



ORIGINAL ARTICLE

IN VITRO INVESTIGATION OF THE POTENTIAL OF *FICUS EXASPERATA* EXTRACTS AGAINST BACTERIAL PATHOGENS ISOLATED FROM CHICKENS**Olasunkanmi Olawuwo^{1*}, Oyinlola Olaokun³, Abimbola Aro², Gift Omokhua-Uyi², Ibukun Famuyide², Prudence Kayoka-Kabongo¹, Lyndy McGaw²**¹Department of Agriculture and Animal Health, University of South Africa, South Africa; ²Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria, South Africa; ³Department of Biology, School of Science and Technology, Sefako Makgatho Health Sciences University, South Africa OPEN ACCESS*Correspondence: lasunstephen1@gmail.com

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Campylobacter species and *Escherichia coli* are leading causes of bacterial diarrhea in humans and livestock globally, often transmitted through food and water with poultry, pigs, and cattle, as reservoirs, are increasingly resistant to antibiotics, necessitating alternative therapeutic strategies. Building on previous reports of the antibacterial, cytotoxic, phytochemical, and antioxidant properties of *Ficus exasperata* leaf extracts as phyto-genic feed additives for chickens, this study expands the scope by evaluating additional clinical *E. coli* isolates with their selectivity index, genotoxicity assays and profiling the methanol extract using standard bioassays and GC-MS. The methanol extract demonstrated broad and best efficacy across all tested bacteria, though the aqueous had the highest total antibacterial activity (2 308 mL/g). The reference strains (ATCC) were more resistant than clinical isolates. The methanol extract showed the lowest cytotoxicity (LC₅₀: 0.99 mg/mL on Vero cells and 2.69 mg/mL on Caco-2 cells) and the highest selectivity indices (Vero: 6.33; Caco-2: 14.17). It was non-mutagenic to *Salmonella typhimurium* TA98 compared to acetone extract. The GC-MS analysis of methanol extract revealed 9-Octadecen-1-ol (24.52%) as the most abundant compound. The methanol extract showed promising potential for further *in vivo* testing as phyto-genic feed additives and alternatives for managing campylobacteriosis and colibacillosis in chickens.

Keywords: antibacterial; *E. coli* isolates; *Ficus exasperata*; GC-MS; methanol extract; selectivity index

INTRODUCTION

Poultry comprises the greatest reservoir of *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, which are the most common causes of bacterial diarrhoea in humans worldwide [1]. Similarly, the disease is zoonotic, and domestic animals such as poultry, pigs, and cattle may act as reservoirs. One of the critical public health problems in recent years is antimicrobial resistance in food-borne pathogens [2, 3]. Reports have shown the resistance of poultry and human *Campylobacter* isolates to the antibiotic ciprofloxacin due to veterinary application of enrofloxacin and other quinolones [3, 4]. According to the 2017 report of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), the most common causes of food-borne zoonotic diseases were *Campylobacter* and *Salmonella* bacteria [5]. In Canada, the annual incidence of food poisonings ranges between 3.1 and 5 million. In Australia, the incidence is 5.4 million [6, 7].

Avian pathogenic *Escherichia coli* (APEC) infection is zoonotic and also pathogenic to chickens. It has been responsible for great economic losses to the poultry industry worldwide [8]. The pathological presentations vary from localized, such as salpingitis or omphalitis, to systemic infections. Antimicrobial drugs can be used to reduce mortality in birds diagnosed with a localized or generalized bacterial infection. However, selection of antimicrobial drugs must be based on the susceptibility of isolated bacteria, as *E. coli* may be resistant to commonly used antibacterial drugs such as tetracycline, sulfonamides, ampicillin, and streptomycin [9, 10]. In humans, the enteropathogenic strains of *E. coli* (EPEC) cause diarrhoeal illness, especially in young infants, presenting symptoms such as watery diarrhoea with low-grade fever and vomiting and more severe cases resulting in death [11].

The ban on the use of antibiotics as growth promoters in livestock by the European Union occurred as a result of the emergence of antibiotic resistance in bacteria colonizing humans [12, 13], due to the development of resistant strains against conventional antibiotics. Therefore, feed companies and researchers are in search of alternative products and strategies that can help to maintain animal gut health in order to prevent or reduce the prevalence of pathogens in the food chain [14].

Ficus exasperata has been reported as a potential source of antimicrobial compounds. Major ethnomedicinal

usage has been reported throughout Africa, with Nigeria, Cameroon, Ivory Coast, and Sierra Leone being the folkloric hubs. Crude extracts have been reported to exhibit a wide spectrum of *in vitro* and *in vivo* pharmacological activities like antidiabetic, anticonvulsant, anti-inflammatory, antimicrobial, hypolipidemic, antioxidant, antiulcer, anxiolytic, and hypotensive [15, 16].

The consumption of anticarcinogens and antimutagens in the diet may be the most effective way of preventing human cancer [17]. Therefore, incorporating non-genotoxic, antimutagenic plants like *Ficus* leaf-based feed additives may contribute not only to poultry health and productivity but also to improved food safety, public health, and protection against diet-related carcinogenesis in humans.

Previous studies by Olawuwo et al. [18, 19] demonstrated the antibacterial and cytotoxic effects of *F. exasperata* leaf extracts against reference strains of *E. coli* and *Campylobacter* species and reported phytochemical and antioxidant activities of the acetone and aqueous extracts. The present study extends this work by including additional clinical isolates of *E. coli* with their selectivity index, genotoxicity assays and chemical profiling of the methanol extract investigating the antibacterial potential and safety of *F. exasperata* in the treatment of colibacillosis and campylobacteriosis in chickens.

MATERIALS AND METHODS

Plant collection

Fresh leaves of *F. exasperata* were collected from Ibadan Metropolis in the Lagelu Local Government Area of Oyo State, the southwestern part of Nigeria. The plant was identified by Mr Esimekhinai, D. P. O with a voucher specimen (UIH - 22626) and was deposited after identification in the herbarium of the Department of Botany, University of Ibadan.

Plant preparation and extraction

The fresh leaves were rinsed, air dried, and ground into powder. Three grams of the powdered material were weighed into centrifuge tubes, and 30 ml of acetone, methanol, and distilled water were added to separate aliquots and allowed to macerate for 24 h. The mixtures were centrifuged at 300×g for 10 min, and filtration was carried out using Whatman No. 1 filter paper. The resultant

extracts were transferred into pre-weighed, labelled glass vials. Resultant extracts were placed under a stream of air to dry completely and stored in a dark room at 4 °C while preparing for the experiment. The resultant extracts were reconstituted in their respective solvents to give the desired concentrations used in the study.

Microbial strains

Of the 11 pathogens used in this study, three were reference strains, American Type Culture Collection (ATCC), while eight were clinical isolates. The reference strains used were *Escherichia coli* (ATCC 25922), *Campylobacter coli* (ATCC 43478), and *Campylobacter jejuni* (ATCC 33560). Clinical isolates were (A) *Escherichia coli* (B 3427/16), (B) *Escherichia coli* (B 3584/16), (C) *Escherichia coli* (B 3412/16), (D) *Escherichia coli* (B 364/16), (E) *Escherichia coli* (B 3584/16), (F) *Escherichia coli* (B 3375/16), (G) *Escherichia coli* (B 3282/16), and (H) *Escherichia coli* (B 3397/16) from chickens. All were obtained from the Department of Veterinary Tropical Diseases (DVTD), Faculty of Veterinary Science, University of Pretoria.

Antibacterial assay

The antibacterial activity of acetone, methanol, and aqueous leaf extracts of *Ficus exasperata* against reference *Campylobacter* strains and clinical *E. coli* isolates from poultry was evaluated in vitro using a serial microdilution assay [20]. Clinical isolates were cultured in Mueller Hinton (MH) broth for *E. coli* and Brain Heart Infusion (BHI) broth for *Campylobacter*, incubated at 37 °C for 18–24 h, and adjusted to 3×10^8 cfu/ml (McFarland No. 1) by absorbance measurement at 560 nm. Plant extracts (10 mg/ml) were prepared and serially diluted in 96-well plates and inoculated with standardized bacterial suspensions; gentamicin (2 mg/ml) and acetone served as positive and negative controls, respectively. Plates containing *E. coli* were incubated at 37 °C for 24 h in a regular incubator, while those with *Campylobacter* were incubated anaerobically. Following incubation, 40 µl of p-iodonitrotetrazolium (INT, 0.2 mg/ml) was added to detect bacterial growth, and the minimum inhibitory concentration (MIC) was recorded as the lowest concentration with clear inhibition. The assay was conducted in triplicate and repeated twice for reliability.

Toxicological assays

In vitro cytotoxicity assay

The cytotoxicity of the plant extracts was evaluated against Vero monkey kidney and human intestinal (Caco-2) cell lines using the MTT assay as described by Mossman [21] and modified by McGaw et al. [22]. Cells grown in Minimal Essential Medium (MEM) supplemented with gentamicin and foetal calf serum were harvested, adjusted to 5×10^4 cells/ml, and seeded into 96-well plates, with columns 1 and 12 containing only MEM to minimize edge effects. After 24 h incubation at 37°C with 5% CO₂, cells were treated in quadruplicate with varying concentrations of plant extracts (prepared from 100 mg/ml stock) for 48 h, while doxorubicin served as a positive control and untreated cells as a negative control. Following treatment, cells were washed with PBS, supplied with fresh MEM, and incubated with MTT solution (5 mg/ml) for 4 h to allow formazan crystal formation, which was then solubilized in dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader, with blank wells (no cells) for correction. Cytotoxicity was expressed as the lethal concentration (LC₅₀), defined as the extract concentration causing a 50% reduction in absorbance relative to untreated controls.

In vitro genotoxicity assay

The plate incorporation assay, also known as the Ames test [23], was used to determine the genotoxicity of the selected plant extracts against *Salmonella typhimurium* strains TA98 and TA100. Acetone and methanol extracts were prepared at concentrations of 5, 0.5, and 0.05 mg/ml in DMSO under sterile conditions. The samples were tested against *S. typhimurium* strains TA98 and TA100 without an exogenous metabolic activation system. Briefly, 100 µl of each extract was added to the test tube, followed by 500 µl of phosphate buffer and 100 µl of a fresh overnight culture (prepared by inoculating 100 µl stock bacteria in 10 ml of Oxoid nutrient broth); then 2 ml of top agar with biotin/histidine was added. The mixture was centrifuged and poured into minimal agar plates. A positive control, 4-nitroquinoline-N-oxide (4-NQO) at a concentration of 2 µg/ml, negative controls of 10% DMSO and sterile distilled water were also prepared. The plates were incubated for 48 h at 37°C and a colony counter was used to count the colonies.

Gas chromatography-mass spectrometry (GC-MS) of methanol plant extract

Analysis of chemical constituents of methanol plant extract of *F. exasperata* with the best biological activities and industrial relevance was carried out in a LECO Pegasus 4D GC-TOFMS (LECO Africa (Pty) Ltd., Kempton Park, South Africa on a gas chromatography (GC) capillary column of dimension Rxi-1MS 30 m x 0.25 mm ID x 0.25 µm film thickness (Restek, Bellefonte, PA, USA). Gas chromatography (GC) UHP Helium (Afrox, South Africa) was used as a carrier gas at a flow rate of 1 ml/min. A 1 µL methanol solution of the sample was injected in a split mode at 10:1 with injector temperature at 250°C. The GC oven temperature was programmed at 40°C (held for 3 min), at 8°C/min to 300°C (hold for 5 min). The constant flow mode mass acquisition from 40-450 Da was maintained. The MS transfer line temperature was 280°C. The ion source temperature was maintained at 230°C. Compound spectra were detected by electron ionization system (70 eV), MS solvent delay 5 min and electron energy 70 eV in the electron ionisation mode (EI+). The relative quantity of the compounds present in the extract was expressed as a percentage based on the peak area produced in the chromatogram. The tentative identification was done by comparison of retention times with standard samples

and by matching their mass spectra at a rate of 10 spectra/s and a detector voltage of 1750 V against mass spectra of the National Institute of Standards and Technology (NIST) 14 Library.

Statistical analysis

Some of the data obtained from experiments were expressed as mean $\pm$ standard deviation (SD) and were subjected to descriptive statistics using the Student's t- test to compare two sample means. A one-way ANOVA for the test of significance between groups that were more than two and Tukey's post-hoc test were used to compare means of all samples using GraphPad Prism, version 5.01, April 2016, statistical software Chicago, IL, USA, where applicable.

RESULTS

Antibacterial activity (MIC) and total antibacterial activity (TAA)

The antibacterial activity of *F. exasperata* is presented in Table 1. The acetone and methanol extracts of *Ficus exasperata* were moderately active, with the lowest average MIC values for all tested organisms of 0.24 and 0.56 mg/

Table 1. Minimum inhibitory concentration (MIC) of acetone, methanol, and aqueous extracts of *F. exasperata* against *Campylobacter* strains and eight selected *Escherichia coli* isolates

Extracts	MIC (mg/ml)										Average MIC	
	<i>E. coli</i> R	<i>E. coli</i> (A)	<i>E. coli</i> (B)	<i>E. coli</i> (C)	<i>E. coli</i> (D)	<i>E. coli</i> (E)	<i>E. coli</i> (F)	<i>E. coli</i> (G)	<i>E. coli</i> (H)	<i>C. c.</i>		<i>C. j.</i>
Acetone	0.78	0.07	0.07	0.15*	0.11*	0.22*	0.18	0.22*	0.46*	0.05	0.30*	0.24*
Methanol	0.78	0.15*	0.07	0.07	0.07	0.62*	0.30*	0.15*	0.15*	2.50	1.25	0.56*
Aqueous	1.87	0.15*	0.03	0.34*	0.03	0.30*	0.62*	0.03	0.03	2.50	1.87	0.71
Gentamicin	>0.01	0.001	0.0006	0.001	0.0006	0.0003	0.0002	0.0002	0.0006	0.62	0.001	0.06

MIC < 0.1 mg/ml – Significantly active (**Bold**), 0.1 ≤ MIC ≤ 0.625 mg/ml – Moderately active*, MIC > 0.625 mg/ml, Gentamicin (2 mg/ml) – positive control, A–H – isolates, R – ATCC. *C. c.* – *C. coli*, *C. j.* – *C. jejuni*.

Table 2. The total antibacterial activity (TAA) in ml/g of acetone, methanol, and aqueous extracts of *Ficus exasperata* against typed *Campylobacter* strains and 8 selected *Escherichia coli* isolates

Total Activity													
Extracts	Yield (%)	<i>E. coli</i> (R)	<i>E. coli</i> (A)	<i>E. coli</i> (B)	<i>E. coli</i> (C)	<i>E. coli</i> (D)	<i>E.coli</i> (E)	<i>E. coli</i> (F)	<i>E. coli</i> (G)	<i>E. coli</i> (H)	<i>C. c.</i>	<i>C. j.</i>	Average
Acetone	3.33	42	476	476	222	303	151	185	151	72	666	111	259
Methanol	9.33	119	622	1 333	1 333	1 333	150	311	622	622	37	74	596
Aqueous	17.00	90	1 133	5 666	500	5 666	566	274	5 666	5 666	68	90	2 308

A–H – isolates, R – ATCC. *C. c.* – *C. coli*, *C. j.* – *C. jejuni*.

ml, respectively. The findings indicated that the methanol extract had the best antibacterial activity against all the tested bacteria.

The total antibacterial activity (TAA) is a function of the extraction yield in mg per 1 gram of plant material and the minimal inhibitory concentration (MIC), expressed in milliliter per gram (ml/g). The results for the TAA values of the extracts against *E. coli* isolates and *Campylobacter* species are presented in Table 2. The aqueous extract showed average TAA values of 2,308 ml/g, followed by the methanol extract with average TAA values of 596 ml/g, while acetone extracts had the lowest % yield with the lowest average TAA values of 259 ml/g.

Cytotoxicity and selectivity index (SI)

The LC_{50} values of the acetone, methanol, and aqueous extracts against Vero kidney cells were 0.09 ± 0.05 , 0.99 ± 0.23 , and 0.27 ± 0.21 mg/ml, respectively, while the LC_{50} values of the acetone, methanol, and aqueous extracts against Caco-2 cells were 1.82 ± 0.56 , 2.69 ± 0.44 , and 0.05 ± 0.00 mg/ml, respectively (Figure 1). The methanol extract had the highest LC_{50} (lowest toxicity) value against both Vero and Caco-2 cells (Figure 1).

The SI values for the acetone, methanol, and aqueous extracts against Vero and Caco-2 cells ranged from 0.10 to 14.14 and 0.02 to 38.42, respectively, with the methanol extract having the highest value. Selectivity index (SI) values for antimicrobial activity were calculated using the formula $SI = LC_{50}/MIC$, with units for LC_{50} and MIC in mg/ml.

Genotoxicity

The methanol extract was non-mutagenic against TA98 at all concentrations but toxic to TA100 at 5 mg/

ml, except for the acetone extract that showed clear mutagenicity with numbers of revertant colonies greater than twice that of the negative controls for the 5 mg/ml (the highest concentration tested) and also increased with increasing concentrations of extracts. The methanol extract was not toxic to *S. typhimurium* TA98 and TA100 though it showed a dose-dependent response against *S. typhimurium* TA100 (Table 4).

Gas chromatography-mass spectrometry (GC-MS) of methanol plant extract

The gas chromatographic-mass spectrometric analysis of the extract was done to elucidate the presence of any potential antibacterial and antioxidant compounds in the methanol extract of *F. exasperata* (Table 5, Figure 2). Out of ninety-eight metabolites, only 58 were identifiable; the most abundant compound with the largest yields exhibiting the highest peak areas in the extract is 9-Octadecen-1-ol (24.52%).

DISCUSSION

Antibacterial activity

Antibacterial activity was classified as active, moderately active, weak, or inactive following the benchmark established by Kuete [24] and Efferth and Kuete [25]. The findings indicated that the methanol extract had the best antibacterial activity against all chicken isolates but relatively weak activity against chicken *Campylobacter* species.

The total antibacterial activity (TAA) is a function of the extraction yield in mg per 1 gram of plant material and the minimal inhibitory concentration (MIC), expressed in ml per gram (ml/g) [26]. The TAA is a useful pharmacological criterion for the selection of plant species, and it also indicates which plant species could be the best source of extract for use by poor communities or for organic production [26].

The ethanol extract of the leaves of *F. exasperata* has been reported to have good inhibitory activity (300 mg/ml) against *E. coli* using a well diffusion assay [27]. The methanol extract of the bark of *Ficus religiosa* was found to be more active against all the enterotoxigenic *E. coli* using disc diffusion [28].

All the leaf extracts of *Ficus deltoidea* have been reported to show inhibitory activity against the fungus,

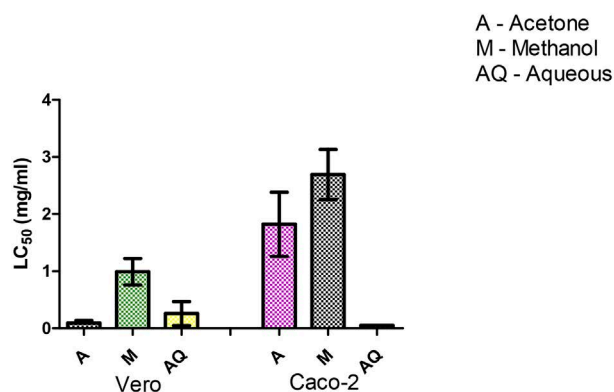


Fig. 1. Cytotoxicity (LC_{50}) of extracts against Vero kidney cells and Caco-2 cells

Table 3. Selectivity index (SI) against Vero kidney cells and Caco-2 cells

	SI Vero cells			SI Caco-2 cells		
	Acetone	Methanol	Aqueous	Acetone	Methanol	Aqueous
<i>E. coli</i> (R)	0.12	1.27	0.14	2.33	3.45	0.03
<i>E. coli</i> (A)	1.29	6.60	1.73	26	17.93	0.33
<i>E. coli</i> (B)	1.29	14.14	8.67	26	38.42	1.66
<i>E. coli</i> (C)	0.60	14.14	0.76	12.1	38.42	0.14
<i>E. coli</i> (D)	0.82	14.14	8.67	16.5	38.42	1.66
<i>E. coli</i> (E)	0.41	1.60	0.87	8.20	4.33	0.16
<i>E. coli</i> (F)	0.50	3.30	0.42	10.1	8.96	0.08
<i>E. coli</i> (G)	0.41	6.60	8.67	8.27	17.93	1.66
<i>E. coli</i> (H)	0.20	6.60	8.67	3.95	17.93	1.66
<i>C. coli</i>	1.80	0.40	0.10	36.40	1.07	0.02
<i>C. jejuni</i>	0.30	0.80	0.14	6.06	2.15	0.02
Average	0.70	6.33	3.53	14.17	17.18	0.67

A selectivity index > 1 is regarded to be safe (**Bold**), R – ATCC

Table 4. Genotoxicity of selected extracts against *Salmonella typhimurium* strains (TA 98 and TA100) presented as mean ± standard deviations

Plant Species	Extracts	<i>Salmonella typhimurium</i> strains					
		TA98			TA100		
		Concentration mg/ml			Concentration mg/ml		
<i>Ficus exasperata</i>		5 mg/ml	0.5 mg/ml	0.05 mg/ml	5 mg/ml	0.5 mg/ml	0.05 mg/ml
		Number of revertant colonies per concentration					
	Acetone	264.50±28.99	46.50±7.78	19.00±1.41	262.50±0.71	282.00±7.07	217.50±17.68
	Methanol	13.00±1.41	5.50±0.71	10.50±2.12	244.50±0.71	217.00±1.41	174.50±3.54
4NQO		133 ± 1.21			491.50±4.95		
10% DMSO	21.50±2.12				260.00±2.83		
Water	13.50±2.12				244.50±6.36		

DMSO – dimethyl sulphoxide, 4NQO – 4-nitroquinoline-N-oxide

gram-positive and gram-negative bacteria strains tested, except for the chloroform and aqueous extracts on *Bacillus subtilis*, *E. coli*, and *Pseudomonas aeruginosa* using disc diffusion [29]. Koona and Rao [30] reported that the methanol extract of the leaf of *Ficus benghalensis* displayed high antibacterial activity against all tested bacteria, while Gayathri and Kannabiran [31] observed that the aqueous extract of the bark of the same plant also had active inhibition (0.04 mg/ml to 0.1 mg/ml) against the tested bacteria.

To the best of our knowledge the findings from this study comprise the first report on the antibacterial activity of *F. exasperata* against different chicken clinical isolates of *E. coli*. The different reported findings on the antibacterial activities of this plant and other related species could be attributed to geographical location, age of plant at harvest, season of harvest, and method of extraction, all of

which affect the yield of active constituents of medicinal plants [32, 33]. This can also be due to differences in laboratory procedures and reagents used [34, 35].

Cytotoxicity and selectivity index (SI)

Three extracts of *F. exasperata* leaves were tested against Vero kidney and Caco-2 cell lines for general and local toxicity, respectively. Makhafola et al. [36] stated that until cellular toxicity tests are conducted, no natural products or crude plant extracts can be considered safe for usage. It is expected that the sensitivity of the cell lines to the extracts will be different because of different metabolic activities and uptake capabilities [37]. The human intestinal cell line (Caco-2) has been reported for its known uptake capabilities [38]. Therefore, the choice of Caco-2 cells was attributable to its uptake capabilities and being

Table 5. Major compounds detected in the methanol crude leaf extract of *F. exasperata*

S/N	RT (s)	Compounds	MW	Molecular formula	% Peak Area	Similarity Index (%)
1	739.6	n-Hexane	86	C ₆ H ₁₄	0.25251	95.5
2	801.3	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	139	C ₇ H ₉ NO ₂	0.037975	72.4
3	816	Hexadecane	226	C ₁₆ H ₃₄	0.075317	89.6
4	987.7	Eicosane	282	C ₂₀ H ₄₂	0.083463	85.2
5	1030.9	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	180	C ₁₁ H ₁₆ O ₂	0.063582	87.5
6	1059.8	1,3-Benzenediol, o-(2-methylbenzoyl)-o'-(3-cyclopentylpropionyl)-	352	C ₂₂ H ₂₄ O ₄	0.048256	81.2
7	1069.4	Diethyl Phthalate	222	C ₁₂ H ₁₄ O ₄	0.13902	94.8
8	1116.7	aR-Turmerone	216	C ₁₅ H ₂₀ O	1.0882	90.5
9	1117.1	n-Tridecan-1-ol	200	C ₁₃ H ₂₈ O	1.0882	84.1
10	1175.7	Tetradecanoic acid	228	C ₁₄ H ₂₈ O ₂	0.20765	83.6
11	1190.3	3-Methylbenzoic acid, 2-formyl-4,6-dichlorophenyl ester	308	C ₁₅ H ₁₀ Cl ₂ O ₃	0.050817	79.8
12	1191.2	2-Undecanethiol, 2-methyl-	202	C ₁₂ H ₂₆ S	0.050817	78.8
13	1196.4	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	196	C ₁₁ H ₁₆ O ₃	0.10085	81.5
14	1220.8	Neophytadiene	278	C ₂₀ H ₃₈	0.95137	91.0
15	1225.4	2-Pentadecanone, 6,10,14-trimethyl-	268	C ₁₈ H ₃₆ O	0.71471	85.1
16	1244.2	Phthalic acid, isobutyl nonyl ester	348	C ₂₁ H ₃₂ O ₄	0.004233	85.6
17	1247.3	1-Hexadecanol	242	C ₁₆ H ₃₄ O	12.262	94.0
18	1247.7	Cyclohexene, 3-propyl-	124	C ₉ H ₁₆	12.392	70.7
19	1272.9	2-Piperidinone, N-[4-bromo-n-butyl]-	233	C ₉ H ₁₆ BrNO	0.2513	79.7
20	1273.5	Pentadecanoic acid, 14-methyl-, methyl ester	270	C ₁₇ H ₃₄ O ₂	0.2513	80.6
21	1279.7	Cyclotetradecane	196	C ₁₄ H ₂₈	0.070195	89.4
22	1287.7	Isophytol	296	C ₂₀ H ₄₀ O	0.086541	73.3
23	1288.7	Benzenepropanoic acid, 3,5-bis(1,1 dimethylethyl)-4-hydroxy-, methyl ester	292	C ₁₈ H ₂₈ O ₃	0.086541	74.4
24	1299.7	n-Hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂	3.4626	92.0
25	1302	Dibutyl phthalate	278	C ₁₆ H ₂₂ O ₄	0.52773	93.8
26	1312.5	Oxalic acid, allyl octadecyl ester	382	C ₂₃ H ₄₂ O ₄	0.11053	79.9
27	1313.2	Hexadecanoic acid, ethyl ester	284	C ₁₈ H ₃₆ O ₂	0.11053	75.2
28	1349.9	Cyclooctene, 3-ethenyl-	136	C ₁₀ H ₁₆	0.12643	78.7
29	1354	9-Octadecen-1-ol, (E)-	268	C ₁₈ H ₃₆ O	24.521	95.4

Table 5: (Continued)

S/N	RT (s)	Compounds	MW	Molecular formula	% Peak Area	Similarity Index (%)
30	1365.8	Hexadecen-1-ol, trans-9-	240	C ₁₆ H ₃₂ O	8.6044	94.1
31	1374.9	9-Octadecenoic acid (Z)-, methyl ester	296	C ₁₉ H ₃₆ O ₂	0.089798	74.5
32	1376.1	3-Tetradecen-5-yne, (Z)-	192	C ₁₄ H ₂₄	0.35967	74.3
33	1382.8	Phytol	296	C ₂₀ H ₄₀ O	2.3539	90.7
34	1400.9	1,8,11,14-Heptadecatetraene, (Z,Z,Z)-	232	C ₁₇ H ₂₈	0.87074	71.6
35	1409.9	Octadecanoic acid	284	C ₁₈ H ₃₆ O ₂	0.78268	87.6
36	1412.5	Decanedioic acid, dibutyl ester	314	C ₁₈ H ₃₄ O ₄	0.51516	89.5
37	1423.9	Dodecanamide	199	C ₁₂ H ₂₅ NO	0.42652	80.7
38	1451.9	cis-9,10-Epoxyoctadecan-1-ol	284	C ₁₈ H ₃₆ O ₂	0.15911	85.7
39	1462	1-Octadecyne	250	C ₁₈ H ₃₄	0.19828	79.5
40	1465.1	cisZ-11,12-Epoxytetradecan-1-ol	228	C ₁₄ H ₂₈ O ₂	0.2618	77.9
41	1473.5	Cyclopentane, heneicosyl-	364	C ₂₆ H ₅₂	0.12349	73.9
42	1473.7	1-Hexadecanol	242	C ₁₆ H ₃₄ O	0.12349	70.2
43	1493.9	Oxirane, tetradecyl-	240	C ₁₆ H ₃₂ O	0.15779	79.4
44	1511.2	4,8,12,16-Tetramethylheptadecan-4-olide	324	C ₂₁ H ₄₀ O ₂	0.21983	92.4
45	1515.8	9-Octadecenamide, (Z)-	281	C ₁₈ H ₃₅ NO	1.344	87.8
46	1524.9	trans-Farnesol	222	C ₁₅ H ₂₆ O	0.14699	72.9
47	1527.7	Dodecanamide	199	C ₁₂ H ₂₅ NO	0.43441	88.9
48	1602.9	Bis(2-ethylhexyl) phthalate	390	C ₂₄ H ₃₈ O ₄	0.12006	90.3
49	1690.6	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	390	C ₂₄ H ₃₈ O ₄	0.058469	77.0
50	1706.1	9-Octadecenamide, (Z)-	281	C ₁₈ H ₃₅ NO	0.53898	86.6
51	1724.1	Squalene	410	C ₃₀ H ₅₀	0.38526	92.2
52	1823.9	γ-Tocopherol	416	C ₂₈ H ₄₈ O ₂	0.002837	79.0
53	1855.2	Cholesta-4,6-dien-3-ol, (3á)-	384	C ₂₇ H ₄₄ O	0.37283	75.3
54	1876.7	DL-α-Tocopherol	430	C ₂₉ H ₅₀ O ₂	0.13892	850
55	1879.7	26-Nor-5-cholesten-3á-ol-25-one	386	C ₂₆ H ₄₂ O ₂	0.57127	74.8
56	1948.7	5-Cholestene-3-ol, 24-methyl-	400	C ₂₈ H ₄₈ O	0.37861	70.3
57	1970.4	Stigmasterol	412	C ₂₉ H ₄₈ O	0.2277	81.6
58	2015.5	á-Sitosterol	414	C ₂₉ H ₅₀ O	4.7134	89.3

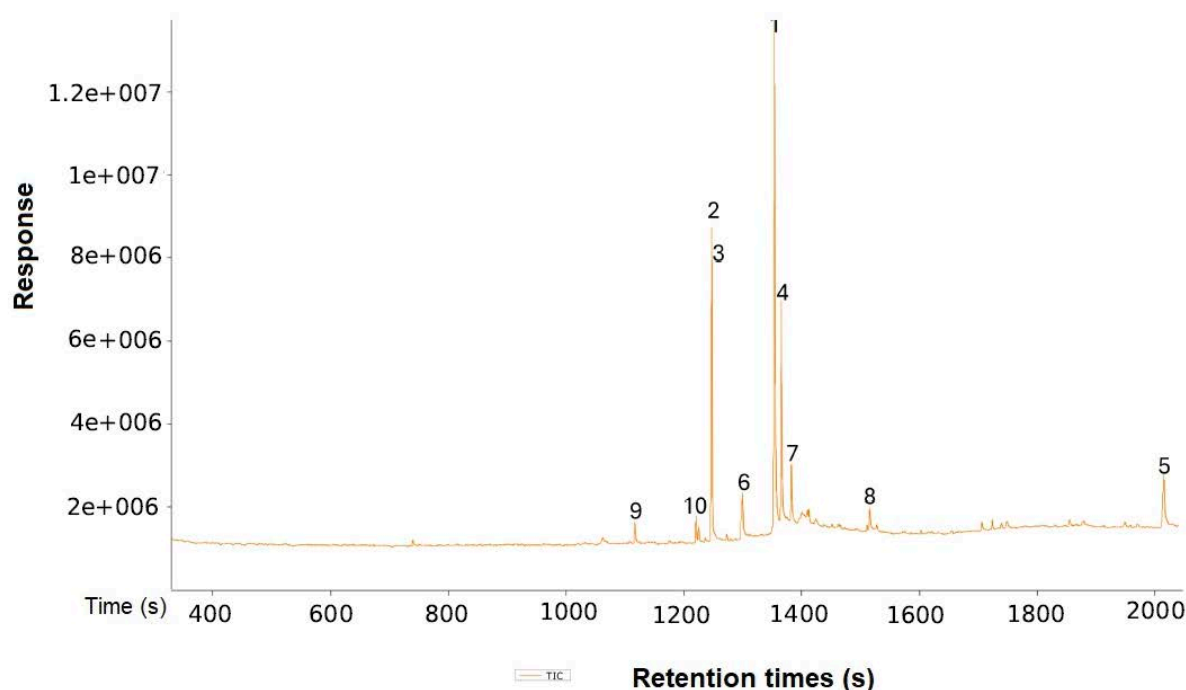


Fig. 2. GC-MS chromatogram of methanolic extract of *F. exasperata*

(1) 9-Octadecen-1-ol (24.52%), (2) Cyclohexene,3-propyl- (12.39%), (3) 1-Hexadecanol (12.26%), (4) Hexadecen-1-ol,trans-9- (8.60%), (5) α -Sitosterol (4.71%), (6) n-Hexadecanoic acid (3.46%), (7) Phytol (2.35%), (8) 9-Octadecenamide,(Z)- (1.34%), (9) α R-Turmerone (1.08%) and (10) n-Tridecan-1-ol (1.08%).

an absorptive surface for the bioactive ingredients in the feed additives.

In another study by Pinto [39], it was demonstrated that these cells, upon differentiation, expressed several morphological and biochemical characteristics of small intestinal enterocytes. In order to reduce the use of experimental animals for toxicity testing, the Caco-2 cell model has been considered for the development of alternative *in vitro* toxicity tests. Moreover, the gastrointestinal tract can be a direct target for several toxicants. The extracts with $LC_{50} > 0.1$ mg/ml are considered not cytotoxic [40]. Two cell lines were evaluated in this study since it may be misleading to determine the safety and utility of a plant extract using just one.

Similar to our findings, Sowemimo et al. [41] reported that the ethanol extract of *F. exasperata* was not toxic to brine shrimps and rat lymphocyte chromosomes with no inhibition in the telomerase activity. Likewise, Bafor and Igbinuwen [42] observed no toxicity on the haematological parameters and changes in physiological activity after oral and intraperitoneal administration of the aqueous extract in mice during the acute toxicity test.

Kudumela et al. [43] and McGaw et al. [44] reported that SI values greater than or equal to 10 are preferred;

however SI above 1 is an indication that the biological activity of the plant extracts is higher than their cellular toxicity. The higher the SI values, the safer the extracts. Therefore, the methanol extract is the safest of all the tested extracts.

Similarly, it has been reported that cell line sensitivity in Caco-2 cells decreased progressively from more distal regions of the gastrointestinal tract: gastric > duodenal > ileal > colonic, although further study would be required to determine whether this is a phenomenon that might be relevant to intestinal uptake rates [37].

Genotoxicity

For an extract to be genotoxic, the number of revertant colonies should be equal to or greater than twice the average of the negative control and a clear dose-dependent response should be observed for the various concentrations tested [23]. The methanol extract was not toxic to *S. typhimurium* TA98 and TA100, though it showed a dose-dependent response against *S. typhimurium* TA100. Our findings in this study are similar to those of Satish et al. [45], they reported that *Ficus benghalensis* stem bark exhibited non-toxicity in *S. typhimurium* strains against induced mutagenicity of sodium azide (NaN_3).

Flavonoids have been reported to possess significant antimutagenic activity, such as glaberrone, quercetin, myricetin, kaempferol, and hesperidin [46]. Natural antimutagens from edible and medicinal plants are of particular importance because they may be useful for human cancer prevention [47].

Gas Chromatography-Mass Spectrometry (GC-MS)

The solvent used for the extraction [48] and the seasonal variation and soil type [49] are among the determining factors for the chemical composition of a plant. It has been reported that phenolic compounds are more soluble in highly polar solvents, such as methanol [50]. Methanol extract from this current study had better antibacterial activity across all the tested pathogens and was industrially relevant and the safest among the tested extracts with good total antibacterial activity (TAA). In addition, it has the highest selectivity index (SI) against Vero and Caco-2 mammalian cells.

Therefore, the methanol extract of *F. exasperata* was chosen for analysis using Gas Chromatography-Mass Spectrometry (GC-MS); it reveals the presence of several bioactive compounds with notable antimicrobial and antioxidant activities and, in some cases, anticancer activities. Among the most abundant compounds identified are 9-Octadecen-1-ol which has demonstrated antimicrobial activity as reported by Mugayi and Mukanganyame [51], suggesting its potential role in the inhibitory effect of the extract against microbial pathogens. Cyclohexene, 3-propyl- was previously identified in the essential oil of *Cyperus articulatus*; this compound exhibited significant antimicrobial (antibacterial and antifungal) and antioxidant activities against a wide range of pathogens, including *E. coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Aspergillus flavus*, and *Trichoderma harzianum* [52], indicating its broad-spectrum bioactivity.

1-Hexadecanol was known for its antimicrobial and antioxidant properties; its compound supports the potential of the methanol extract of *F. exasperata* in combating oxidative stress and microbial infections [53]. Hexadecen-1-ol, trans-9- was identified in *Indigofera longeracemosa*; this compound showed specific antimicrobial activity with agar disc diffusion, particularly against fish pathogens such as *Aeromonas hydrophila* (3 ± 0.2 mm) and *Aeromonas sobria* (9 ± 0.1 mm), although it was inactive against *Vibrio harveyi* [54]. Its presence in *F. exasperata*

suggests selective antimicrobial capabilities. The α -Sitos-terol was detected in the methanol extract of *Heliotropium bacciferum* Forssk, this compound has been widely reported for its antibacterial and antifungal effects [55].

Generally, these compounds highlight the multifaceted therapeutic potential of the methanol extract of *F. exasperata*, especially its antimicrobial and antioxidant properties. Their individual and synergistic actions could explain the biological efficacy of the extract and support its possible use in pharmaceutical or feed additive formulations targeting microbial infections and oxidative stress.

CONCLUSION

This study demonstrated the antibacterial potential of *F. exasperata* leaf extracts against antibiotic-resistant *Campylobacter* species and *E. coli*. The methanol extract showed superior efficacy, low cytotoxicity, high selectivity indices, and no mutagenic effects, indicating good safety. Although the aqueous extract had higher total antibacterial activity, it was less biologically robust overall. Clinical isolates were more susceptible than reference strains, highlighting practical relevance. GC-MS analysis identified 9-Octadecen-1-ol as the dominant compound likely contributing activity. Despite *in vitro* limitations, the methanol extract showed promise as a phytochemical feed additive, warranting further *in vivo* and formulation studies.

Data Availability Statement

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Generative AI Statement

I acknowledge using Grammarly (<https://grammarly.com/>) to improve the grammar and punctuation of this research paper.

Authorship

Conceptualization, supervision and reviewing: Olanekanmi Olanuwu, Ibukun Famuyide and Lyndy McGaw; Data analysis: Olanekanmi Olanuwu, Abimbo

la Aro and Oyinlola Olaokun; Manuscript writing, reviewing and editing: Olasunkanmi Olawuwo, Ibukun Famuyide, Abimbola Aro, Gift Omokhua-Uyi, Prudence Kayoka-Kabongo and Lyndy McGaw.

Conflict of Interest

All authors of this study declare no conflict of interest.

Ethical Consideration

The presented work did not avail human participation and animal use. However, the study, protocol number REC 232-19, was approved by the faculty ethics committee, Faculty of Veterinary Science, University of Pretoria.

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REFERENCES

1. Olson, C. K., Ethelberg, S., van Pelt, W., Tauxe, R. V., 2008: Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. *Campylobacter*, 121–38. DOI: [10.1128/9781555815554.ch9](https://doi.org/10.1128/9781555815554.ch9)
2. Gupta, A., Nelson, J. M., Barrett, T. J., Tauxe, R.V., Rositer, S. P., Friedman, C. R., et al., 2004: Antimicrobial resistance among *Campylobacter* strains, United States, 1997–2001. *Emerg. Infect. Dis.*, 10, 6, 1102–1109. DOI: [10.3201/eid1006.030635](https://doi.org/10.3201/eid1006.030635)
3. EFSA, 2014: The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012. *EFSA Journal*, 12, 3, 3590. DOI: [10.2903/j.efsa.2014.3590](https://doi.org/10.2903/j.efsa.2014.3590)
4. Engberg, J., Neimann, J., Nielsen, E. M., Aarestrup, F. M., Fussing, V., 2004: Quinolone-resistant *Campylobacter* Infections: Risk Factors and Clinical Consequences. *Emerg. Infect. Dis.*, 10, 6, 1056–1063. DOI: [10.3201/eid1006.030669](https://doi.org/10.3201/eid1006.030669)
5. Guillier, L., Gourmelon, M., Lozach, S., Cadel-Six, S., Vignaud, M. L., Munck, N., et al., 2019: Tracing the source of bacterial strains based on accessory genes. Application to *Salmonella* Typhimurium environmental strains. *bioRxiv*, 814459. DOI: [10.1101/814459](https://doi.org/10.1101/814459)
6. Keerthirathne, T. P., Ross, K., Fallowfield, H., Whiley, H., 2016: A review of temperature, pH, and other factors that influence the survival of *Salmonella* in mayonnaise and other raw egg products. *Pathogens*, 5, 4, 63. DOI: [10.3390/pathogens5040063](https://doi.org/10.3390/pathogens5040063)
7. Rohde, A., Hammerl, J. A., Al Dahouk, S., 2016: Detection of foodborne bacterial zoonoses by fluorescence *in situ* hybridization. *Food Control*, 69, 297–305. DOI: [10.1016/j.foodcont.2016.05.008](https://doi.org/10.1016/j.foodcont.2016.05.008)
8. Hussein, A. H. M., Ghanem, I. A. I., Eid, A. A. M., Ali, M. A., Sherwood, J. S., Li, G., et al., 2013: Molecular and phenotypic characterization of *Escherichia coli* isolated from broiler chicken flocks in Egypt. *Avian Dis.*, 57, 3, 602–611. DOI: [10.1637/10503-012513-Reg.1](https://doi.org/10.1637/10503-012513-Reg.1)
9. Miles, T. D., McLaughlin, W., Brown, P. D., 2006: Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. *BMC Vet. Res.*, 2, 7, 1–9. DOI: [10.1186/1746-6148-2-7](https://doi.org/10.1186/1746-6148-2-7)
10. Vandemaele, F., Assadzadeh, A., Derijcke, J., Vereecken, M., Goddeeris, B. M., 2002: Avian pathogenic *Escherichia coli* (APEC). *Tijdschr Diergeneesk.*, 127, 19, 582–588.
11. Nataro, J. P., Kaper, J. B., 1998: Diarrheagenic *Escherichia coli*. *Clin. Micro. Rev.*, 11, 1, 142–201. DOI: [10.1128/cmr.11.1.142](https://doi.org/10.1128/cmr.11.1.142)
12. Schwarz, S., Kehrenberg, C., Walsh, T. R., 2001: Use of antimicrobial agents in veterinary medicine and food animal production. *Int. J. Antimicrob. Agents*, 17, 6, 431–437. DOI: [10.1016/S0924-8579\(01\)00297-7](https://doi.org/10.1016/S0924-8579(01)00297-7)
13. Ezerskis, Z., Robouch, P., von Holst, C., 2012: EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003.
14. Santini, C., Baffoni, L., Gaggia, F., Granata, M., Gasbarri, R., Di Gioia D., et al., 2010: Characterization of probiotic strains: An application as feed additives in poultry against *Campylobacter jejuni*. *Int. J. Food Microbiol.*, 141, S98–S108. DOI: [10.1016/j.ijfoodmicro.2010.03.039](https://doi.org/10.1016/j.ijfoodmicro.2010.03.039)
15. Ahmed, F., Ahmed, M. K. K., Abedin, M. Z., Karim, A. A., 2012: Traditional uses and pharmacological potential of *Ficus exasperata* Vahl. *Syst. Rev. Pharm.*, 3, 1, 15–23.

16. Singh, D., Singh, B., Goel, R. K., 2011: Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: A review. *J. Ethnopharmacol.*, 134, 3, 565–583. DOI: [10.1016/j.jep.2011.01.046](https://doi.org/10.1016/j.jep.2011.01.046)
17. Farombi, E. O., 2004: Diet-related cancer and prevention using anticarcinogens. *Afr. J. Biotechnol.*, 3, 12, 651–661. Available from: <https://www.ajol.info/index.php/ajb/article/view/15034>
18. Olawuwo, O. S., Abdalla, M. A., Mühling, K. H., McGaw, L. J., 2021: Proximate analysis of nutrients and in vitro radical scavenging efficacy in selected medicinal plant powders with potential for use as poultry feed additives. *S. Afr. J. Bot.*, 146, 103–110. DOI: [10.1016/j.sajb.2021.09.038](https://doi.org/10.1016/j.sajb.2021.09.038)
19. Olawuwo, O. S., Famuyide, I. M., McGaw, L. J., 2022: Antibacterial and antibiofilm activity of selected medicinal plant leaf extracts against pathogens implicated in poultry diseases. *Front. Vet. Sci.*, 9. DOI: [10.3389/fvets.2022.820304](https://doi.org/10.3389/fvets.2022.820304)
20. Eloff, J. N., 2007: A Sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, 64, 8, 711–713. DOI: [10.1055/s-2006-957563](https://doi.org/10.1055/s-2006-957563)
21. Mosmann, T., 1983: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65, 1–2, 55–63. DOI: [10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4)
22. McGaw, L. J., Elgorashi, E. E., Eloff, J. N., 2007: Cytotoxicity of african medicinal plants against normal animal and human cells. In: Kuete, V., editor. *Toxicological Survey of African Medicinal Plants*, 181–233. DOI: [10.1016/B978-0-12-800018-2.00008-X](https://doi.org/10.1016/B978-0-12-800018-2.00008-X)
23. Maron, D. M., Ames, B. N., 1983: Revised methods for the Salmonella mutagenicity test. *Mutation Research/Environmental Mutagenesis and Related Subjects*, 113, 3–4, 173–215. DOI: [10.1016/0165-1161\(83\)90010-9](https://doi.org/10.1016/0165-1161(83)90010-9)
24. Kuete, V., 2010: Potential of cameroonian plants and derived products against microbial infections: A Review. *Planta Medica*, 76, 14, 1479–1491. DOI: [10.1055/s-0030-1250027](https://doi.org/10.1055/s-0030-1250027)
25. Efferth, T., Kuete, V., 2010: Cameroonian medicinal plants: pharmacology and derived natural products. *Front Pharmacol.*, 1, 123, 1–19. DOI: [10.3389/fphar.2010.00123](https://doi.org/10.3389/fphar.2010.00123)
26. Eloff, J. N., McGaw, L. J., 2006: Plant extracts used to manage bacterial, fungal, and parasitic infections in Southern Africa. In: Ahmad, I, Aqil, F., Owais, M., editors. *Modern Phytomedicine*, John Wiley & Sons, Ltd., pp. 97–121. DOI: [10.1002/9783527609987.ch5](https://doi.org/10.1002/9783527609987.ch5)
27. Odunbaku, O. A., Ilusanya, O. A., Akasoro, K. S., 2008: Antibacterial activity of ethanolic leaf extract of *Ficus exasperata* on *Escherichia coli* and *Staphylococcus albus*. *Sci. Res. Essay*, 3, 11, 562–564.
28. Uma, B., Prabhakar, K., Rajendran, S., 2009: In vitro Antimicrobial activity and phytochemical analysis of *Ficus religiosa* L. and *Ficus bengalensis* L. against diarrhoeal enterotoxigenic *E. coli*. *Ethnobotanical Leaflets*, 2009, 4.
29. Abdsamah, O. A., Zaidi, N. T. A., Sule, A. B., 2012: Antimicrobial activity of *Ficus deltoidea* Jack (mas cotek). *Pak. J. Pharm. Sci.*, 25, 3, 675–678.
30. Koona, S. J., Rao, B. S., 2012: In vitro evaluation of antibacterial activity of crude extracts of *Ficus benghalensis* Linn., the banyan tree leaves. *Indian J. Nat. Prod. Resour.*, 3, 2, 281–284.
31. Gayathri, M., Kannabiran, K., 2009: Antimicrobial activity of *Hemidesmus indicus*, *Ficus bengalensis* and *Pterocarpus marsupium* roxb. *Indian J. Pharm. Sci.*, 71, 5, 578–81. DOI: [10.4103/0250-474X.58182](https://doi.org/10.4103/0250-474X.58182)
32. Calixto, J. B., 2000: Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz. J. Med. Biol. Res.*, 33, 2, 179–189. DOI: [10.1590/S0100-879X2000000200004](https://doi.org/10.1590/S0100-879X2000000200004)
33. Evans, W.C., 2009: *Trease and Evans' Pharmacognosy*. Elsevier Health Sciences; 16th ed. 2009. pp. 3322.
34. Bonini, P., Plebani, M., Ceriotti, F., Rubboli, F., 2002: Errors in laboratory medicine. *Clin. Chem.*, 48, 5, 691–698. DOI: [10.1093/clinchem/48.5.691](https://doi.org/10.1093/clinchem/48.5.691)
35. Wallach, J. B., 2007: *Interpretation of Diagnostic Tests*. Lippincott Williams & Wilkins., pp. 1202.
36. Makhafola, T. J., Samuel, B. B., Elgorashi, E. E., Eloff, J. N., 2012: Ochnaflavone and Ochnaflavone 7-O-Methyl Ether two antibacterial biflavonoids from *Ochna pretoriensis* (Ochnaceae). *Nat. Prod. Comm.*, 7, 12. DOI: [10.1177/1934578X1200701216](https://doi.org/10.1177/1934578X1200701216)
37. Froscio, S. M., Fanok, S., Humpage, A. R., 2009: Cytotoxicity screening for the cyanobacterial toxin cylindrospermopsin. *J. Toxicol. Environ. Health*, 72, 5, 345–349. DOI: [10.1080/15287390802529906](https://doi.org/10.1080/15287390802529906)
38. Meunier, V., Bourrié, M., Berger, Y., Fabre, G., 1995: The human intestinal epithelial cell line Caco-2; pharmacological and pharmacokinetic applications. *Cell Biol. Toxicol.*, 11, 3, 187–194. DOI: [10.1007/BF00756522](https://doi.org/10.1007/BF00756522)
39. Pinto, M., Robine-Leon, S., Appay, M. D., Keding, M., Triadou, N., Dussaulx, E., et al., 1983: Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biol. Cell*, 47, 323–330.
40. Sambuy, Y., De Angelis, I., Ranaldi, G., Scarino, M. L., Stammati, A., Zucco, F., 2005: The Caco-2 cell line as

- a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biol. Toxicol.*, 21, 1–26. DOI: [10.1007/s10565-005-0085-6](https://doi.org/10.1007/s10565-005-0085-6)
41. Sowemimo, A. A., Fakoya, F. A., Awopetu, I., Omobuwa-jo, O. R., Adesanya, S. A., 2007: Toxicity and mutagenic activity of some selected Nigerian plants. *J. Ethnopharm.*, 113, 3, 427–432. DOI: [10.1016/j.jep.2007.06.024](https://doi.org/10.1016/j.jep.2007.06.024)
 42. Bafor, E. E., Igbinuwen, O., 2009: Acute toxicity studies of the leaf extract of *Ficus exasperata* on haematological parameters, body weight and body temperature. *J. Ethnopharm.*, 123, 2, 302–307. DOI: [10.1016/j.jep.2009.03.001](https://doi.org/10.1016/j.jep.2009.03.001)
 43. Kudumela, R. G., Masoko, P., 2018: In vitro assessment of selected medicinal plants used by the Bapedi community in South Africa for treatment of bacterial infections. *J. Evid.-Based Integr. Med.*, 23. DOI: [10.1177/2515690X18762736](https://doi.org/10.1177/2515690X18762736)
 44. McGaw, L. J., Steenkamp, V., Eloff, J. N., 2007: Evaluation of *Athrixia* bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. *J. Ethnopharm.*, 110, 1, 16–22. DOI: [10.1016/j.jep.2006.08.029](https://doi.org/10.1016/j.jep.2006.08.029)
 45. Satish, A., Punith Kumar, R., Rakshith, D., Satish, S., Ahmed, F., 2013: Antimutagenic and antioxidant activity of *Ficus benghalensis* stem bark and *Moringa oleifera* root extract. *Int. J. Chem. Anal. Sci.*, 4, 2, 45–48. DOI: [10.1016/j.ijcas.2013.03.008](https://doi.org/10.1016/j.ijcas.2013.03.008)
 46. Miyazawa, M., Hisama, M., 2003: Antimutagenic activity of flavonoids from *Chrysanthemum morifolium*. *Biosci. Biotechnol. Biochem.*, 67, 10, 2091–2099. DOI: [10.1271/bbb.67.2091](https://doi.org/10.1271/bbb.67.2091)
 47. Malik, S., Kaur, K., Prasad, S., Jha, N. K., Kumar, V., 2022: A perspective review on medicinal plant resources for their antimutagenic potentials. *Environ. Sci. Pollut. Res.*, 29, 62014–62029. DOI: [10.1007/s11356-021-16057-w](https://doi.org/10.1007/s11356-021-16057-w)
 48. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., Lightfoot, D. A., 2017: Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6, 4, 42. DOI: [10.3390/plants6040042](https://doi.org/10.3390/plants6040042)
 49. Saravanakumar, A., Ganesh, M., Peng, M. M., Aziz, A. S., Jang, H. T., 2015: Comparative antioxidant and antimycobacterial activities of *Opuntia ficus-indica* fruit extracts from summer and rainy seasons. *Frontiers in Life Sci.*, 8, 2, 182–191. DOI: [10.1080/21553769.2015.1028655](https://doi.org/10.1080/21553769.2015.1028655)
 50. Osorio-Tobón, J. F., 2020: Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *J. Food Sci. Technol.*, 57, 12, 4299–4315. DOI: [10.1007/s13197-020-04433-2](https://doi.org/10.1007/s13197-020-04433-2)
 51. Mugayi, L. C., Mukanganyama, S., 2024: Antimycobacterial and antifungal activities of leaf extracts from *Trichilia emetica*. *Scientifica*, 1, 8784390. DOI: [10.1155/sci5/8784390](https://doi.org/10.1155/sci5/8784390)
 52. Azzaz, N. A. E., El-Khateeb, A. Y., Farag, A. A., 2014: Chemical Composition and Biological Activity of the Essential Oil of *Cyperus articulatus*. *Int. J. Acad. Res.*, 6, 5, 265–269.
 53. Omotoso, D. R., Olubowale, V. O., Aina, F. M., Daramola, O. O. O., 2024: Phytochemical Profiling of *Basella alba* Using Gas Chromatography-Mass Spectrometry. *Trop. J. Nat. Prod. Res.*, 8, 6, 7561–7565.
 54. Subash, N., Raju, G., 2014: GC-MS analysis and antibacterial activity of stem of *Indigofera longeracemosa* Boiv. *Ex Baill. Nat. Pharm. Technol.*, 4, 2, 1–6.
 55. Abdelkhalek, A., Elshaer, M., Al-Askar, A., Kowalcze-wski, P., Behiry, S., 2024: Antimicrobial activities and metabolites profiling of *Heliotropium bacciferum* Forssk. methanolic extract. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 52, 3, 13602. DOI: [10.15835/nbha52313602](https://doi.org/10.15835/nbha52313602)