria

	TCM					
	200mg/mL	**100mg/mL**	**50mg/mL**	**25mg/mL**	**Vanc**	**CAF**
C. freundii(ATCC 43864)	14.33 ±0.333^{a***b***c***d**}	11.67 ±0.333^{a***b***c***e**}	8.33±0.333^{a***d***e***}	6.83 ±0.167^{a***d***e***}	22.33 ±0.33	22.67 ±0.33
K. pneumonia (ATCC 700603)	15.67 ±0.667^{a***b***c***}	14.00 ±0.577^{a***b***c***}	9.67±0.333 ^{a***d***e***}	8.17 ±0.167^{a***d***e***}	24.00 ±0.57	21.67 ±0.66
S. typhi (ATCC 6539)	18.0 ±0.00^{a***b***c***d***}	15.33 ±0.333^{a***b***c***e***}	10.33 ±0.333^{a***b***d***e***}	8.17 ±0.167^{a***c***d***e***}	22.67 ±0.66	22.33 ±0.33
E. coli (ATCC 25922))	16.25 ±0.333^{a***b***c***d**}	14±0.00^{a***b***c**e**}	11.33±0.333 ^{a***b***d**e***}	7.5 ±0.289^{a***c***d***e***}	24.00 ±0.57	23.33 ±0.33
P. aeruginosa	17.67 ±0.333^{a***b***c***d**}	15.33± 0.333^{a***b***c***e**}	12±0.00 ^{a***d***e***}	10.67±0.667 ^{a***d***e***}	22.33 ±0.66	22.33 ±0.66
S. aureus(ATCC 29213)	16.25 ±0.577^{a***b***c***d**}	15±0.00^{a***b***c***e***}	10.33±0.333 ^{a***b***d***e***}	7 ±0.577^{a***c***d***e***}	18.33 ±0.33	22.33 ±0.33

Notes: Values are expressed as Mean ± S.E.M (n=3), analysis was performed with One-Way ANOVA followed by Tukey's test; [a]Compared to vancomycin, [b]Compared to 25 mg/mL, [c]To 50 mg/mL, [d]To 100 mg/mL, [e]To 200 mg/mL; **P<0.01, ***P<0.001. The negative control has shown no antibacterial activity.
Abbreviations: CAF, chloramphenicol; Vacho, vancomycin.

Table 4 Zone of Inhibition (in mm) of the Different Concentrations of PE Fraction of *A. schimperi* Leave Against the Tested Bacteria

	PE					
	200mg/mL	**100mg/mL**	**50mg/mL**	**25mg/mL**	**Vanc**	**CAF**
C. freundii(ATCC 43864)	13.67 ±0.33^{a***b***c*e**}	11.67 ±0.33^{a***b***c*e**}	10.00 ±0.00^{a***b*d*e***}	8.33 ±0.33^{a***c*d***e***}	22.33 ±0.33	22.67 ±0.33
K. pneumonia (ATCC 700603)	17.00 ±0.00^{a***b***c***d**}	14.67 ±0.33^{a***b***c**e**}	12.00 ±0.00^{a***b*d**e***}	10.33 ±0.33^{a***c**d***e***}	24.00 ±0.57	21.67 ±0.66
S. typhi (ATCC 6539)	16.00 ±0.00^{a***b***c***d**}	14.00 ±0.00^{a***b***c***e**}	11.67 ±0.33^{a***b**d***e***}	9.67 ±0.33^{a***c**d***e***}	22.67 ±0.66	22.33 ±0.33
E. coli (ATCC 25922))	17.67 ±0.33^{a***b***c***d**}	15.33±0.33^{a***b***e**}	13.67±0.33^{a***b**e***}	11.00 ±0.57^{a***c**d***e***}	24.00 ±0.57	23.33 ±0.33
P. aeruginosa	17.33± 0.33^{a***b***c***d**}	14.67 ±0.33^{a***b***c**e**}	12.00 ±0.57^{a***b**d**e***}	9.33 ±0.33^{a***c**d***e***}	22.33 ±0.66	22.33 ±0.66
S. aureus(ATCC 29213)	17.33 ±0.33^{a***b***c***d**}	15.00 ±0.00^{a***b***c***e**}	12.00 ±0.57^{a***b**d**e***}	9.67 ±0.33^{a***c**d***e***}	18.33 ±0.33	22.33 ±0.33

Notes: Values are expressed as Mean ± S.E.M (n=3), analysis was performed with One-Way ANOVA followed by Tukey's test; [a]Compared to positive control, [b]To 25 mg/mL, [c]To 50 mg/mL, [d]To 100 mg/mL, [e]To 200 mg/mL; *P<0.05, **P<0.01, ***P<0.001. The negative control has shown no antibacterial activity.
Abbreviations: CAF, chloramphenicol; Vacho, vancomycin.

Acute Toxicity Particles in Mice After Oral Administration

The mice were observed continuously for 1h after administration of the plant fraction intermittently for 4 h, over a period of 24 h and for 14 days for gross behavioral changes and other signs of toxicity manifestations. After the seventh day of the first administration, the same fractionates were given to the same mice but by doubling the doses. Then again, they were observed for any signs of behavioral change, exudates or external lesions for the following 4 hours and for the next 5 days. No immediate signs or lesions were seen. The present results indicate that

Table 5 Zone of Inhibition (in mm) of the Different Concentrations of EA Fraction of *A. schimperi* Leave Against the Tested Bacteria

	EA					
	200mg/mL	**100mg/mL**	**50mg/mL**	**25mg/mL**	**Vanc**	**CAF**
C. freundii(ATCC 43864)	14.33 ±0.333^{a***b***c***d**}	11.67 ±0.333^{a***b***c***e**}	8.33±0.333^{a***d***e***}	6.83± 0.167^{a***d***e***}	22.33 ±0.33	22.67 ±0.33
K. pneumonia (ATCC 700603)	15.67 ±0.667^{a***b***c***}	14±0.577^{a***b***c***}	9.67±0.333^{a***d***e***}	8.17 ±0.167^{a***d***e***}	24.00 ±0.57	21.67 ±0.66
S. typhi (ATCC 6539)	18.25± 0.00^{a***b***c***d***}	15.33 ±0.333^{a***b***c***e***}	10.33 ±0.00^{a***b***d***e***}	8.18 ±0.167^{a***c***d***e***}	22.67 ±0.66	22.33 ±0.33
E. coli (ATCC 25922))	16.67 ±0.333^{a***b***c***d**}	14.5 ±0.00^{a***b***c***d**}	11.88 ±0.333^{a***b***d***e***}	7.5 ±0.289^{a***c***d***e***}	24.00 ±0.57	23.33 ±0.33
P. aeruginosa	17.67± 0.333^{a***b***c***d**}	15.33± 0.333^{a***b***c***e**}	12±0.57^{a***d***e***}	10.67 ±0.667^{a***d***e***}	22.33 ±0.66	22.33 ±0.66
S. aureus(ATCC 29213)	16.25 ±0.333^{a***b***c***d*}	15±0.33^{a***b***c***e*}	10.33 ±0.333^{a***b***d***e***}	7 ±0.577^{a***c***d***e***}	18.33 ±0.33	22.33 ±0.33

Notes: Values are expressed as Mean ± S.E.M (n=3), analysis was performed with One-Way ANOVA followed by Tukey's test; [a]Compared to positive control, [b]To 25 mg/mL, [c]To 50 mg/mL, [d]To 100 mg/mL, [e]To 200 mg/mL; *P<0.05, **P<0.01, ***P<0.001. The negative control has shown no antibacterial activity.
Abbreviations: CAF, chloramphenicol; Vacho, vancomycin.

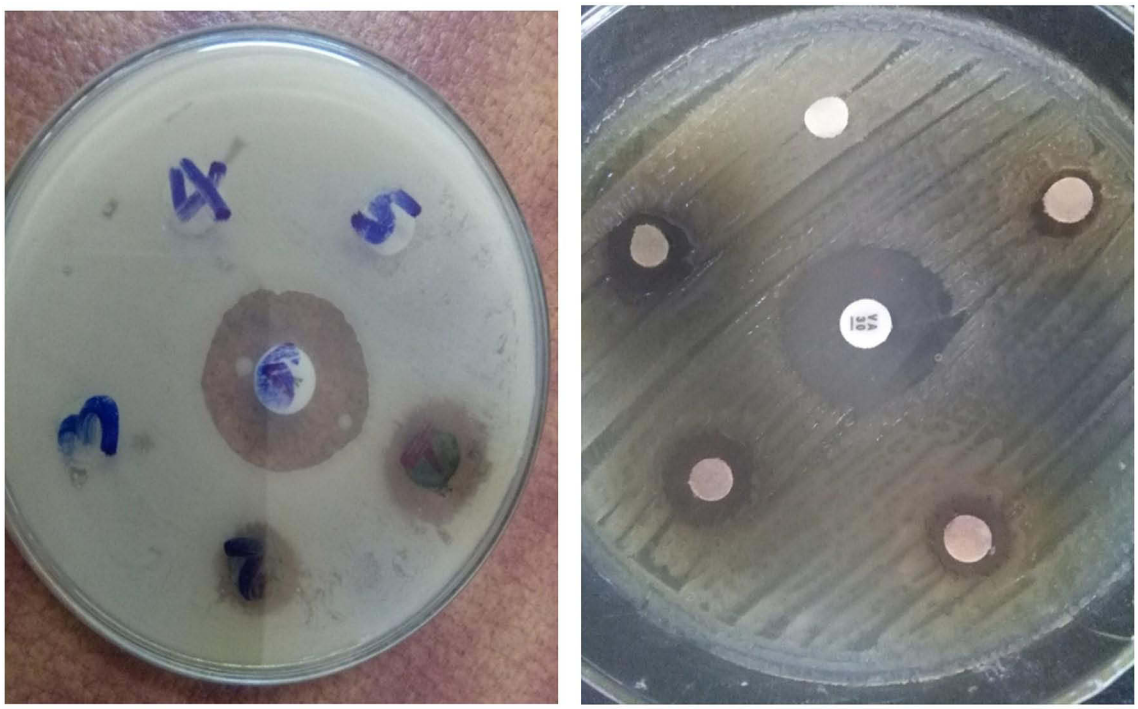

Figure 1 Zone of inhibition of *A. schimperi* TCM (**A**) and EA (**B**) fraction against *S. typhi*(+ve CAF disc as a positive control and 5%DMSO as a negative control).

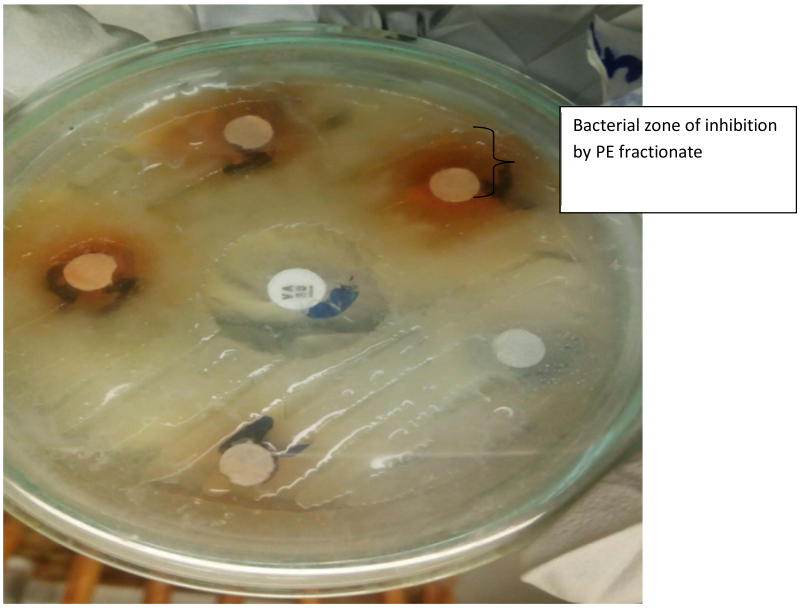

Figure 2 Agar well diffusion test by PE fractionate of the leaves of *A. schimperi* against *K. pneumoniae*. (+ve CAF disc as a positive control and 5% DMSO as a negative control).

Table 6 The MIC (in mg/mL) of the Solvent Fractions of the Leaves of *A. schimperi* Against Tested Bacteria

Bacteria	DCM Fraction		PE Fraction	
	MIC	MBC	MIC	MBC
K. pneumoniae	4.17±0.00	6.95±1.39	11.13±2.78	11.13±2.78
S. aureus	13.91±2.78	16.70±0.00	13.91±2.78	19.416±7.34
C. freundii	22.24±5.54	22.24±5.54	33.34±0.00	33.34±0.00
E. coli	11.13±2.78	13.91±2.78	13.91±2.78	16.70.±0.00
P. aeruginosa	13.91±2.78	13.91±2.78	11.13±2.78	13.91±2.78
S. typhi	11.13±2.78	13.91±2.78	11.13±2.78	11.13±2.78

Note: The values are the average of triplicate tests.
Abbreviation: MIC, minimum inhibitory concentration.

Table 7 The MIC (in mg/mL) of the Solvent Fractions of the Leaves of *A. schimperi* Against Tested Bacteria

	TCM Fraction		EA Fraction	
	MIC	MBC	MIC	MBC
E. coli	16.7±0.000	22.24±5.54	22.24±5.54	16.7±0.000
S. typhi	8.35±0.000	8.35±0.000	4.17±0.000	4.17±0.000
S. aureus	27.79±5.54	27.79±5.54	27.79±5.54	33.34±0.00
C. freundii	44.45±11.11	55.56±11.11	44.45±11.11	55.56±11.11
K. pneumoniae	27.79±5.00	33.34±0.00	33.34±0.000	33.34±0.000
P. aeruginosa	11.14±2.79	11.14±2.79	8.35±0.000	11.14±2.79

Note: The values are the average of triplicate tests.
Abbreviation: MIC, minimum inhibitory concentration.

the leave fractions of *A. schimperi* showed no sign of abnormality at 2000mg/kg.

Disscusion

The present study was conducted with the objective of assessing the biologically active secondary metabolites of the plant *A. schimperi* and determining which fractions of the leaf are responsible for its antimicrobial effect. According to the present study, the zone of inhibition of the EA and TCM fractions showed a greater zone of inhibition than that of the DCM and PE fractions at given concentrations against the growth of each test bacterium.

A study conducted in Tanzania on *A. schimperi* leaf acetone extract inhibited the growth of *Salmonella enteritidis, Bacillus cereus, Klebsiella pneumoniae, Escherichia coli, Enterococcus faecalis, Streptococcus thermophilus* and *Lactobacillus bulgaricus* with an MIC value ranging between 0.7812 and 25 mg/mL against microbes tested in this study.[29] Likewise, a study conducted in Ethiopia on *A. schimperi* methanolic and water extracts showed the growth inhibition of *S. aureus, S. pyogenes, E. coli, P. aeruginosa* and *P. vulgaris*.[14]*Citrobacter freundii* appears to be the least susceptible bacteria for all fractions of the plant, with the respective mean zone of inhibition at the highest concentration (200mg/mL).

Phytochemical compounds commonly associated with combating microbial resistance and having antimicrobial activity in medicinal plants are flavonoids, alkaloids, tannins, terpenoids, essential oils, saponins, and phenols. Even though, at this point in time, it is difficult to judge the mechanism of action of the bioactivity of the solvent fractions of the study plant, it is plausible to speculate about their antibacterial effect based on the different mode of action of the bioactive secondary metabolites detected in the phytochemical analysis of this study.[30]

The possible mechanism of antibacterial activities of alkaloid compounds might be disruption of cell membranes or inhibition of protein synthesis of bacteria. Phenols affect bacterial metabolism, possibly by precipitating of proteins.[25] The antimicrobial activity of saponins on microorganisms is due to their interaction with the sterol moiety, which is present in the membrane of microbes and causes destruction of microbial cell membranes, causing leakage of cell contents.[31] On the other hand, flavonoids inhibit a perplexing number and variety

of prokaryotic enzymes and methylation of DNA is another way of antimicrobial activity of flavonoids.[32]

According to the present study, the result of the antibacterial activity test indicated that both the solvent fractions were found to have greater antibacterial effects against all the test bacteria with varying degrees of antibacterial activity spectrum. The reason could be due to the differences in the composition and concentrations of the secondary metabolites in the fractions. *Staphylococcus aureus* was the least susceptible bacteria to the chloroform and ethyl acetate fraction at equal concentrations compared to the other tested bacteria.[33]

Furthermore, the differences in the antibacterial spectrum of the ethyl acetate and the chloroform fraction against the test bacteria might be linked to the differences in the composition and the concentration of the secondary metabolites in the respective fractions.[31]

The groups of phytochemical compounds commonly associated with combating microbial resistance and having antimicrobial activity in medicinal plants are flavonoids, alkaloids, tannins, tritepenoids, essential oils, saponins, glycosides, and phenols.[34]

The presence of flavonoids in ethyl acetate and chloroform faction of the leaf of *A. schimperi* could contribute their own share for the observed antibacterial activities. The possible mechanism of action for the antibacterial effects of flavonoids includes damage or disruption of the cell membranes and inhibition of the synthesis of nucleic acids, which can lead to the death of the susceptible bacterium.[35]

Phenols are the other phytochemicals that might be involved in the antibacterial activities of medicinal plants used for different infectious diseases. The extracts of phenolic compounds have been found to have a growth inhibition effect on different bacteria (*E. coli, S. aureus and Salmonella typhi*).[36]

Tannins are the other compounds that have been found to have antimicrobial activities against the growth of bacteria. The antibacterial role of tannin constituents has been shown to inhibit and decrease the load and growth of aerobic, mouth cavity colonizing bacteria.[37]

According to the qualitative test of this study anthraquinones have been detected in the fraction of the leaves of *A. schimperi*. Thus, the antibacterial activities of the ethyl acetate and the chloroform fractions of the present study might be related to the presence of anthraquinones. This antibacterial action of anthraquinones has been strengthened by the growth inhibitory effect of different anthraquinone compounds such as emodin (isolated from *Rheum officinale*).[38]

The plausible antibacterial activity of anthraquinones has been associated with their interaction with the bacterial cell wall and cell membrane components, which can lead to the death of the bacteria as a result of leakage of cytoplasmic components and loss of cell integrity.[39]

Conclusion and Recommendations

Acokanthera schimperi is one of medically important plants which have variety of effects on treating infectious diseases. The present study revealed that the solvent fractions of the leaves of *A. schimperi* have antibacterial activities against the growth of the selected bacterial species with varying antibacterial spectrum. Therefore, the study provides a scientific basis for the traditional claimed use of the medicinal plant for the treatment of bacterial infections.

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Disclosure

The authors report no conflicts of interest in this work.

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