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Influence of selected plant extracts on bacterial motility, aggregation, hydrophobicity, exopolysaccharide production and quorum sensing during biofilm formation of enterohaemorrhagic *Escherichia coli* O157:H7

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ABSTRACT

Diarrhoeagenic *Escherichia coli* is a serious threat to human and animal health, with antimicrobial drug resistance contributing to the problem. Alternative mechanisms of prevention and treatment of *E. coli*-induced diarrhoea include those targeting biofilm production and quorum sensing, properties that have been associated with some investigated plant extracts. Plants were selected from various genera, including *Vachellia, Senegalia, Morus, Leucaena, Salix, Grewia, Ziziphus, Searsia, Dichrostachys* and *Ceratonia*, based on their known antimicrobial activity. The antibacterial effects of leaf extracts against multidrug-resistant *E. coli* O157:H7 were investigated using a broth microdilution method, and cytotoxicity to normal mammalian cell lines was studied using a tetrazolium colorimetric assay. Subsequently, antibiofilm activity and inhibition of extracellular polymeric substance (EPS) production by plant extracts against *E. coli* O157:H7 was investigated using crystal violet as a staining dye after 0 and 24 h incubation and carbohydrate estimation 5 % phenol-sulfuric assay. The biosensor strain *Chromobacterium violaceum* ATCC 12472 was used to study the anti-quorum sensing potential of the selected plant extracts. Cell motility, cell surface hydrophobicity (CSH), and aggregation were also investigated. All plant extracts had weak antibacterial activity (MIC > 0.62 mg/ml) with relatively low cytotoxicity. The acetone extracts of *Salix babylonica* and *Leucaena leucocephala* prevented bacterial cell attachment (0 h) by 81.21 % and 89.36 %, respectively. Approximately 28 % of plant extracts eradicated established biofilms by more than 50 %, however, they were unable to inhibit EPS production above 30 %. The half-maximal concentration of extracts generally inhibited violacein production (ranging from 0.01 to 0.02 mg/ml), with *V. erioloba* acetone extract being the most effective at quorum quenching. Some plant extracts exhibited a significant difference ($p<0.05$) in motility, aggregation and hydrophobicity compared to untreated cells. This study highlights the potential of selected plant extracts to act via different mechanisms of action to decrease virulence of enteric *E. coli*, motivating further investigation of the bioactive compounds in the leaves.

1. Introduction

Disease outbreaks caused by enterohaemorrhagic *E. coli* (EHEC), among which is the pathotype O157: H7, are considered a serious problem in many countries including Japan, Europe and North America (Pakbin et al., 2021). Diarrhoeagenic *E. coli* is also becoming more frequent in African countries, including Ethiopia, Nigeria and South Africa (Ateba and Mbewe, 2011; Gambushe et al., 2022). In South Africa, bacterial and viral diarrhoea accounts for 19 % of infant deaths and a total of 46 % of infant mortality for the entire African continent

Abbreviations: EPS, Extracellular polymeric substances; CSH, cell surface hydrophobicity; EHEC, enterohaemorrhagic *E. coli*; HUS, haemolytic uremic syndromes; Stx, Shiga toxin; AHLs, acylated homoserine lactones; QS, quorum sensing; MDR, multidrug resistant; MIC, minimum inhibitory concentration; INT, *p*-iodonitrotetrazolium; MH, Mueller Hinton; ATCC, American Type Culture Collection; CFU, colony-forming unit; MTT, 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide; DMEM, Dulbecco's Modified Eagle's Medium; FCS, foetal calf serum; DMSO, dimethyl sulfoxide; LC_{50}, Lethal concentration 50%; PBS, phosphate-buffered saline; OD, optical density; MQSIC, minimum quorum sensing inhibitory concentration; SI, selectivity index; LB, Luria-Bertani; SAT, salt aggregation test; CV, crystal violet; TSB, tryptic soy broth; NCI, United States National Cancer Institute

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(Awotiwon et al., 2016). Cattle are asymptomatic carriers of *E. coli* O157:H7 and consumption by humans of food and water contaminated with faeces can result in diarrhoea (Ateba and Bezuidenhout, 2008).

When food or water contaminated with *E. coli* O157:H7 is ingested by humans, the pathogen adheres to and colonizes the colon epithelial cells, releasing Shiga toxin (Stx). Stx is a virulence factor of EHEC that causes physiological damage to the vascular system of the intestinal mucosa, causing watery diarrhoea that may advance to more severe haemorrhagic colitis (Liu et al., 2023). Once in the blood circulation, the Stx binds to the granulocytes and platelets and is transferred to the kidney, resulting in haemolytic uremic syndrome (Liu et al., 2023).

Diarrhoeagenic *E. coli* strains are an economic burden to the livestock industry, affecting broilers, swine, cattle, and other farm animals (Gomes et al., 2016). The conventional way to control bacterial infections is prophylactic use of antibiotics within the feed, as well as therapeutic antibiotic use when indicated. The European Union has prohibited the use of antibiotics as a feed additive for growth promotion (Vondruskova et al., 2009). Unfortunately, over-prescribing and incorrect use of antibiotics commonly used to treat bacterial infections have led to global antibiotic resistance in several microbe species (Ventola, 2015; Serwecińska, 2020). Strains of *E. coli,* including O157:H7 isolates from humans and animals, have been reported to be multi-drug resistant (MDR) to antibiotics, including tetracycline, chloramphenicol, ampicillin, amoxicillin-clavulanate, cephalexin, ciprofloxacin, cefotaxime, gentamicin, amikacin and others (Bischoff et al., 2002; Ateba et al., 2008; Abdalla et al., 2021). Mupfunya et al. (2021) reported an increasing trend in *E. coli* antimicrobial resistance in the Mnisi community in the Mpulamanga province, South Africa. Humans are exposed to bacterial resistance genes from consumption of residual antibiotic-contaminated animal products or exposure to antibiotic-contaminated environments.

Resistance is a defense strategy developed by microorganisms to overcome the effectiveness of drugs, thereby surviving exposure to antibiotic drugs (Tanwar et al., 2014). Pathogenic bacteria are difficult to treat because of their ability to form biofilm amongst other mechanisms. A biofilm is defined as a community of aggregated cells organized as microcolonies attached to a biotic or abiotic surface and embedded in an organic polymer matrix (Song et al., 2018). According to Chen et al. (2018), biofilms are 10−1 000 times more resistant to antibiotics than planktonic bacteria. *E. coli* 0157:H7 has been extensively studied for its ability to form biofilm on various surfaces including vegetables, fruits, colon epithelial cells and surfaces in meat processing facilities (Zhao et al., 2023). The formation of biofilm is regulated by four steps, including (i) reversible and irreversible attachment to a surface (ii) bacterial adhesion to the surface and release of extracellular polymeric substances (EPS), (iii) colony formation and maturation of biofilm, and lastly (iv) dispersion of the cells to new niches, contributing to a new biofilm life cycle (Sharma et al., 2019; Lu et al., 2019). The EPS matrix is composed of eDNA, proteins, and polysaccharides, which allows for stronger adhesion of bacteria to the host cells, and formation of a protective barrier against antibiotics (Song et al., 2018). This matrix is extremely difficult to disrupt. Antimicrobial resistance in veterinary and human medicine may continue for a long time (Andersson and Hughes, 2010), indicating the need to discover and develop a new class of antibiotic effective against infectious diseases with antibiofilm potential.

Bacterial cell attachment to a host surface depends on Brownian movement, van de Waals forces, and electrostatic forces. Additionally, cell surface hydrophobicity (CSH) is crucial for the attachment or detachment of cells to an abiotic or biotic surface and subsequent biofilm formation (Krasowska and Sigler, 2014). Furthermore, enteropathogenic *E. coli* is an autoaggregator, meaning that the microorganism facilitates bacteria-bacteria interaction to adhere to the surface of host intestinal cells to initiate diarrhoea (Sherlock et al., 2005; Nwoko and Okeke, 2021). Autoaggregation properties also allow the bacterial cells to protect themselves against foreign substances such as antibiotics or unfavorable nutritional conditions (Trunk et al., 2018). On the other hand, cell motility such as twitching and swarming allows a bacterial cell to migrate and interact with the host during the pathogenesis of infectious diseases. Swarming and twitching motility of bacteria due to surface appendages such as flagella and pili allows bacteria to escape to a favorable environment (Wadhwa and Berg, 2022).

Maturation of biofilm is controlled by the signalling process called quorum sensing (QS) (Camele et al., 2019; Sharma et al., 2019). This cell-to-cell communication recognizes the bacterial population density by sensing and measuring the accumulation of specific self-produced signal molecules known as autoinducers that regulate gene expression and determine virulence (Lu et al., 2019). Gram-positive bacteria use acylated homoserine lactones (AHLs) as autoinducers, which activate violacein production in *Chromobacterium violaceum* (González and Keshavan, 2006; Pei et al., 2023). The QS system regulates several activities in bacteria, including pathogenicity, virulence, bioluminescence, motility, symbiosis and antibiotic resistance, as well as EPS production (Bouyahya et al., 2017).

Traditional medicine has been widely used for the treatment of gastrointestinal infections including diarrhoea for humans and animals (Elisha et al., 2017; Adeyemo et al., 2022). South Africa is rich in shrubs or trees containing structurally diverse phytochemicals that may be potential antimicrobial agents, as well as possessing antidiarrhoeal and anti-biofilm formation potential against *E. coli* strains (Elisha et al., 2017; Derebe et al., 2018; Famuyide et al., 2019). Plants synthesize a variety of secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, steroids, terpenoids and tannins (Motlhatlego et al., 2020). Numerous studies have reported that certain of these phytochemicals are able to reduce biofilm in vitro and in vivo (Mishra et al., 2020).

The Fabaceae (Leguminosae) is the largest plant family with species widely distributed in southern Africa. This includes plant species such as *Vachellia* spp. and *Senegalia* spp., *Dichrostachys cinerea, Ceratonia siliqua* and *Leucaena leucocephala,* which have been traditionally used in South Africa for treatment of gastrointestinal ailments in humans and animals (Shandukani et al., 2018; Husein et al., 2020; Mazimba et al., 2022). In addition, *Searsia lancea* (Anacardiaceae) has been reported to have in vitro antibacterial activity against diarrhoeagenic *E. coli* (Ahmed et al., 2014; Adeyemo et al., 2022). *Ziziphus mucronata* (Rhamnaceae), *Morus alba* (Moraceae), *Grewia* species (Malvaceae) and *Salix babylonica* (Salicaceae) have previously shown good antibacterial activity against *E. coli* causing diarrhoea (González-Alamilla et al., 2019; Mongalo et al., 2020; Bhandari et al., 2021 Mongalo et al., 2022).

There is sufficient evidence in the literature that the selected plants have antimicrobial and antidiarrhoeal effects against *E. coli*. However, antibiofilm and anti-quorum sensing effects as well as other mechanisms of the plant extracts have not been fully explored. Thus, it can be hypothesised that extracts of the selected plant species are able to potentially prevent or eradicate biofilm formed by *E. coli* 0157:H7 and inhibit the QS signaling pathway involved in diarrhoea. The plant extracts may also have the ability to inhibit bacterial motility and EPS production, additional beneficial traits for an anti-diarrhoeal medication.

2. Materials and methods

2.1. Plant collection, preparation and extraction

Leaves of fifteen plant species and pod material of *Vachellia nilotica* were collected from communal rangelands in Pretoria North, Gauteng, South Africa between April 2017 and September 2020. These plants were selected based on their pharmacological and

traditional use in treatment of diarrhoea (Table 1). For authentication and identification, voucher specimens were prepared and lodged in the H.G.W.J. Schweickerdt Herbarium at the University of Pretoria (PRU, South Africa). Selection of the suitable extraction solvent is crucial in discovering the most effective antibacterial crude extracts or compounds. Methanol, acetone and other organic solvents have been used successfully to isolate antimicrobial phytochemicals (Eloff, 1998a; Cowan, 1999). The collected material was oven-dried (40 °C) and ground to fine powder. In glass jars, ten grams (g) of each dried material were separately extracted with 100 mL of acetone and methanol. The glass jars were vigorously shaken for 24 h on an orbital shaker. Then, they were filtered through Whatman No. 1 filter paper. The filtrate was transferred into pre-weighed glass vials and placed under a stream of air to evaporate to dryness.

2.2. Preparation of inoculum

E. coli O157:H7 ATCC 43888 and other reference strains including *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were purchased from the American Type Culture Collection (ATCC). They were maintained in cryotubes containing a 15 % glycerol solution and stored in a freezer at − 80 °C.

2.3. Microdilution assay

The antibacterial assays were carried out using the broth serial microdilution method in U-bottom sterile 96 microplates (Eloff, 1998b). *E. coli* strains were prepared by inoculating a single colony of each bacterial strain from an agar plate into sterilised Mueller Hinton (MH) broth (Sigma Aldrich, South Africa) and grown overnight in a shaking incubator (120 rpm) at 37 °C. The cultures were standardized to 0.5 McFarland standard equivalent to 1.5×10^8 CFU/ml. One hundred μl of sterile water was added to each well, and this was followed by the addition of 100 μl aliquots of 10 mg/ml plant extracts (dissolved in acetone/methanol) in the first well and this was serially diluted two-fold along the ordinate. Subsequently, 100 μl of each of the appropriately adjusted bacterial cultures were added to all the wells except the broth and culture control. Gentamicin (Virbac) and acetone/ methanol were used as positive and negative controls, respectively. Plates were incubated aerobically in an incubator at 37 °C. To each well of the incubated plates, 40 μl of 0.2 mg/ml *p*-iodonitrotetrazolium (INT) was added. Then the plates were further incubated at 37 °C for 30 min before reading the minimum inhibitory concentration (MIC). The last well with clear inhibition of bacteria reflected as a decrease in red colour was recorded as the MIC.

2.4. In vitro cytotoxicity assay

The cytotoxicity of the 32 plant extracts was investigated against Vero cells (African green monkey kidney cells ATCC® CCL-81™) using the 3-(4,5-dimethylthiazol)−2,5-diphenyl tetrazolium bromide (MTT) assay (Mosmann, 1983). The cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco™, NYC) supplemented with 5 % foetal calf serum (FCS) and 1 % gentamicin in a 5 % CO_2 incubator at 37 °C until confluence. Cell suspensions (1×10^5 cells/ml) were seeded in wells of 96-well microtitre plates for 24 h at 37 °C. Plant extracts (100 mg/ml) were dissolved in methanol or acetone and diluted to concentrations of 100, 75, 50, 25, 12.5 and 7.5 μg/ml using DMEM. After cell attachment, 100 μl of plant extracts were added to each well containing cells and incubated at 37 °C in a 5 % CO_2 incubator for 48 h. Doxorubicin (Pfizer laboratories) and acetone or methanol served as positive and negative controls, respectively. Subsequently, plates were washed with 100 μl phosphate-buffered saline (PBS), and 200 μl of fresh medium was added into each well. Then, 30 μl of MTT (5 mg/ml in PBS) was added to each well and the plates were incubated for 4 h at 37 °C. The content in the wells was aspirated and 50 μl of dimethyl sulfoxide (DMSO) was added to the wells to solubilize the formed formazan crystals. The absorbance was measured on a microplate reader at a wavelength of 570 nm. Each extract concentration was determined in triplicate, and the assay was repeated three times. The extract concentration causing 50 % inhibition of cell viability (LC_{50}) was obtained from using a regression line between cell viability and respective concentrations. Selectivity index (SI) values for antimicrobial activity were calculated using the following formula;

$$Selectivity\ index(SI) = LC_{50}/MIC$$

2.5. Biofilm forming potential

Effective conditions for *E. coli* strains to form biofilm were determined using the method of Mohsenipour and Hassanshahian (2015) with modifications from Adeyemo et al., al.(2022). Growth parameters such as incubation temperature (37 °C and 30 °C) and growth medium with four combinations i.e. i) only tryptic soy broth (TSB), ii) TSB supplemented with 1.2 % glucose, iii) only Mueller-Hinton Broth (MHB) and iv) MHB supplemented with 2 % glucose were investigated in this study. Briefly, cultures equivalent to 0.5 McFarland standard (1.5×10^8 CFU/ml) were prepared from an overnight culture of test bacteria grown in MHB only or supplemented with 2 % or 1.2 % glucose or TSB only. The standardized bacteria were further diluted 1:100 to obtain an inoculum of 1.5×10^6 CFU/ml. A 100 μl aliquot of the diluted inoculum was dispensed into the well. Duplicate plates were covered with parafilm and incubated for 24 h at 37 °C or 30 °C. The biofilm formation of the bacteria was determined quantitatively using a crystal violet stain. The biofilm-forming ability was then classified based on the following: (a) non-biofilm former if ODtest ≤ ODc, (b) weak biofilm former if ODc < ODtest ≤ 2 × ODc, (c) moderate biofilm former if 2 × ODc < ODtest ≤ 4 × ODc, and (d) strong biofilm former if ODtest > 4 × ODc, where ODc is the mean ODmedia control + (3 × standard deviation of ODmedia ctrl) and ODtest is the mean optical density of the tested bacterial strain. Only the bacteria with strong biofilm-forming capacity were considered for biofilm formation inhibition and eradication of pre-formed biofilm.

2.6. Inhibition of bacterial biofilm formation

The inhibition of biofilm formation by acetone and methanol extracts was assessed (Sandasi et al., 2010; Mohsenipour and Hassanshahian, 2015) with modifications (Adeyemo et al., 2022). The prevention of biofilm attachment (planktonic) (0 h) and disruption of pre-formed biofilm (24 h) were investigated. Briefly, for 0 h, 100 μl standardized bacterial culture (OD_{590} = 0.02 equivalent to 1×10^6 CFU/ml) prepared in TSB was inoculated into sterile flat bottomed 96-well microtitre plates followed by the addition of 100 μl plant extract (sub-MIC of 0.16 mg/ml) and incubated for 24 h at 37 °C without shaking. For 24 h, the biofilm was allowed to form at 37 °C before exposure to plant extracts. Extraction solvents (methanol and acetone) and gentamicin served as negative and positive controls, respectively. The biofilm biomass was assayed using the modified crystal violet (CV) staining method (Baloyi et al., 2019). The absorbance was measured at 590 nm on a microplate reader (Biotek, USA). The readings were recorded in quadruplicate. The percentage inhibition was calculated using the following formula;

$$\text{Percentage inhibition} = \frac{\text{ODcontrol} - \text{ODtest}}{\text{ODcontrol}} \text{ x } 100$$

2.7. Inhibition of EPS production by extracts during biofilm development of E. coli O157:H7

The effects of plant extracts on EPS production during biofilm development were investigated (Rubini et al., 2018). Aliquots of

Table 1
Tree/shrub species collected from Onderstepoort, Pretoria based on traditional use and pharmacological activities.

Family	Scientific name	Common name	Traditional use	Pharmacological activity	Voucher number	References
Anacardiaceae	*Searsia lancea* (L.f.) F.A. Barkley	Karee (E), Moshabele (T)	Diarrhoea	Antibacterial, anti-biofilm, anti-quorum sensing	PRU131536	(Ahmed et al., 2014; Maroyi, 2017; Adeyemo et al., 2022)
Boraginaceae	*Ehretia rigida* (Thunb.) Druce	Puzzle bush (E), morobe (T)		Antibacterial	PRU131533	(Mnikathi, 2021)
Fabaceae	*Ceratonia siliqua* L.	Carob (E)		Antibacterial	PRU125471	(Fadhil Abdul-Hussein, 2018; Abdul-kareem et al., 2020)
	Dichrostachys cinerea (L.) Wight & Arn	Sickle bush (E), ugagane (Z)	Diarrhoea, stomachache, dysentery, stomach and intestinal disorder	Antibacterial	PRU131904	(Shandukani et al., 2018)
	Leucaena leucocephala (Lam.) de Wit	White popinac (E), white leadtree, ubobo (Z)	Diarrhoea	Antibacterial, Anti-diarrhoeal	PRU131537	(Shandukani et al., 2018; Husein et al., 2020; Mazimba et al., 2022)
	Senegalia galpinii (Burtt Davy) Seigler & Ebinger	Monkey-thorn (E), molopa (NS)	Diarrhoea, haemorrhage	Antibacterial	PRU125472	(Adhikari and Rangra, 2023)
	Vachellia erioloba (E.Mey.) P.J.H.Hurter	Camel thorn (E)	Diarrhoea	Antibacterial	PRU131903	(Marius et al., 2018)
	Vachellia gerrardii (Benth.) P.J.H. Hurter subsp. *gerrardii*	Red thorn (E), moki (T)	Diarrhoea, dysentery, colic, stomachache	Antibacterial	PRU125477	(Omwenga et al., 2017; Hinai et al., 2020)
	Vachellia karroo (Hayne) Banfi & Glasso	Sweet thorn (E), mooka (T)	Diarrhoea, dysentery, haemorrhage, stomachache	Antibacterial	PRU125472	(Olajuyigbe and Afolayan, 2012; Maroyi, 2017; Adhikari and Rangra, 2023)
	Vachellia nilotica (L.) P.J.H. Hurter & Mabb. subsp. *kraussiana* (Benth) Kyal. & Boatwr	Scented-pod acacia (E), motsha (T)	Diarrhoea	Antibacterial, anti-diarrhoeal, anti-qorum sensing	PRU125475	(Misar et al., 2007; Ezeamagu et al., 2013; Akintunde et al., 2015; Alain et al., 2022)
	Vachellia sieberiana (DC.) Kyal. & Boatwr. var. *woodii* (Burtt Davy) Kyal. & Boatwr	Paperbark thorn (E), morumosetlha (T)	Diarrhoea, haemorrhage	Antibacterial, anti-diarrhoeal	PRU125469	(Dawurung et al., 2019; Ngaffo et al., 2020)
	Vachellia tortilis (Forssk.) Gallaso & Banfi	Umbrella thorn (E), mosu (T)	Diarrhoea, dysentery, gastric irritation, stomachache, indigestion	Antibacterial	PRU12473	(Van der Merwe et al., 2001; Hinai et al., 2020)
Malvaceae	*Grewia monticola* Sond.	Silver raisin (E), mogwana (T)	Stomach and intestinal disorders		PRU131534	(Wickens, 1980)
Moraceae	*Morus alba* L.	White mulberry (E)		Antibacterial	PRU131532	(Bhandari et al., 2021; Chen et al., 2021)
Rhamnaceae	*Ziziphus mucronata* Willd. subsp. *mucronata*	Buffalo thorn (E), mokgalo (T)	Diarrhoea, dysentery	Antibacterial	PRU94270	(Mongalo et al., 2022)
Salicaceae	*Salix babylonica* L.	Weeping willow (E)		Antibacterial	PRU131536	(Hutchings, 1996; González-Alamilla et al., 2019)

E, Z, T, NS denotes English, Zulu, Tswana and Northern Sotho.

100 μl of inoculum of 1 $\times$ 10^6 CFU/ml were added into wells of 96-flat bottom microplates. The plates were incubated for 24 h at 37 °C to initiate biofilm formation. Afterward, the different extracts at a concentration of 0.16 mg/ml (sub-MIC) were exposed to the biofilm community and incubated at 37 °C for 24 h. The unattached cells were removed, and the cells were resuspended into 60 μl of PBS (pH 7). To quantify the exopolysaccharides, the carbohydrates test was employed (Masuko et al., 2005). To the bacterial cells, 50 μl of concentrated sulphuric acid and 50 μl of 5 % phenol were added. The plates were incubated for 24 h at room temperature. The absorbance was measured at 490 nm on a microplate reader. The experiments were replicated thrice.

2.8. Inhibition of violacein production in Chromobacterium violaceum ATCC12472

Since most Gram-negative bacteria have N-acyl homoserine lactone (AHL) quorum-sensing systems, the use of *C. violaceum* to evaluate the quorum-sensing inhibitory activity of *E. coli* is highly applicable(Pei et al., 2023). The QS assay was performed as previously reported (Vasavi et al., 2013) with modifications in 48-well plates. Different concentrations of plant extracts (0.65–0.10 mg/ml) were resuspended in Luria-Bertani (LB) medium, and 500 μl was added into microplate wells. Thereafter, 500 μl of an overnight culture (1.5 $\times$ 10^8 CFU/ml) of *C. violaceum* grown in LB at 30 °C was aliquoted into each well. The plates were incubated on a shaker at 30 °C for 24 h. The minimum inhibitory concentration (MIC) (no violet pigment and no turbidity), and minimum quorum sensing inhibitory concentration (MQSIC) (turbidity and violet pigment or violaceum production) were determined. After incubation, the plates were centrifuged at 978 x g for 11 min, and the supernatant was discarded. To the pellets, 50 % dimethyl sulfoxide in methanol was added, and the plates were left in the incubator at 30 °C for 1 h. The absorbance was determined at 575 nm. The half-maximal inhibitory (IC_{50}) concentrations were determined.

2.9. Inhibition of swarming and twitching motility of E. coli

Flagella-directed swarming motility assays were performed using Luria-Bertani (LB) medium supplemented with 5 % glucose and 5 % of agar (Magnini et al., 2021). The agar for twitching motility included 1 % tryptone, 0.5 % NaCl, 0.5 % yeast extract, and 1 % agar (Semmler et al., 1999). A tiny hole was stabbed into the agar with a sterile pipette tip. The bacterial cells were standardised to 1 McFarland (3.0 $\times$ 10^8 cfu/ml) were grown to an OD_{600} of 1.0, and approximately 3 μl of culture was introduced into the hole on the agar using a sterile pipette tip. Thereafter, in the same hole the bacterial cells were treated with 2 μl of 0.16 mg/ml of plant extracts and methanol was added as a control. The motility was assessed by measuring the diameter (mm) traveled by the bacteria on the semi-solid agar after 24, 48 and 72 h. The experiment was repeated twice.

2.10. Inhibition of E. coli aggregation by salt aggregation assay (SAT)

The ability of *E. coli* O157:H7 to aggregate under the influence of plant extracts was determined using a modification of the standard salt aggregation test (SAT) (Rosenberg et al., 1980; Rosenberg, 1984; Voravuthikunchai and Limsuwan, 2006). Briefly, cultures of *E. coli* strains on nutrient agar were suspended in phosphate buffer at pH 7 and adjusted to the 0.5 McFarland turbidity standard (1.5 $\times$ 10^8 CFU/ml). Aliquots of 50 μl of plant extracts (0.16 mg/ml) were added to 50 μl of the standardized bacterial cell suspension in U-shaped microtitre plate wells. Subsequently, 100 μl of ammonium sulfate solutions (final concentration 0.05, 0.25, 0.5, 0.75, and 1.5 M) were added. The microtitre plates were then gently shaken (80 rpm) for 5 min and incubated at room temperature for 30 min before bacterial aggregation was estimated. The presence of aggregation was determined by sedimentation. The SAT result was defined as positive when bacterial aggregation was visible and negative when no aggregation was observed. The SAT titer was defined as the lowest concentration of ammonium sulfate solution at which microbes still yielded visible aggregation. Strains autoaggregating in potassium phosphate buffer and/or expressing SAT titers of 0.05 and 0.25 were considered highly aggregative or hydrophobic, and strains with titers of 0.5 to 1.5 were considered low aggregative. Strains were considered non-aggregative if they did not produce a positive SAT result even at a 1.5 M concentration of ammonium sulfate (Voravuthikunchai and Limsuwan, 2006). The experiment was repeated three times.

2.11. Inhibition of cell surface hydrophobicity (CSH) using the bacterial adhesion to hydrocarbon (BATH) assay

To determine the effects of extracts on bacterial cell surface adhesion to hydrocarbons, the method of Rosenberg et al. (1980) was followed with modifications. Briefly, 1 ml of *E. coli* 0157:07 inoculum (1.5 $\times$ 10^8 CFU/ml) was treated with 0.2 ml of 0.16 mg/ml plant extracts and untreated bacteria were grown in LB overnight at 37 °C. After incubation, the cells were resuspended in a phosphate buffer (0.1 M, pH = 7) to obtain an OD of 0.3 (1 McFarland or 3.0 $\times$ 10^8 CFU/ml) at a wavelength of 550 nm (A0). To 1 ml of cell suspension, 0.4 ml of toluene (hydrocarbon) was added. This suspension was equilibrated by vortexing for 1 min. The tubes were then placed in an incubator at 37 °C for 15 min for phase separation, after which the aqueous phase was removed, and its absorbance was measured at 600 nm (A1). The results were expressed as the percentage of bacterial adherence to hydrocarbons (A) as compared with the control using the following equation:

$$A = (A0 - A1/A0)x100$$

2.12. Statistical analysis

Results are shown as the mean of three determinations were calculated with Microsoft Excel. The statistical value of the difference $p<0.05$ between the treatment and control groups was interpreted by one-way analysis of variance (ANOVA) using the SPSS package (version 10.0) and Tukey's post hoc test where appropriate. Microsoft Excel was employed to illustrate the graphs.

3. Results

3.1. Antibacterial and cytotoxic effects of extracts against three E. coli strains

In general, the results presented in Table 2 showed that the selected plant methanol and acetone extracts had antibacterial effects with MIC ranging from 0.63 to 2.5 mg/ml against the three *E. coli* strains. In addition, the lethal concentration (LC_{50}) of the 32 plant extracts against mammalian Vero cells was high compared to that of doxorubicin, the positive control. Noticeably, the methanol extracts of *V. gerrardii* and *Z. mucronata* had the lowest cytotoxicity compared to other extracts. The LC_{50} values of these extracts were higher than 1 mg/ml (the highest concentration tested), so for purposes of calculating the SI, values, LC_{50} was taken as being equal to 1 mg/ml. The SI is inversely proportional to the MIC, thus the better the antibacterial activity the higher the SI.

3.2. Biofilm forming capacity of E. coli

Results in Table 3 indicate that the *E. coli* strains tested are weak (ATCC 25922) to strong biofilm formers (ATCC 35218 and O157:H7) when grown with TSB and incubated at 37 °C for 24 h. *E. coli* 0157:H7

Table 2
MIC (mg/ml), selectivity index (in brackets), and cytotoxicity (LC_{50} in mg/ml).

Plant species	MIC (mg/ml)						LC_{50} (mg/ml)	
	ATCC 295225		ATCC 35218		ATCC 0157:H7			
	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone
C. siliqua	1.25 (0.33)	0.63 (1.14)	1.25 (0.33)	1.25 (0.57)	1.25 (0.33)	1.25 (0.57)	0.41	0.71
D. cinerea	0.63 (0.93)	0.63 (1.15)	1.25 (0.93)	0.63 (1.15)	1.25 (0.46)	1.25 (0.58)	0.58	0.72
E. rigida	1.25 (0.54)	1.25 (0.13)	1.25 (0.54)	1.25 (0.13)	1.25 (0.54)	1.25 (0.13)	0.67	0.16
G. monticola	1.25 (0.58)	1.25 (0.28)	1.25 (0.58)	1.25 (0.28)	1.25 (0.58)	1.25 (0.28)	0.72	0.35
L. leucocephala	1.25 (0.14)	2.50 (0.24)	1.25 (0.14)	1.25 (0.48)	1.25 (0.14)	1.25 (0.48)	0.18	0.60
M. alba	2.50 (0.28)	1.25 (0.38)	1.25 (0.55)	1.25 (0.38)	1.25 (0.55)	1.25 (0.38)	0.69	0.47
S. babylonica	1.25 (0.60)	1.25 (0.45)	2.50 (0.30)	1.25 (0.45)	2.50 (0.30)	2.50 (0.22)	0.75	0.56
S. galpinii	0.63 (0.64)	1.25 (0.42)	1.25 (0.32)	0.63 (0.83)	1.25 (0.32)	1.25 (0.42)	0.40	0.52
S. lancea	1.25 (0.59)	1.25 (0.10)	1.25 (0.59)	1.25 (0.10)	1.25 (0.59)	1.25 (0.10)	0.74	0.13
V. erioloba	1.25 (0.47)	1.25 (0.67)	1.25 (0.47)	0.63 (0.67)	1.25 (0.47)	1.25 (0.34)	0.59	0.42
V. gerrardii	1.25 (0.80)	2.50 (0.42)	0.63 (1.60)	1.25 (0.42)	1.25 (0.80)	1.25 (0.42)	1.00	0.53
V. karroo	0.63 (0.70)	1.25 (0.78)	1.25 (0.35)	0.63 (0.78)	1.25 (0.35)	1.25 (0.39)	0.44	0.49
V. nilotica	1.25 (0.07)	1.25 (0.13)	1.25 (0.07)	1.25 (0.13)	2.50 (0.04)	1.25 (0.13)	0.09	0.16
V. sieberiana	2.50 (0.28)	1.25 (0.06_	1.25 (0.57)	1.25 (0.06)	1.25 (0.57)	1.25 (0.06)	0.71	0.07
V. tortilis	1.25 (0.59)	1.25 (0.18)	1.25 (0.59)	1.25 (0.18)	1.25 (0.59)	2.50 (0.09)	0.74	0.22
Z. mucronata	2.50 (0.40)	0.63 (0.34)	1.25 (0.80)	1.25 (0.34)	1.25 (0.80)	1.25 (0.34)	1.00	0.43
Gentamicin	0.01		0.01		0.003		nd	
Doxorubicin	Nd		nd		nd		0.01	

nd denotes not determined.
Outstanding (MIC<0.02 mg/ml), excellent (MIC = 0.021 to 0.04 mg/ml), very good (MIC= 0.041–0.08 mg/ml), good (MIC = 0.081 to 0.16 mg/ml), average (MIC = 0.161 to 0.32 mg/ml) and weak (MIC>0.32 mg/ml) Eloff (2021).

Table 3
Ability of three *E. coli* strains to form biofilm under controlled conditions (different media and temperature).

	Optical density values (Mean±SD)							
Strain	37 °C TSB	37 °C MHB	37 °C TSB+1.2 % GLU	37 °C MHB+2 % GLU	30 °C MHB	30 °C TSB	30 °C MHB+2 % GLU	30 °C TSB+ 1.2 % GLU
***E.coli* ATCC 25922**	0.15±0.00*	0.25±0.03*	0.09±0.01*	0.07±0.00*	0.24±0.02**	0.19±0.01**	0.08±0.00*	0.09±0.00*
***E.coli* ATCC 35218**	0.42±0.00***	0.34±0.02**	0.19±0.01**	0.07±0.00*	0.34±0.04***	0.19±0.01**	0.07±0.00*	0.10±0.01*
***E.coli* O157:H7**	0.37±0.01***	0.18±0.01*	0.07±0.00*	0.07±0.00*	0.08±0.00*	0.24±0.01**	0.06±0.00°	0.07±0.02*

Glu-represents glucose.
MHB represents Mueller-Hinton Broth.
TSB represents Tryptic Soy Broth.
* denotes WBF (weak biofilm former).
** denotes MBF (moderate biofilm former).
*** denotes strong biofilm former.

was observed to be NBF (non-biofilm formers) at 30 °C when cultured in MH broth supplemented with 2 % glucose.

3.3. *Effect of plant extracts on the inhibition of cell attachment (0 h) and cell colonization (24 h) and EPS production against* E. coli *O157:H7*

Table 4 shows the inhibition and disruption of biofilm formation which was investigated by crystal violet staining at different stages of biofilm formation of *E. coli* O157:H7 on a polystyrene surface, and EPS inhibition using the carbohydrate quantification assay. The results obtained demonstrated that *D. cinerea, S. galpinii* and *V. sieberiana* methanol extracts prevented cell attachment (0 h) by more than 80 %. On the other hand, *E. rigida* and *V. tortilis* methanol extracts and *V. nilotica* acetone extract showed more than 80 % inhibition of pre-formed biofilm (24 h). Both extracts of *E. rigida* showed good anti-biofilm potential after 0 h and 24 h incubation. Generally, there was a significant difference ($p<0.05$) in the inhibition of biofilm between plant species and extraction solvents. Methanol extracts exhibited better anti-biofilm capabilities at 0 h and 24 h compared to acetone extracts, and it generally proved difficult to eradicate biofilm after 24 h. The selected plant extracts were ineffective inhibitors of EPS production during biofilm formation as shown in Table 4. The negative values indicate that the plant extract promoted the production of EPS instead of having an inhibitory effect. However, acetone

Table 4
Effect of acetone and methanol plant extracts on biofilm formation (0 h and 24 h) and EPS inhibition of *E. coli* 0157:H7.

Plant extracts	Biofilm inhibition				EPS inhibition	
	Methanol		Acetone		Methanol	Acetone
	0 h	24 h	0 h	24 h	24 h	24 h
C. siliqua	47.54	29.89	53.81	12.12	−48.05	18.33
D. cinerea	**80.31**	−1.70	42.78	−8.03	0.83	17.92
E. rigida	63.41	**89.36**	62.35	53.56	−48,05	−22.60
G. monticola	44.36	48.09	23.94	−52.98	−33.88	18.63
L. *leucocephala*	14.14	35.78	45.06	59.55	−90.93	0.56
M. alba	−1.00	52.22	15.32	38.05	−48.05	−72.38
S. babylonica	30.91	78.69	12.25	26.31	−37.16	−0.30
S. galpinii	**89.91**	33.68	40.37	−35.35	−1.33	28.49
S. lancea	26.64	78.52	32.82	−54.41	−28.07	−8.86
V. erioloba	54.65	2.45	42.34	−11.23	−1.35	9.42
V. gerrardii	41.75	49.18	42.98	8.71	17.37	3.86
V. karroo	51.12	−25.48	14.44	11.57	−48.05	−33.05
V. nilotica	38.63	19.15	41.17	**86.01**	−5.93	24.60
V. sieberiana	**85.40**	70.16	46.02	18.66	−37.72	−8.91
V. tortilis	41.27	**81.21**	−8.76	−36.40	−48.05	6.34
Z. mucronata	42.76	26.31	27.44	23.83	17.80	−47.73
Gentamicin	76.00	66.00	76.00	66.00	31.00	
Average	48.70	43.15	35.90	12.12	−26.17	−5.60

Negative value denotes promotion of biofilm formation and EPS production.
Values of inhibition of biofilm below 0 % were classified as biofilm promoting, between 0 and 49 % as weak biofilm inhibitors and 50–69 % as good biofilm inhibitors, and 70–100 % as outstanding biofilm inhibitors.

Table 5
Effects of acetone and methanol plant extracts as anti-QS against *C. violaceum.*

Plant	Methanol			Acetone		
	MIC (mg/ml)	MQSIC (mg/ml)	$MQSIC_{50}$ (mg/ml)	MIC (mg/ml)	MQSIC (mg/ml)	$MQSIC_{50}$ (mg/ml)
C. siliqua	0.31	0.16	0.10^{abcde}	0.31	0.16	0.09^{de}
D. cinerea	0.16	0.08	0.05^{abc}	0.31	0.16	0.06^{bcd}
E. rigida	0.63	0.31	0.15^{abcde}	0.16	0.08	0.03^{ab}
G. monticola	0.08	0.04	0.002^{a}	0.31	0.16	0.14^{ef}
L. leucocephala	0.31	0.16	0.11^{de}	0.63	0.31	0.24^{h}
M. alba	0.31	0.16	0.04^{abc}	0.16	0.08	0.04^{abc}
S. babylonica	0.31	0.16	0.12 ^{cde}	0.63	0.31	0.20 g^{h}
S. galpinii	0.63	0.31	0.21^{ef}	0.63	0.31	0.15f^{g}
S. lancea	0.08	0.04	0.002^{a}	0.63	0.31	0.16 g
V. erioloba	0.16	0.08	0.01^{ab}	0.08	0.04	0.003^{a}
V. gerrardii	0.31	0.16	0.10^{abcd}	0.31	0.16	0.09^{de}
V. karroo	0.08	0.04	0.01^{ab}	0.08	0.04	0.02^{ab}
V. nilotica	0.63	0.31	0.18^{ef}	0.31	0.16	0.08^{cd}
V. tortilis	0.08	0.04	0.01^{a}	0.63	0.31	0.22^{h}
V. sieberiana	0.16	0.08	0.03^{ab}	0.16	0.08	0.05^{bcd}
Z. mucronata	0.63	0.31	0.27^{f}	0.16	0.08	0.03^{ab}
Vanillin	0.31	0.16	0.08^{abcd}	0.31	0.16	0.08^{bcd}

Same letter(s) indicate not statistically significant at $p<0.05$ using Tukey (HSD) post hoc test.

extracts of *V. nilotica* (pod) and *S. galpinii* (leaf) had weak activity of 24 % and 28 % respectively.

3.4. Effect of plant extracts on violacein production against C. violaceum

Antiquorum sensing against *C. violaceum* was studied by quantifying violacein production after exposure to treatment with 32 plant extracts (Table 5). The MIC ranged from 0.08 to 0.63 mg/ml. The minimum quorum sensing inhibitory concentration (MQSIC) is the lowest concentration indicated by bacterial growth and lack of violacein pigmentation. The selected plants were able to inhibit the QS activity of *C. violaceum*. The MQSIC observed was ≥0.16 mg/ml for most plant extracts apart from six acetone extracts and eight methanol extracts with MQSIC ≤0.08 (mg/ml). The acetone extract of *V. erioloba* (0.003 mg/ml) and methanol extracts of *S. lancea* (0.002 mg/ml) and *G. monticola* (0.002) exhibited significantly ($p<0.05$) low $MQSIC_{50}$ compared to other selected plants.

3.5. Effect of 32 plant extracts on E. coli O157:H7 twitching and swarming motility

The effects of acetone and methanol extracts at a concentration of 0.16 mg/ml on the twitching motility and swarming of *E. coli* O157: H7 were investigated for 72 h and results are presented in Table 6. Five plant extracts, namely *V. gerrardii, V. erioloba, V. nilotica, S. babylonica* and *S. lancea* caused a significant decrease in twitching motility in comparison to the untreated cells. The acetone extracts of *E. rigida, S. babylonica,* and *V. gerrardii* had a significant impact on both types of motilities. A significant difference ($p<0.05$) was observed after 72 h incubation.

3.6. Effect of plant extract on cell surface hydrophobicity (CSH)

The effect of the 32 plant extracts on cell surface hydrophobicity against *E. coli* O157:H7 was measured by a BATH test. The results in Table 7 show that most of the plant extracts decreased the

Table 6
Effect of plant extracts on *E. coli* O157:H7 swarming and twitching motility after 24 h, 48 h, and 72 h incubation.

Plant species	Swarming (migration zone mm)						Twitching (migration zone mm)					
	Methanol			Acetone			Methanol			Acetone		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
C. siliqua	13.67^{ef}	14.33^{cde}	17.67^{abcd}	14.60^{e}	15.60^{ef}	17.00^{d}	15.67^{fg}	17.00^{ef}	19.33^{efg}	15.33^{f}	16.33^{def}	17.67^{def}
D. cinerea	18.00 g	19.67^{i}	25.33^{ef}	15.33^{e}	16.33^{fg}	17.67^{d}	21.00^{i}	23.00^{h}	27.00^{h}	15.00^{f}	17.67^{fgh}	21.00^{h}
E. rigida	0.00^{a}	11.00^{b}	13.67^{a}	10.00^{c}	12.00^{bc}	13.67^{b}	13.00^{de}	17.00^{ef}	20.00^{fg}	11.00^{cd}	15.33^{d}	18.00^{efg}
G. monticola	15.00^{f}	16.33^{efg}	17.33^{abc}	15.00^{e}	15.67^{ef}	17.33^{d}	15.33^{fg}	17.00^{ef}	25.33^{h}	15.67^{f}	17.33^{efg}	19.67^{fgh}
L. leucocephala	20.33^{h}	22.00^{j}	25.67^{ef}	14.33^{e}	15.33^{ef}	19.00^{d}	12.33^{cd}	16.00^{de}	$18.33cd^{efg}$	15.00^{f}	18.00 g^{h}	21.00^{h}
M. alba	10.67^{be}	13.00^{bc}	15.00^{abc}	19.00^{f}	21.33^{i}	25.33^{f}	15.00^{f}	17.00^{ef}	18.67^{defg}	19.67^{h}	21.67^{jk}	24.67^{i}
S. babylonica	12.33^{cde}	14.00^{cde}	18.33^{bcd}	7.00^{b}	8.67^{a}	10.67^{a}	13.00^{de}	13.67^{bc}	15.67^{bcd}	6.67^{b}	11.67^{c}	12.67^{ab}
S. galpinii	12.67^{de}	16.00^{efg}	$21.00hd^{e}$	9.67^{c}	16.00^{efg}	17.00^{d}	13.00^{de}	14.00^{cd}	16.67^{bcde}	10.67^{f}	12.00^{c}	14.00^{bc}
S. lancea	20.00^{h}	24.00^{j}	26.00^{f}	18.00^{f}	21.00^{i}	23.33^{f}	0.00^{a}	5.00^{a}	14.67^{b}	0.00^{a}	5.00^{a}	16.00^{cde}
V. erioloba	11.33^{bcd}	18.00 ^{ghi}	24.67^{ef}	18.00^{f}	21.33^{i}	22.00^{e}	18.67^{h}	19.33 ^{g}	20.67 ^{g}	0.00^{a}	5.00^{a}	10.67^{a}
V. gerrardii	0.00^{a}	8.00^{a}	14.00^{ab}	0.00^{a}	10.33^{ab}	12.00^{ab}	14.33^{ef}	15.33^{cde}	17.67^{bcdefg}	0.00^{a}	5.33^{a}	12.33^{ab}
V. karroo	14.67^{f}	17.33^{fgh}	19.33^{cd}	20.23^{f}	22.00^{i}	24.67^{ef}	15.67^{fg}	16.67^{ef}	18.33^{cdefg}	20.33^{h}	22.00^{k}	25.00^{i}
V. nilotica	13.33^{ef}	14.67^{cde}	17.33^{abcd}	5.67^{b}	9.00^{a}	10.00^{a}	12.00^{cd}	15.67^{cde}	17.33^{bcdef}	5.67^{b}	10.00^{b}	15.33^{cd}
V. sieberiana	12.67^{de}	14.67^{cde}	17.00^{abcd}	15.33^{e}	17.33^{fgh}	18.67^{d}	10.33^{c}	16.00^{de}	20.33^{fg}	15.00^{f}	16.00de	17.67^{def}
V. tortilis	11.00^{bcd}	15.00^{cde}	17.33^{cd}	13.00^{de}	14.00^{cd}	16.67^{cd}	10.33^{c}	11.67^{b}	15.33^{bc}	12.67^{de}	17.33^{efg}	20.33 ^{gh}
Z. mucronata	9.67^{b}	13.00^{bc}	15.00^{abc}	11.00^{cd}	13.00^{c}	14.00^{bc}	5.33^{b}	6.00^{a}	8.67^{a}	14.00^{ef}	17.33^{efg}	20.00^{fgh}
Gentamicin	11.00^{bcd}	$15.33d^{ef}$	18.67^{cd}	11.00^{cd}	15.33^{ef}	18.67^{d}	10.00^{c}	15.33^{cde}	18.67^{defg}	11.67^{cd}	15.33^{d}	18.00^{efg}
Negative control	14.67^{f}	18.67^{hi}	25.67^{f}	15.33^{e}	18.67^{h}	25.67^{f}	15.67^{fg}	20.33 ^{g}	25.33^{h}	15.67^{f}	20.33^{ij}	26.00^{i}
Untreated cells	15.00^{cdef}	18.00 ^{ghi}	25.00^{ef}	15.00^{e}	18.00 ^{gh}	25.00^{ef}	17.00 g^{h}	19.00 g	25.00^{h}	17.00 ^{g}	19.00^{hi}	25.00^{i}

Same letter(s) indicate not statistically significant at $p<0.05$ using Tukey (HSD) post hoc test.

Table 7
Percentage of adhesion to hydrocarbons obtained for *E. coli* O157:H7 after treatment with plant extracts.

Plant	% BATH	
	Acetone	Methanol
C. siliqua	49.25^{ef}	50.83^{efg}
D. cinerea	48.67^{ef}	42.86^{c}
E. rigida	30.04^{b}	33.00^{ab}
G. monticola	40.00^{c}	51.00^{efg}
L. leucocephala	47.75^{efg}	46.67^{cdef}
M. alba	41.60^{cd}	49.50^{defg}
S. babylonica	49.50^{f}	51.63^{fg}
S. galpinii	44.00^{cde}	49.83^{defg}
S. lancea	48.50^{ef}	45.88^{cde}
V. erioloba	22.25^{a}	48.62^{def}
V. gerrardii	48.25^{efg}	48.21^{def}
V. karroo	48.60^{ef}	44.84^{cd}
V. nilotica	42.57^{cd}	37.17^{b}
V. sieberiana	40.36^{c}	49.38^{defg}
V. tortilis	51.50^{fg}	51.34^{fg}
Z. mucronata	45.17^{def}	48.64^{def}
Gentamicin	30.00^{b}	30.00^{a}
Negative control	54.46 ^{gh}	54.46 ^{gh}
Untreated cells	57.00^{h}	57.00^{h}

Column sharing the same letters are not significantly different at ($p<0.05$) according to Tukey(HSD) post hoc test.

hydrophobicity of *E. coli* O157:H7 significantly ($p<0.05$) except for methanol extracts of *V. tortilis* (51.38 %), *S. babylonica* (51.50 %) and *G. monticola* (51.00 %) when compared to untreated cells (57.00 %). Both the acetone (30.04 %) and methanol (33.00 %) extracts of *E. rigida* exhibited the best ability to alter hydrophobicity surface properties along with *V. eriobola* (22.25 %) acetone extract, with results similar to those of the positive control, gentamicin (30.00 %).

3.7. Effect of plant extracts on aggregation of E. coli O157:H7

Table 8 shows the results of the effect of 32 plant extracts at sub-MIC of 0.16 mg/ml against *E. coli* O157:H7. This study revealed that *E. coli* O157:H7 was autoaggregative in all the salt concentrations used before exposure to plant extracts. After treatment, the plant extracts including *G. monticola, M. alba, V. karroo* and *V. nilotica* prevented aggregation (non-aggregate) of *E. coli* O157:H7 at all salt concentrations. On the hand, acetone extracts of *C. siliqua, S. babylonica, Z. mucronata* and methanol extract of *E. rigida, L. leucocephala, S. galpinii* and *V. sieberiana* promoted aggregation of *E. coli* O157:H7. Finally, both methanol and acetone extracts of *V. gerrardii* indicated weak aggregation at only the highest salt concentration.

4. Discussion

The use of antibiotics such as quinolones, ciproflaxin, furazolidine and trimethoprim in treatment of EHEC infections is not recommended because they increase the production and release of Stx from bacteria (Mühlen and Dersch, 2020; Liu et al., 2023). *E. coli* (STEC) O157:H7 is resistant to several classes of antibiotics (Amézquita-López et al., 2016; Greig et al., 2023). Thus, there is no effective protective measure or therapeutic antibiotic available against EHEC—HUS. There is an urgent need to discover alternative antibiotics. The present study investigated the efficacy of acetone and methanol extracts from sixteen plant species against quorum sensing and biofilm formation of *E. coli* O157:H7 strains associated with diarrhoea in humans.

It is important to determine the MIC of a substance against an organism in order to choose an appropriate concentration that may affect the virulence factors involved in pathogenicity such as biofilm formation without killing the organism. Acetone and methanol extracts of *D. cinerea* had weak antibacterial activity against *E. coli* ATCC 25922. These results confirmed that the methanolic extract had

Table 8
Effect of acetone and methanol extracts of 16 plants on auto aggregation of *E. coli* O157:H7.

Plant species	Acetone					Methanol				
	Concentration $(NH_4)SO_4$ M					Concentration $(NH_4)SO_4$ M				
	0.05 M	0.25 M	0.5 M	0.75 M	1.25 M	0.05 M	0.25 M	0.5 M	0.75 M	1.25 M
C. siliqua	*****	****	***	**	*	–	–	–	–	–
D. cinerea	–	–	–	**	*	–	–	–	–	–
E. rigida	–	–	–	**	*	*****	****	***	**	*
G. monticola	–	–	–	–	–	–	–	–	–	–
L. leucocephala	–	–	–	–	*	*****	****	***	**	*
M. alba	–	–	–	–	–	–	–	–	–	–
S. babylonica	*****	****	***	**	*	–	–	–	–	*
S. galpinii	–	–	–	–	–	*****	****	***	**	*
S. lancea	–	–	–	**	*	–	–	–	–	–
V. erioloba	–	–	–	–	–	–	–	–	–	*
V. gerrardii	–	–	–	–	*	–	–	–	–	*
V. karroo	–	–	–	–	–	–	–	–	–	*
V. nilotica	–	–	–	–	–	–	–	–	–	–
V. tortilis	–	–	–	–	–	*****	****	***	**	*
V. sieberiana	–	–	–	–	–	*****	****	***	**	*
Z. mucronata	*****	****	***	**	*	–	–	–	–	–
Gentamicin	–	–	–	–	*	–	–	–	–	*
Negative control	*****	****	***	**	*	*****	****	***	**	*
Untreated cells	*****	****	***	**	*	*****	****	***	**	*

* Weak aggregation at 1.25 M.
** Moderate aggregation at 0.75 M.
*** Average aggregation at 0.50 M.
**** High aggregation at 0.25 M.
***** Outstanding aggregation at 0.05 M
—Denotes non-aggregation at 1.25, 0.75, 0.50, 0.25 and 0.05 M.

a weak MIC as previously reported by Mudzengi et al. (2017) where the MIC of a methanolic leaf extract of *D. cinerea* ranged from 7.86 to 13.60 mg/ml against *E. coli* ATCC 25922. However these MIC values are relatively higher than those reported in this study. The discrepancy may be attributed to the seasonal variation and geographical location, which may have effects on antibacterial activities of plants (Adeosun et al., 2022).

Furthermore, most of the selected plants had weak to average antibacterial activity against the *E. coli* strains. According to Mogana et al. (2020), Gram-negative bacteria including those from the Enterobacteriaceae family display greater resistance to plant extracts, which provides a scientific reason for the weak antibacterial activity observed in the current study. There are possibilities that the plant extracts slowed bacterial growth (bacteriostatic) instead of killing the bacteria (bactericidal). Contrary to our results, Adeyemo et al. (2022) reported that the acetone extract of *S. lancea* had good antibacterial activity (MIC = 0.05 mg/ml) against *E. coli* ATCC 25922. However, the *S. lancea* methanol extract as reported by Vambe et al. (2018) displayed MIC of 1.5 mg/ml against multi-drug resistant *E. coli,* which is similar to results obtained in our study. Numerous *Vachellia* and *Senegalia* species have been reported to have good antimicrobial activity against *E. coli* (Maroyi, 2017; Sadiq et al., 2017). The discrepancy in MIC values may be attributed to the part of plant used, harvest time, extraction solvent, seasonal variation and bacterial strain.

Although traditional medicine is often associated with lower toxicity than conventional medicine, it is crucial to establish the toxicity of plant extracts before usage. According to the United States National Cancer Institute (NCI) criteria, a plant with LC_{50} ≤0.02 mg/ml is considered to be highly cytotoxic to mammalian cells. This suggests that all selected plant extracts were not cytotoxic to the Vero cells used in this study. Notably, the methanol extract of *V. gerrardii* and *Z. mucronata* were least toxic (LC_{50} = 1 mg/ml) compared to the other extracts and doxorubicin. Furthermore, these results are in line with a previous study that reported the extracts of *Z. mucronata* to have no cytotoxic effects against Vero cells (Mongalo et al., 2020). The ethanolic extract of *Acacia* (*Vachellia*) *gerrardii* decreased the cell viability to < 50 % at ≥ 0.025 mg/ml against Madin Darby Canine Kidney (MDCK) cell clone 7 (Omwenga et al., 2017). In vivo toxicity studies are important before reaching any conclusions on the toxicity of an extract, as this depends on host gut interactions and bioavailability (Famuyide et al., 2019). The selectivity index (SI) values of the extracts were obtained by dividing MIC (mg/ml) by LC_{50} (mg/ml). A higher SI (>1) is preferable because it implies that a plant extract has antibacterial activity at a concentration that is not toxic to mammalian cells. The acetone extracts of *C. siliqua, D. cinerea* and the methanol extract of *V. gerrardii* had the highest selectivity index values against *E. coli* ATCC 25922 and *E. coli* ATCC 35218 respectively compared to other extracts. A plant extract with high selectivity index (≥ 1) increases its potential to be developed into alternative antimicrobial agents (Dzoyem et al., 2016).

The formation of biofilm is dependent on nutrient availability, temperature, osmotic pressure, and pH among other factors (Mirghani et al., 2022). In this study, various biofilm formation conditions were tested including different culture media (MHB and TSB) supplemented with glucose (MHB+2 % glucose and TSB+1.2 % glucose). The effect of variation in incubation temperature (30 °C and 37 °C) was also investigated by measuring the optical density (OD) at 590 nm after culture. The results indicated that both temperature and growth media play a significant role in biofilm development. The strongest biofilm formation was reported at 37 °C incubation using TSB and MHB without glucose supplementation as growth media. According to Nyonyo et al. (2013), media composition, particularly in the presence of glucose, for instance, TSB+0.25 % glucose, promotes biofilm formation. In this study, biofilm formation was reduced due to high glucose content of 1.2 % in the medium. It is more likely that there is a threshold for concentration of glucose for *E. coli* to initiate biofilm formation.

Biofilms were studied on a hydrophobic polystyrene surface of a microtitre plate because this is a better substrate for biofilm formation than a hydrophilic glass or metal surface. Studies have highlighted that natural products are able to decrease biofilm formation of *E. coli* O157:H7 on various surfaces (Ryu and Beuchat, 2005; Yaron and Römling, 2014). Extraction solvents including water, methanol, ethanol, chloroform, ether, dichloromethanol and acetone have been used for the extraction of natural compounds from various sources for anti-biofilm activity experiments (Mishra et al., 2020). This study demonstrated that methanol extracts are better than extracts prepared using acetone in destroying or preventing biofilm formation after 0 and 24 h. Alam et al. (2020) demonstrated a strong positive association among antibiofilm activity of methanolic extracts and polar compounds, indicating the key role of phytochemicals in bacterial biofilm inhibition.

Most plant extracts were generally not able to prevent cell attachment or adherence, thus allowing biofilm to form. The methanol extract of *V. karroo* inhibited cell attachment by 51.12 %, while (Baloyi et al., 2022) reported that *V. karroo* methanol extract did not prevent cell attachment of *E. coli* ATCC 10536. Although many extracts had the ability to moderately inhibit preformed biofilm, only a few showed outstanding activities. This included the acetone extract of *V. nilotica* (86.01 %), and methanol extracts of *E. rigida* (89.36 %) and *V. tortilis* (81.21 %). Both the methanol and acetone extracts of *E. rigida* significantly prevented biofilm formation and eradicated pre-existing biofilm by more than 50 %. Previous studies confirm that plant extracts have anti-biofilm potential against different strains of *E. coli* (Adeyemo et al., 2022; Famuyide et al., 2019) including *E. coli* 0157:H7 (Cho et al., 2013, Hu et al., 2019, Abbas et al., 2014). According to Adeyemo et al. (2022) *S. lancea* acetone extract prevented 76.97 % and eradicated 74.46 % of biofilm by *E. coli* ATCC 25922, however, in this study the methanol extract was active (78.52 %) against *E. coli* 0157:H7 after 24 h. Generally it was more difficult to eradicate preformed biofilm than it was to prevent cell attachment or adhesion. Recent reports confirmed that it is less difficult to inhibit cell attachment than disrupt established biofilm (Famuyide et al., 2019; Adeyemo et al., 2022). Some of the selected plants therefore have a pronounced antibiofilm effect against multidrug-resistant *E. coli*.

The adhesive nature of the biofilm matrix allows microbes to remain on a surface and attach. According to Mombeshora et al. (2021), a reduction in EPS production is crucial for the disruption of the entire formed biofilm. Our results confirm that it is extremely difficult to disrupt the EPS matrix, as only a few extracts of the 32 tested had weak activity. This supports the role of the EPS matrix in enhancing resistance of microbes to antimicrobial substances (Di Martino, 2018).

According to Wu et al. (2020), *E. coli* is highly motile in liquid and semi-agar liquid because of two peritrichous flagella and is hence a good model organism to study various cell movements. Additionally, the same motile bacteria also often form non-motile multicellular aggregates bound by an extracellular matrix called biofilm. The *E. coli* 0157:H7 used in this study was highly motile and our results clearly demonstrated that some plant extracts significantly ($p< 0.05$) decreased swarming and twitching motility against *E. coli* O157:H7. This is in support of the study of Lee et al. (2014), indicating that 14 plant extracts and compounds were able to reduce swarming and swimming motility of *E. coli* enterohemorrhagic O157:H7 (EHEC). Likewise, Martínez-Graciá et al. (2015) reported that plant extracts inhibited the growth rate, swarming motility, and virulence gene expression of *E. coli* O157:H7. Both methanol and acetone extracts of *V. gerrardii* displayed good anti-motility (swarming and twitching). Another study showed that the hydroethanolic leaf extract of *Vachellia* (*Acacia*) species at 32–512 μg/ml significantly reduced cell motility against *E. coli* O157:H7.

This study revealed that EHEC O157:H7 is a highly aggregative bacteria at SAT concentration of 0.05 M. Likewise, (Rozgonyi et al., 1985) showed that a strongly aggregating bacteria is also highly hydrophobic, meaning that *E. coli* O157:H7 was hydrophobic before exposure to plant extracts. The study showed that methanol and acetone extracts of the three *Vachellia* species, namely *V. gerrardii, V. karroo* and *V. nilotica,* prevented the aggregation of EHEC O157:H7 at all SAT concentrations. On the other hand, Fabaceae species ethanolic extracts, including *Vachellia (Acacia) catechu* did not exhibit any effect on CSH and aggregation of EHEC O157:H7 (Voravuthikunchai and Limsuwan, 2006).

E. coli has been reported to have a hydrophobic surface property when assessed by other methods (Kragh et al., 2016). The current study confirms that *E. coli* O157:H7 is hydrophobic in an aqueous environment. This study provides the first evidence on the effects of the selected plant extracts on cell adhesion or attachment during initiation of biofilm. *Vachellia erioloba* acetone extract demonstrated a significant decrease in cell adhesion hydrophobicity of 22.25 %. In support, Voravuthikunchai and Limsuwan (2006) reported that the ethanolic extracts of Thai medicinal plants, including *Vachellia* (*Acacia*) species used for the treatment of diarrhoea, reduced cell surface hydrophobicity of *E. coli* O157:H7.

In the colony formation stage of biofilm, quorum-sensing (QS) signals, which comprise a cell-to-cell communication mechanism in bacteria, regulate the production of biofilm matrix compounds (Zhao et al., 2023). The ability of *C. violaceum* to produce violacein, a water-insoluble purple pigment, has been used to study anti-quorum sensing mechanisms. The anti-quorum sensing potential of the selected plant extracts is poorly investigated. The findings of the present study demonstrate the potential use of plant extracts as inhibitors of QS. According to Samreen et al. (2022), the methanolic extract of *V. nilotica* at sub-inhibitory concentrations (125, 62.5, and 31.25 μg/ml) showed a 14 mm zone inhibition of violaceum production against *C. violaceum* ATCC 12472. In addition, the green pods of *V. nilotica* extracts (unhydrolyzed and hydrolysed) and fractions (unhydrolyzed and hydrolysed ethyl acetate) had anti-QS potential using the disk diffusion assay, and also showed a high presence of polar compounds, i.e., gallic acid, ellagic acid, epicatechin and rutin (Singh et al., 2009). Likewise, the methanolic pod extract of *V. nilotica* in the current study showed MQSIC and IC_{50} values of 0.31 and 0.18 mg/ml, respectively. Furthermore, Bodede et al. (2018) established that methanol leaf extracts of *Senegalia* species from South Africa efficiently inhibited quorum sensing by reducing violacein production against *C. violaceum* ATCC 12472 strain. The contrast in anti-quorum sensing activity of certain plant extracts can be attributed to the variation in plant species, method of analysis, part of plant used and extraction solvent, among other criteria.

5. Conclusion

Some of the methanol extracts tested in this study had excellent antibiofilm potential against EHEC (*E. coli* O157:H7) biofilm forming strain, despite having poor antibacterial activity against planktonic forms of bacteria. The extracts were relatively safe to normal mammalian cells. Amongst the plants investigated, *E. ridiga* and *V. sieberiana* were effective in inhibiting cell attachment (0 h) and eradicating matured biofilms (24 h). Additionally, some plant extracts showed good anti-QS activity, anti-motility and anti-aggregative, particularly *V. erioloba*. This study provides evidence that some plant extracts may serve as sources of novel anti-biofilm and anti-quorum sensing agents to protect against *E. coli* diarrhoeal infections in humans and animals. In future, non-toxic and effective extracts may be incorporated into formulations for development of therapeutic antimicrobial drugs. Plant leaves may also be utilized as phytogenic animal feed additives to reduce the incidence of EHEC diarrhoea in animals. Further investigations will include fractionation and identification of antibiofilm compounds from active plant extracts.

Author contributions

MML, LJM, IMF, DJP, EEE and KGK were involved in conceptualization and design of this manuscript. MML, ROA, DJP and FNM, contributed to data collection. MML and FNM conducted data analysis and statistics. Review of the manuscript was done by all authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Compliance with ethical standards

The study was approved by Research Ethics Committee of the Faculty of Veterinary Science, University of Pretoria (Certificate REC032–21).

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