PHYTOCHEMICAL COMPOSITION AND POTENTIAL ANTIBACTERIAL ACTIVITY OF THE TROPICAL SPIDERWORT (*COMMELINA BENGHALENSIS* L.)

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Abstract. Plant-based antimicrobials are preferred due to the lack of many side effects often related to synthetic ones. *Commelina benghalensis* is a perennial herb native to the tropics of Africa and Asia; traditionally used to treat various types of illnesses by faith healers. The aims of this study are to identify the phytochemical compounds of plant parts of *C. benghalensis*, namely leave and stems, and determine the antibacterial activity of extracts. The objectives were to identify the composition and nature of various chemicals in *C. benghalensis* using phytochemical tests, Thin Layer Chromatography (TLC), Energy-Dispersive X-Ray (EDX), Gas Chromatography-Mass Spectrometry (GC-MS) and the antibacterial efficiency using methanolic leaf and stem extracts against various strains of bacteria. Phytochemical analysis revealed the presence of phenols, alkaloids, terpenes, mucilage and gum, amino acids, carbohydrates, flavonoids, saponins, sterols, fixed oils and fat. Important phytochemicals with medicinal properties were identified using GC-MS, these included vitamin E, phytol, stigmasterol and squalene. The methanolic leaf and stem extracts subjected to antibacterial analysis inhibited Gram-positive bacterial strains such as *Bacillus subtillus*, *Staphylococcus aureus* and *Methicillin*-resistant *Staphylococcus aureus*, as well as Gram-negative strains which included *Escherichia coli* and *Pseudomonas aeruginosa*. Further studies on *C. benghalensis* should include isolating and elucidating the structures of theses bioactive compounds responsible for the pharmacological and antimicrobial activity.
Keywords: *antimicrobial, Energy-Dispersive X-Ray Spectroscopy, Gas Chromatography-Mass Spectrometry, inhibition, medicinal plant*

Introduction

Throughout the globe, traditional systems of healing have relied heavily on plant natural products (Veeresham, 2012). African traditional medicine is one of the most diverse medical systems (Gurib-Fakim, 2006; Mahomoodally, 2013). There is a rich biodiversity that combines with culturally diverse healing practices across various districts (Gurib-Fakim, 2006; Mahomoodally, 2013). Traditional medicines have dominated the health care system in Africa (Steenkamp, 2003; Abdullahi, 2011). This is due to inadequate access to modern medicines for the management and treatment of diseases in rural, low- and middle-income communities (Steenkamp, 2003; Abdullahi, 2011) coupled with the elevating costs of western drugs of which most of the general populace is unable to afford (Afolayan and Adebola, 2004). South Africa has the richest

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temperate flora (19581 indigenous species) in the world (Nielsen et al., 2012), with 3000 species used as medicinal plants of which 350 species are the most commonly traded and used in the form of medication (van Wyk et al., 1997; Nielsen et al., 2012). Medicinal plants are often traded as raw materials or partially chopped, as a dry powder or in the form of a mixture often with water (Mander et al., 2007) and they are used to treat a variety of illnesses and ailments. Plant extracts are also commonly used to treat various infectious diseases (Buwa and van Staden, 2006).

In recent years, microorganisms have become resistant to conventional antimicrobial agents (Silva and Fernandes Jr, 2010). The resistance is due to induced mutations in the genetic composition of the microorganism caused by antimicrobial agents (drugs, synthetic drugs or antibiotics) (Gupta et al., 2016). Antimicrobial resistance in South Africa has attained a distressing magnitude; with research indicating that more than half of all the hospitals studied had acquired *S. aureus* infections that originated from methicillin resistance (Bramford et al., 2010; van Vuuren and Mulharhi, 2017). This resistance to antibiotics has driven scientists to discover other forms of antibacterial drugs (Ahmed et al., 2019). Plant-based antimicrobials seem to have an appealing quality, and they appear to lack many side effects often related to synthetic antimicrobials (Oikeh et al., 2016). The medicinal properties of plants are attributed to various secondary metabolites/phytochemicals such as alkaloids, terpenoids, phenols and tannins. They may act on their own, additively or synergistically for the improvement of one's health (Gurib-Fakim, 2006; Vanitha et al., 2019). These phytochemicals are produced in response to environmental stresses (Singer et al., 2003). For example, phytochemicals produce cytotoxicity towards pathogens or neurotoxicity towards herbivores (Briskin, 2000). These traits could prove to be useful in human health as antimicrobial medicines, antidepressants, muscle relaxants, anaesthetics or sedatives (Briskin, 2000). Reported traditional medicinal plants with antimicrobial effects need to be investigated as the phytochemicals present may not bring about resistance in microorganisms, and their activity could be diversified with structural modification (Khan et al., 2011a; Parimala and Shoba, 2014; Wintola and Afolayan, 2015).

The tropical spiderwort (*Commelina benghalensis;* Commelinaceae*)*, is a perennial herb native to Africa (Hasan et al., 2010). *Commelina benghalensis* has been given numerous names in the different dialects spoken in South Africa, i.e. the Benghal dayflower, Benghal wandering Jew (English); Idambiso, Idangabane, Idemadema and Idlebendlele, (IsiZulu); Blouselblommetjie (Afrikaans.); Indabane (Ndebele); Uhlotshane (isiXhosa); Lala-tau, Khopo-e-nyenyane and Khotsoana (Sesotho); Ndamba (Tshivenda) (Tshiila, 2016). According to South African National Biodiversity Institute (SANBI) , *C. benghalensis* is widespread and highly abundant in South Africa, with an incredibly low risk of extinction (Foden and Potter, 2005). However, *C. benghalensis* is considered an invasive weed in certain parts of the world, posing a threat to the production of crops (Webster et al., 2005). According to Walker and Everson (1985), in Queensland, the plant has been found in cultivations and associated with dry–beans, maize, sorghum and peanuts where it competes with crops and thus lowers the harvest yield. Traditionally, the whole plant or stems are used in South Africa to treat infertility in women and in the treatment of various skin conditions (Steenkamp, 2003; Lebogo et al., 2014). Throughout Africa, the plant is used to treat, for example, colds, flu, gonorrhoea, conjunctivitis, Malaria and jaundice (Novy, 1997; Yetein et al., 2013). Previous reports highlighted the use of *C. benghalensis* to treat various illnesses as it possesses analgesic, anti-inflammatory and anti-oxidant properties (Ibrahim et al., 2010; Anusuya et al., 2012;

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Hossain et al., 2014; Chowdhury et al., 2015). In consideration of the ethnobotanical uses of *C. benghalensis*, it is proposed that this plant has antibacterial properties. This study is aimed at identifying the phytochemical compounds of plant parts, namely leaves and stems, and determining the antibacterial activity of extracts in order to correlate to its medicinal use. The objectives of this study were to assess the composition and nature of various chemicals in *C. benghalensis* using phytochemical tests, TLC, EDX and GC-MS and to investigate the antimicrobial activities using leaf and stem methanolic extracts of *C. benghalensis* against various strains of bacteria in order to validate the plants use as a source of traditional medicine. In terms of the antibacterial activity, the hypothesis put forth states that *C. benghalensis* will produce moderate to strong activity against the various bacterial strains tested.

Materials and methods

Plant material collection

Commelina benghalensis leaves and stems were collected at the University of Kwa-Zulu Natal (UKZN), Westville campus in Durban. The species was identified using herbarium specimens and thereafter a voucher specimen (18259) was deposited in the Ward Herbarium in the School of Life Science in UKZN.

Phytochemical analyses

In preparation for extracts, leaves and stems were air dried at room temperature (23°C) for a duration of two months. Once dry, the material was ground separately into a fine powder using a 600 W glass jug blender (Russell Hobbs 23821-56, Russell Hobbs Inc., United Kingdom). The solvents used for extraction were hexane, chloroform, and methanol. The powdered material (10 g) was placed into a round bottom flask, connected to a Soxhlet apparatus, and hexane (100 mL) was added. This was left to boil for 3 h at 40°C. The extract was filtered using Whatman® No. 1 filter paper and the extract was retained. The process was repeated 4 times. Extractions using chloroform and methanol followed consecutively using the same procedure. Extractions were carried out on the leaf and stem powdered material. Phytochemical analyses were carried out on the leaf and stem extracts and the procedure was as follows:

Analysis for mucilage and gum

Ruthenium Red test: one mL of extract was treated with 0.5% Ruthenium Red (2 drops). A change in solution colour (pink to red) indicated a positive reaction.

Analyses for carbohydrates

Molisch's test: a drop of alcoholic α-naphthol solution was mixed with 1 mL of extract in a test tube. Concentrated sulphuric acid (0.5 mL) was dispensed along the side of the test tube. The production of violet rings indicated a positive reaction. Fehling's test: one ml of Fehling's A and B (each) was mixed with 1 mL extract thereafter boiled in a water bath. The development of a red precipitate indicated a positive reaction. Benedicts's test: one mL of extract was mixed with 1 mL Benedict's reagent. This solution was boiled in a water bath (2 min). The formation of an orange-red precipitate indicated a positive reaction.

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Analysis for sterols

Sterol test: two mL of extract was treated with 3 mL of chloroform. After mixing, sulphuric acid (2-3 drops) was decanted down the side of the test tube. The development of a fluorescent green ring below the layers and a red ring between the layers indicated a positive reaction.

Analysis for phenols

Phenol test: one mL of extract was treated with 2 drops of ferric trichloride. A change in solution colour or the development of a black or green precipitate indicated a positive reaction.

Analyses for alkaloids

Wagners's test: one mL of extract was treated with 2 drops of Wagner's reagent. A red-brown precipitate indicated a positive reaction. Dragendoff's test: one mL of extract was mixed with 0.5 mL Dragendoff's reagent. A reddish precipitate indicated a positive reaction. Mayer's test: one mL of Mayer's reagent was mixed with 1 mL of extract. The formation of a yellow precipitate indicated a positive reaction.

Analysis for proteins and amino acids

Ninhydrin test: one mL of extract was treated with a drop of the Ninhydrin reagent. A change in solution colour (purple) indicated a positive reaction.

Analysis for flavonoids

Lead Acetate test: five mL of extract was mixed with 1 mL of 5% lead acetate. A white precipitate indicated a positive reaction.

Analyses for saponins

Foam test: two mL of water and 0.5 mL of extract was shaken in a test tube. Foam that persisted for 10 min was indicative of a positive reaction. Froth test: ten mL of water and 3 mL of extract was shaken for 15 min. The development of a 1 cm layer of froth indicated a positive reaction.

Analysis for steroids/terpenoids

Chloroform test: Two mL of chloroform was mixed with 5 mL extract. Concentrated sulphuric acid (3 mL) was dispensed along the side of the test tube to create a layer between the solvent and extract. The development of a reddish-brown colour indicated a positive reaction.

Fixed oils and fats

Oils and Fats test: two drops of extract were dribbled onto Whatman® No.1 filter paper. Oil absorbed on the filter paper was indicative of a positive reaction.

Thin layer chromatography (TLC)

Leaf and stem extracts (hexane, chloroform, and methanol) were spotted onto pre-coated silica gel TLC plates (Merck) using glass capillaries. The plate was left to stand in

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a solution made up of 2 mL ethyl acetate and 8 mL of toluene. Solvents were left to run up the plate to a distance of 8 cm. Ultraviolet-Visible (UV) light (254 and 366 nm) was used to view the plate. Thereafter, the plate was dipped in a solution of anisaldehyde and sulphuric acid, heated in an oven (90°C) for 5 min and photographed. The active compounds retention factors (R_f) were calculated using the following equation:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by the solvent front}} \quad \text{(Eq.1)}$$

Gas chromatography-mass spectrometry (GC-MS)

The QP-2010 Ultra Shimadzu (Japan) GC-MS was used to analyse methanolic leaf and stem extracts. This was aided with a Rx_5Sil Ms capillary (Restek). Helium acted as a carrier gas and had a flow rate of 0.96 mL/min, a total flow rate of 4.9 mL/min, a linear velocity of 36.7 cm/sec, a pressure of 44.9 kPa and purge flow rate of 3.0 mL/min. The injection port temperature was set to 250°C. The initial oven temperature was set to 50°C and held for 1 min and thereafter was increased to a final temperature of 310°C and held for 10 min. The sample was injected with the splitless mode. The total running time for sample analysis was 37 min. The chemical constituents in the methanolic extracts were identified by means of comparisons with the polychlorinated biphenyls (PCB) standards retention times as located in the National Institute of Standards and Technology (NIST) library.

Energy dispersive X-ray spectroscopy (EDX)

Fresh leaves and stems were collected and placed separately into a mortar. Thereafter, liquid nitrogen slush was poured into the mortar and the plant material was crushed using a pestle. The resultant plant powder was dried in an oven for 48 h. A pinch of each plant powdered material was placed onto separate aluminium stubs containing carbon conductive tape. The stubs were coated with gold using the Polaron SC500 Sputter Coater (United Kingdom). The Zeiss Ultra Plus FEG-SEM EDX detector (5 kV) was used for elemental identification.

Antibacterial screening

Methanolic extraction of plant material

To prepare the methanolic extracts, leaves and stems were air dried at room temperature (23°C) for two months. The material was ground separately into a fine powder using a 600 W glass jug blender (Russell Hobbs 23821-56, Russell Hobbs Inc., United Kingdom) once dry. Ten grams of the powdered material was placed into a round bottom flask, connected to a Soxhlet apparatus, 100 mL of methanol was added and left to boil for 3 h at 40°C. The solution was filtered using Whatman® No. 1 filter paper and the extract was retained. This procedure was repeated 4 times for each plant powdered material. Leaf and stem methanolic extracts (5 mL) were dispensed into separate, pre-weighed pill vials and left to dry overnight in a 26°C oven. One mg of the dried extracts from the pill vials were transferred into Eppendorf centrifuge tubes to which 1.5 mL of distilled water was added. The final concentration of the methanolic leaf and stem extracts taken for antibacterial screening was 0.67 mg/ml. These were taken forward to analyse their effect against 9 bacterial strains which included Gram-positive: *Bacillus subtillus*,

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Staphylococcus aureus, S. aureus (ATCC 29213), *Methicillin*-resistant *Staphylococcus aureus* (clinical type) and *Methicillin*-resistant *S. aureus* (environmental type) and Gram-negative: *Escherichia coli*, *E. coli* (ATCC 25218), *Pseudomonas aeruginosa* and *P. aeruginosa* (ATCC 25215).

Agar preparation

A litre of distilled water was used to dissolve 38 g of Mueller-Hinton agar (MHA) (Biolab, South Africa). This was mixed on a stirrer and autoclaved (model HL-320) for 1 h at 121°C. The media was poured into sterile Petri plates (90 mm diameter) and allowed to set. Broth for the bacterial cultures was made using 16 g of nutrient agar mixed into 1 L of distilled water. The broth was autoclaved for 1 h at 121°C. The Gram-positive and -negative bacterial strains were grown overnight in the nutrient broth at 30 and 37°C, respectively, on a mechanical shaker (model SM-3600) in an incubator. The 0.5 McFarland standard was used to standardize the absorbance of the bacterial strains using the Cary 60 UV-Vis.

Well diffusion assay

Under aseptic conditions, bacterial strains were smeared onto the MHA plates using an L-shaped metal spreader. A sterilized metal borer was used to puncture wells (5 mm) into the agar plates to which 90 µl of the prepared methanolic extract was pipetted into. Petri plates were sealed and placed in an incubator for 24 h. Antibacterial activity was identified by the observation of zones of inhibition against the bacterial strains.

Results and discussion

Qualitative phytochemical analyses of bioactive compounds in various solvent extracts of C. benghalensis

The extraction efficiency for various plant materials can differ due to numerous factors. These include the plant chemical composition in which the assorted plant parts containing different availability of bioactive compounds that can be extracted (Hsu et al., 2006); the type of solvents used in an extraction procedure heavily influence the success of the extraction, determination and the isolation of bioactive plant compounds; therefore, there is a need to try various solvents in order to determine percentage yield of the extracts as well as to screen these extracts for phytochemicals (Abegunde and Ayodele-Oduola, 2013). In this study, hexane was initially used in the extraction procedure to remove fatty acids or oily components; followed by chloroform which has medium polarity, and this extracts the polar bioactive compounds withing the plant material; and lastly methanol extracted the polar components as this solvent has high polarity. The Soxhlet extraction procedure is one of the most extensively used extractions methods to date. The major advantages of this procedure are that due to the use of high temperatures and recycling of fresh solvent, there is an increase in mass transfer rate (Malik and Mandal, 2022), there is a low initial investment cost, the solvent will be in constant contact with the sample and there is also a lack of filtration required (Ghenabzia et al., 2023). In terms of the material used in the extraction procedure, generally, better results are produced by finer sized particles due to the enhanced penetration of the solvents and solute diffusion thus increasing the extraction efficacy (Zhang et al., 2018).

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Qualitative phytochemical analyses revealed the presence of important bioactive compounds in *C. benghalensis* leaf and stem extracts (*Table 1*). These compounds groups were mucilage and gums, carbohydrates, terpenoids, phenols, alkaloids, amino acids, flavonoids, saponins, sterols and fixed oils and fats. The major outcomes of the phytochemical tests in *Table 1* show that for the solvent extraction of the leaves, hexane appeared to have high concentrations of alkaloids, fats and oils; chloroform produced high levels of alkaloids and primary metabolite carbohydrates, while the methanol extract produced increased levels of terpenoids. The solvent extraction for the stems, hexane again shows high concentrations of alkaloids. Chloroform also produces high levels of alkaloids and carbohydrates, and high levels of phenols were found in the methanol extract. Phytochemicals are essential for proper functioning and survival of plants. They regulate growth, control fertilization and pollination, and also provide protection against microorganisms, herbivores and competitors (Molyneux et al., 2007). Previous studies on phytochemical analyses of *C. benghalensis* highlighted the presence of similar groups (Mukherjee and Ray, 1986; Hasan et al., 2009; Ibrahim et al., 2010; Prakash et al., 2010; Tiwari et al., 2013; Alaba and Chichioco-Hernandez, 2014; Ndam et al., 2014; Tadesse et al., 2016; Omogbehin et al., 2018; El-Hamid and El Bous, 2019; Ghosh et al., 2019a,b; Kansagara and Pandya, 2019). Ghosh et al. (2019a) reported phytochemicals such as betacyanin, coumarin, phlobatannins, quinones and xanthoprotein aside from those mentioned in our study (*Table 1*).

Table 1. *Phytochemical screening of C. benghalensis leaf and stem extracts using different solvents*

Plant constituents	Phytochemical test	Leaves			Stems		
		Hexane	Chloroform	Methanol	Hexane	Chloroform	Methanol
Mucilage and Gum	Ruthenium red	-	-	+	-	-	++
Carbohydrates	Molisch Fehlings Benedicts	- + +	- +++ -	- - -	- + +	- +++ -	- - -
Terpenes	Chloroform	-	-	+++	-	-	++
Phenols	Ferric trichloride	+	+	+	+	+	+++
Alkaloids	Wagners Dragendorff Mayers	- +++ +++	- +++ ++	+ + -	-- +++ +++	- +++ ++	- + -
Amino acids	Ninhydrin	-	+	++	-	+	++
Flavonoids	Lead acetate	-	-	+	-	-	+
Saponins	Foam Froth	- -	- -	+ +	- -	- ++	+ +
Sterol	Chloroform and sulphuric acid	-	-	++	-	-	+
Fixed oils and Fats	Filter paper	+++	++	++	++	-	-

*Reaction intensity: (-) absence of phytochemicals, (+) presence of phytochemicals, (++) moderate concentrations of phytochemicals, (+++) high concentrations of phytochemicals

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Due to the presence of phytochemicals, plants can perform various pharmacological effects (Shakya, 2016). Mucilage and gums contain demulcent properties that have many uses. These include suppression of cough, acts as a cryoprotectant to help heal gastric ulcers, to regulate sugar levels absorbed by the intestinal tract, treat fevers, colds, dysentery and diarrhoea (Mohanty and Mohan, 2014; Haruna et al., 2016; Chowdhury et al., 2017). Carbohydrates act as an immunomodulating agent, prebiotic and anti-oxidant carrier (Tzianabos, 2000; Dimitrova et al., 2015). Terpenes, saponins and sterols have anticancer, antibacterial, anti-inflammatory and antioxidant properties (Brahmkshatriya and Brahmkshatriya, 2013; Gu et al., 2014; Moses et al., 2014). Amino acids and alkaloids are recognized for their antitussive, anti-malarial, antifungal, anti-inflammatory, acetylcholinesterase (AchE) inhibitor and analgesic properties (Coëffier et al., 2010; Simera et al., 2010; Adnyana et al., 2013). Phenols and flavonoids possess antioxidant, anticancer, anti-inflammatory, antispasmodic and antimicrobial properties (Djeridane et al., 2005; Ghayur et al., 2007; García-Lafuente et al., 2009).

Bioactive compound analysis of C. benghalensis using TLC

Thin layer chromatography is a technique used for the separation of compounds. The bands represent the separated compounds, and they take on different colours with progression up the TLC plate (*Figures 1* and *2*). The leaf extracts (lane 1-3) showed 8 bands for hexane, chloroform exhibited 11 bands and methanol possessed 2 bands. The stem extracts (lane 4-6) displayed 6 bands for hexane, chloroform had 10 and there were no bands present for methanol. The leaf extracted exhibited more bands than stem extracts and chloroform displayed the greatest number of bands for both leaf and stem. The fluorescent bands appeared yellow and orange under Ultra -Violet light (UV) at 254 nm, dark brown under green UV-light at 366 nm, and orange and red under blue UV-light at 366 nm. The R_f values for leaf extracts ranged from 0.023 to 0.849 and stem extracts ranged from 0.023 to 0.844 (*Table 2*). The higher R_f value is indicative of less polar compounds as they move higher up the TLC plate (Bele and Khale, 2011).

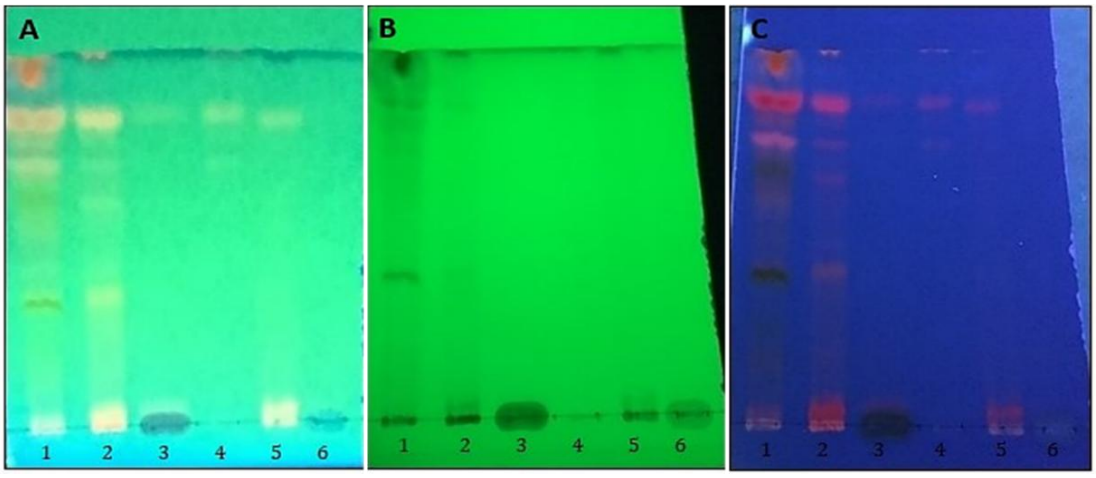

Figure 1. *Thin Layer Chromatography profile of extracts from leaves and stems of C. benghalensis untreated by anisaldehyde viewed under (A) UV light at 254 nm, (B) green UV at 366 nm and (C) blue UV at 366 nm. Leaf extracts were represented in lanes 1 (hexane), 2 (chloroform) and 3 (methanol); stem extracts were represented in lanes 4 (hexane), 5 (chloroform) and 6 (methanol)*

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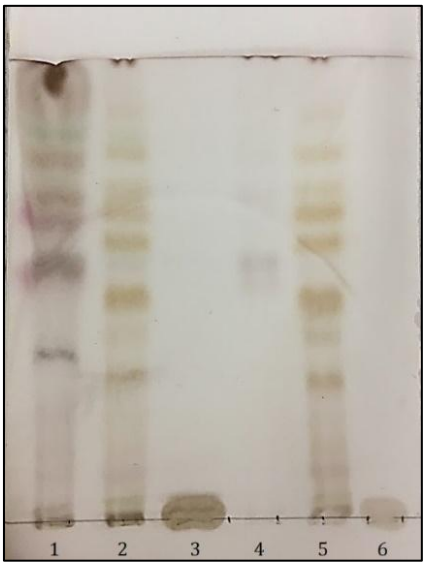

Figure 2. *Thin Layer Chromatography profile of extracts from leaves and stems of C. benghalensis treated in anisaldehyde under visible light. Leaf extracts were represented in lanes 1 (hexane), 2 (chloroform) and 3 (methanol); stem extracts were represented in lanes 4 (hexane), 5 (chloroform) and 6 (methanol)*

Table 2. *Mobility of compounds in C. benghalensis expressed by Rf values from TLC profiling*

Bands	Leaves			Stems		
	Hexane	**Chloroform**	**Methanol**	**Hexane**	**Chloroform**	**Methanol**
1	0.33	0.023	0.686	0.465	0.023	−
2	0.291	0.093	0.837	0.512	0.093	−
3	0.465	0.221	−	0.593	0.198	−
4	0.523	0.419	−	0.663	0.314	−
5	0.628	0.465	−	0.767	0.384	−
6	0.686	0.547	−	0.841	0.523	−
7	0.779	0.581	−	−	0.605	−
8	0.849	0.628	−	−	0.674	−
9	−	0.686	−	−	0.756	−
10	−	0.709	−	−	0.884	−
11	−	0.826	−	−	−	−

Bioactive compound screening of C. benghalensis extracts using GC-MS

Gas chromatography-mass spectrometry is a method used to separate volatile and semi-volatile compounds by means of gas-liquid chromatography and identify these compounds using mass spectrometry (Hussain and Maqbool, 2014). Phytochemical compounds present in the leaf and stem methanol extracts with a peak area percentage greater than 1 were identified (*Tables 3 and 4*) along with their pharmacological properties (*Table 5*). The leaf extract contained 26 phytochemical compounds (*Table 3*) and 21 in the stem extract (*Table 4*). In the leaf extract (*Table 3*), β-Sitosterol had the highest peak area percentage of 9.78% with the retention time of 29.253 min. The lowest peak area percentage (1.06%) in the leaf extract (*Table 3*) was produced by 2-

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methylhexacosane and 13-Docosenamide with a retention time of 24.547 and 25.169 min, respectively. The highest peak area percentage in the stem extract (*Table 4*) was produced by 1-Butanol, 3-methyl-, formate (15.21 %) with a retention time of 7.832 min, while the lowest was acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester with 1.01 % and retention time of 29.926 min. Sumithra and Purushothaman (2017) evaluated the phytochemical constituents of ethanolic leaf extract of *C. benghalensis* using GC-MS. Among their results, they found phytochemicals such as 3-dodecene, 1-hexodeconol, phenol 2,4 bis (1,1dimethyl ethyl), hexadecen1ol, trans9, 9eicosene, 9,10 anthracenedione, tetracosane, Tetratriacontane, Tetracosane 11decyl and compounds also found in *Table 4*. 3, namely, 1,4Benzenedicarboxylic acid, bis(2ethylhexyl) ester and 13Docosenamide, (Z). Each of these phytochemicals possesses various medicinal properties (*Table 5*).

Table 3. *Phytochemical compounds with a peak area % > 1 in the methanolic extract of C. benghalensis leaf using GC-MS*

Peak no.	Retention time	Peak area (%)	Phytochemical compound	CAS no.	Molecular weight
77	29.253	9.78	β-Sitosterol	83-46-5	414
15	19.289	8.66	9-Octadecen-1-ol, (Z)-	1 43-28-2	268
48	25.384	4.56	Squalene	111-02-4	410
17	19.512	4.51	n-Nonadecanol-1	1454-84-8	284
11	17.525	4.17	n-Heptadecanol-1	1454-85-9	246
1	7.706	4.14	1-Butanol, 3-methyl-, formate	110-45-2	116
66	27.575	3.75	Vitamin E	59-02-9	430
74	28.699	3.58	Stigmasterol	83-48-7	412
20	19.744	2.95	Phytol	150-86-7	296
44	24.891	2.58	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	6422-86-2	390
72	28.482	2.33	Ergost-5-en-3-ol, (3.β)-	4651-51-8	400
31	21.965	1.83	9-Octadecenamide, (Z)-	301-02-0	281
27	21.173	1.76	1,2-15,16-Diepoxyhexadecane		254
93	31.840	1.76	Friedelan-3-one	559-74-0	426
61	27.033	1.74	γ-Tocopherol	7616-22-0	416
67	27.652	1.52	Cholestane-3,5-diol, 5-acetate, (3.β, 5.α)-	41721-93-1	446
6	14.713	1.41	1,2,3,5-Cyclohexanetetrol, (1.α, 2.β, 3.α, 5.β)	53585-08-3	148
78	29.396	1.39	Octadecanoic acid	2778-96-3	536
25	20.940	1.36	Tributyl acetylcitrate	77-90-7	402
64	27.338	1.27	1-Heptacosanol	2004-39-9	396
85	30.384	1.14	B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.β)-	1615-94-7	426
84	30.247	1.09	α-Amyrin	638-95-9	426
41	24.547	1.06	2-methylhexacosane		380
46	25.169	1.06	13-Docosenamide, (Z)-	112-84-5	337

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Table 4. *Phytochemical compounds with a peak area % > 1 in the methanolic extract of C. benghalensis stem using GC-MS*

Peak no.	Retention time	Peak area (%)	Phytochemical compound	CAS no.	Molecular weight
76	29.249	7.38	β-Sitosterol	83-46-5	414
16	19.512	6.37	n-Nonadecanol-1	1454-84-8	284
10	17.525	5.97	n-Heptadecanol-1	1454-85-9	256
72	28.696	5.71	Stigmasterol	83-48-7	412
70	28.483	3.01	Ergost-5-en-3-ol, (3.β)-	4651-51-8	400
42	24.381	2.10	Z,Z,Z-8,9-Epoxyeicosa-5,11,14-trienoic acid, methyl ester		334
1	7.832	15.21	1-Butanol, 3-methyl-, formate	110-45-2	116
14	19.289	11.64	9-Octadecen-1-ol, (Z)-	143-28-2	268
6	14.702	1.80	1,2,3,5-Cyclohexanetetrol, (1.α, 2.β, 3.α, 5.β)-	53585-08-3	148
15	19.342	1.78	9-Octadecen-1-ol, (Z)-	143-28-2	268
25	21.137	1.74	1,2-15,16-Diepoxyhexadecane		254
91	31.835	1.69	Friedelan-3-one	559-74-0	426
24	20.941	1.52	Tributyl acetylcitrate	77-90-7	402
52	25.384	1.37	Squalene	111-02-4	410
63	27.336	1.28	1-Heptacosanol	2004-39-9	396
77	29.393	1.26	Distearyl thiodipropionate	693-36-7	682
84	30.377	1.19	B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.β)-	1615-94-7	426
57	26.287	1.10	9-Octadecenoic acid (Z)-	22393-85-7	478
66	27.647	1.07	Cholesterol	57-88-5	386
88	31.413	1.03	(E)-Dodec-2-enyl ethyl carbonate		526
80	29.926	1.01	Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester		278

Energy dispersive X-ray spectroscopy (EDX) on leaves and stems of C. benghalensis

The results of the energy dispersive X-ray of freeze-dried leaves and stems of *C. benghalensis* are shown in the EDX spectra in *Figures 3* and *4*, respectively. The elemental composition of the freeze-dried leaves and stems are shown in *Table 6*. As depicted in *Figure 3*, the leaves of *C. benghalensis* revealed the presence of magnesium (Mg), silicon (Si), chlorine (Cl), potassium (K), calcium (Ca), carbon (C), oxygen (O) and sodium (Na). The stems of *C. benghalensis* contain the same elements found in the leaves aside from Na and in addition of aluminium (Al), phosphorus (P), sulphur (S) and copper (Cu) (*Figure 4*). Carbon had the highest elemental weight percentage composition in both leaves and stem samples, 49.42 and 35.52%, respectively (*Table 6*). The lowest weight percentage in the leaves was obtained by Cu with 0.15%, while the lowest in the stems was S with 0.25%. Plants retain elements in various ways. The roots readily take up various minerals and metallic ions that are dissolved in water in the soil, whereas the leaf blades absorb elements from rainfall (Kirmani et al., 2017). Trace elements present in *C. benghalensis* highlights its importance as a medicinal plant. For example, Na helps to maintain the balance of physical fluid systems in the body and for the function of muscle and nerves (Constantin and Alexandru, 2011).

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Table 5. *Pharmacological activity of the phytochemical compounds identified in the methanolic extracts of C. benghalensis leaf and stem*

Phytochemical compound	Pharmacological action	Reference
1-Butanol, 3-methyl-, formate	Antimicrobial	Sermakkani and Thangapandian, 2012
1,2,3,5-Cyclohexanetetrol, (1.α, 2.β, 3.α, 5.β)	Anti-inflammatory, antioxidant and antimicrobial	Sarumathy et al., 2011
n-Heptadecanol-1	Antiacne agent	Kubo et al., 1994
9-Octadecen-1-ol, (Z)-	Antimicrobial	Gayathri and Sri, 2018
n-Nonadecanol-1	Cytotoxic, antimicrobial	Arora et al., 2017
Phytol	Anti-inflammatory, anxiolytic-like properties, cancer preventive, diuretic anticancer, antimicrobial	Sermakkani and Thangapandian, 2012; Costa et al., 2014
Tributyl acetylcitrate	Anti-Feeding effect on larvae	Hameed et al., 2016
1,2-15,16-Diepoxyhexadecane	Antitumor, anti-inflammatory	Shareef et al., 2016
9-Octadecenamide, (Z)-	Antimicrobial	Khan et al., 2019
2-methylhexacosane	Decreases blood cholesterol level, antimicrobial	Khatua et al., 2016
1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Antimicrobial, antifouling	Sumithra and Purushothaman, 2017
13-Docosenamide, (Z)-	Antimicrobial, anti-nociceptive	Sumithra and Purushothaman, 2017; Khan et al., 2019
Squalene	Antitumor, skin ointment, antibacterial, immunostimulant, anti-inflammatory, chemopresentitive, cancer preventative, pesticide, lipogenase-inhibitor, cosmetics	Yamuna et al., 2017; Rao and Anisha, 2018
γ-Tocopherol	Cardioprotective, anticancer, anti-inflammatory, hypocholesterolemic, antioxidant	Ponnamma and Manjunath, 2012
1-Heptacosanol	Anticancer, antimicrobial, antioxidant, nematicidal	Arora and Saini, 2017
Vitamin E	2E1 inhibitor, antidote, anticancer, endocrine tonic, antitumor, antioxidant	Rao and Anisha, 2018
Ergost-5-en-3-ol, (3.β)-	Jaundice, liver disease, hypocholesterolmic, antioxidant	Parveen et al., 2016; Arora et al., 2017
Stigmasterol	Antiviral, cancer preventive, antihepatotoxic, hypocholesterolaemic antioxidant	Ponnamma and Manjunath, 2012; Padmashree et al., 2018
β-Sitosterol	Reduction of blood levels of cholesterol, antioxidant, anti-hypercholesterolemia, anticancer	Kalaivani et al., 2012
Octadecanoic acid	Antibacterial, anti-inflammatory	Hussein et al., 2016; Sosa et al., 2016
α-Amyrin	Anticancer, anti-inflammatory-antioxidant, antimicrobial	Bharathy and Uthayakumar, 2013
Friedelan-3-one	Anticandidal activities, antipyretic, hepatoprotective, antifeedant, anti-inflammatory, anticancer, analgesic, antibacterial	Lakshmi and Nair, 2017
Distearyl thiodipropionate	Antioxidant	Karahadian and Lindsay, 1988
Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester	Antimicrobial	Ruvanthika et al., 2017

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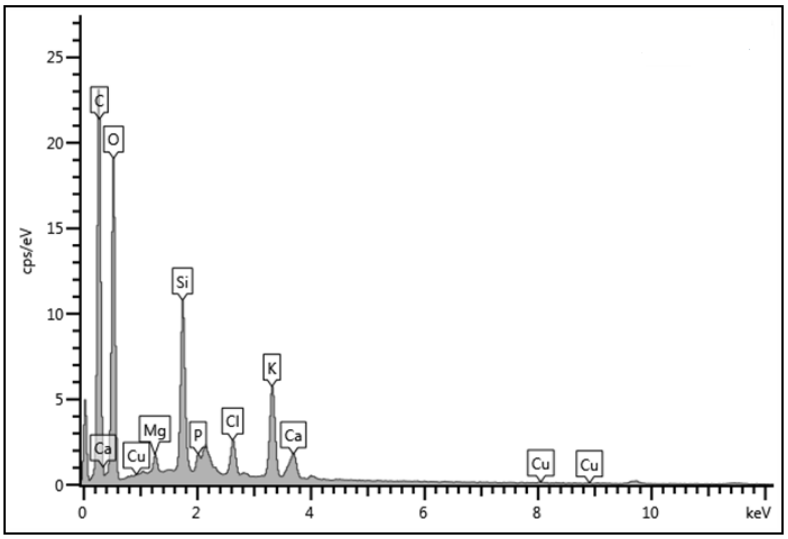

Figure 3. *EDX spectrum of freeze-dried C. benghalensis leaves*

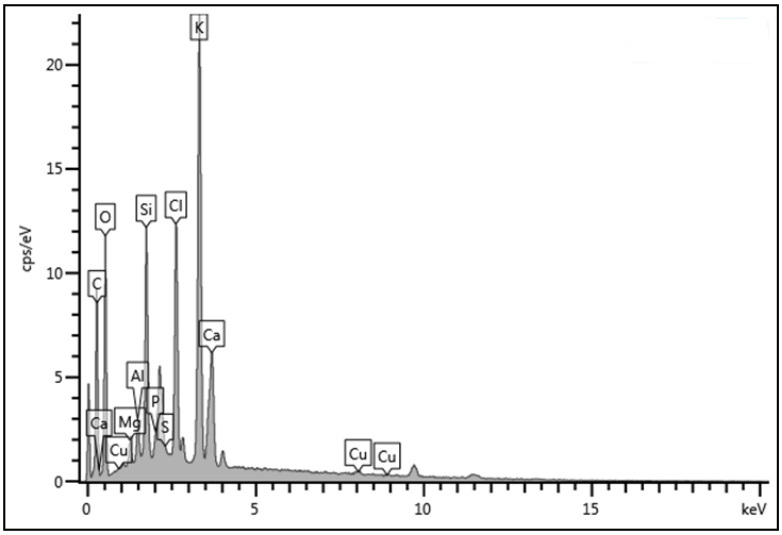

Figure 4. *EDX spectrum of freeze-dried C. benghalensis stem*

Potassium is a vital element, as it is responsible for maintaining blood pressure, heart function, adrenal and kidney function also bone strength and health (Weaver, 2013). Magnesium is traditionally used as a laxative or antacid and is involved in over 300 enzyme systems for muscle contraction, nerve function, hormone receptor binding, protein synthesis and blood pressure regulation (Schwalfenber and Genius, 2017). Silicon is associated with connective tissue, mineralization of bone matrix, collagen formation, bone development and hormonal control (Boguszewska-Czubara and Pasternak, 2011). Piste et al. (2012) revealed that Ca is an important trace element in the human body as it functions in building strong bones and teeth, muscle contraction, regulating heartbeat, nerve impulse, blood clotting and balancing fluid within cells. Plants readily assimilate such elements through the roots. Metallic ions get dissolved in water and retained. Additional sources of these elements for plants are rainfall, atmospheric dusts and plant protection agents, which could be adsorbed through the leaf blade.

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Table 6. *Weight percentage of freeze-dried leaves and stem of C. benghalensis from EDX analysis*

Compound	Weight (%)	
	Leaves	Stems
Magnesium	0.43	0.54
Aluminium	-	0.78
Silicon	3.17	3.91
Phosphorus	0.33	0.49
Sulphur	-	0.25
Chlorine	0.89	5.50
Potassium	2.92	13.25
Calcium	0.87	3.87
Copper	0.15	0.55
Carbon	49.54	35.52
Oxygen	41.69	35.32

Antibacterial activity of leaves and stems of C. benghalensis

Medicinal plants still play a vital role in covering the basic health needs of those in developing countries as they act as therapeutic agents (Parekh and Chanda, 2008). The use of plants as medication has increased over the past few decades. It is probable that bacterial infections will be treated with antibacterial efficient phytochemicals (Balandrin et al., 1985; Parekh and Chanda, 2008). *Table 7* summarizes antibacterial inhibition of methanolic extracts of *C. benghalensis* leaves and stems. The leaf methanolic extract showed inhibition of bacterial strains *B. subtillus* (*Figure 5 A*), *S. aureus* (ATCC 29213) (*Figure 5 C*), Methicillin-resistant *S. aureus* (environmental and clinical) (*Figure 5 D* and *E*), *Escherichia coli* (*Figure 5 F*), *E. coli* (ATCC 25218) (*Figure 5 G*) and *P. aeruginosa* (*Figure 5 H*). The stem methanolic extracts inhibited the same bacterial strains as the leaf extract in addition to *S. aureus* (*Figure 5 B*). No inhibition was observed in the leaf and stem extracts against *P. aeruginosa* (ATCC 25215) (*Figure 5 I*).

Table 7. *Antibacterial activity using methanolic extracts of C. benghalensis leaf and stem against various pathogens*

Pathogen	Leaves	Stem
Bacillus subtillus	+	+
Staphylococcus aureus	-	+/-
Staphylococcus aureus (ATCC 29213)	+/-	+
Methicillin-resistant Staphylococcus aureus (environmental)	+	+
Methicillin-resistant Staphylococcus aureus (clinical)	+/-	+/-
Escherichia coli	+/-	+/-
Escherichia coli (ATCC 25218)	+/-	+/-
Pseudomonas aeruginosa	+/-	+/-
Pseudomonas aeruginosa (ATCC 25215)	-	-

Inhibition intensity: (+) Clear zone of inhibition, (-) no inhibition, (+/-) effect on bacteria

Khan et al. (2011b) used the disc diffusion method to investigate the antibacterial activity of *C. benghalensis*. They showed that the ethanolic extract had inhibited the

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bacterial growth of *Enterococcus faecalis*, *Shigella dysenteriae*, *P. aeruginosa*, *E. coli*, *Staphylococcus pyogenes*, *Staphylococcus saprophyticus*, *S. aureus* and *Streptococcus agalactiae*. Biqiku et al. (2016) verified the antimicrobial properties of *C. benghalensis* as the ethanolic extract exhibited inhibition of *S. aureus*, *S. pyogenes*, *Streptococcus mutans*, *Candida albicans* and *P. aeruginosa*. Jerin et al. (2019) performed the disc diffusion antibacterial analysis on *C. benghalensis* ethanolic leaf extracts. It was found that from the two strains tested, there was no zone of inhibition produced by the extract on *E. coli* but a zone of inhibition could be seen for *S. aureus* of 10 mm at 500 µg/disk, while the antibiotic Kanamycin produced a zone of inhibition of 32 mm at 30 µg/disk. In another research study, the ethanolic leaf extracts of *C. benghalensis* at various concentrations were tested against *S. aureus*, *C. albicans* and *E. coli* (Cuéllar et al., 2010). Their research indicated that with decreasing concentrations of ethanolic extracts, the zones of inhibition had reduced substantially against the three microbial species.

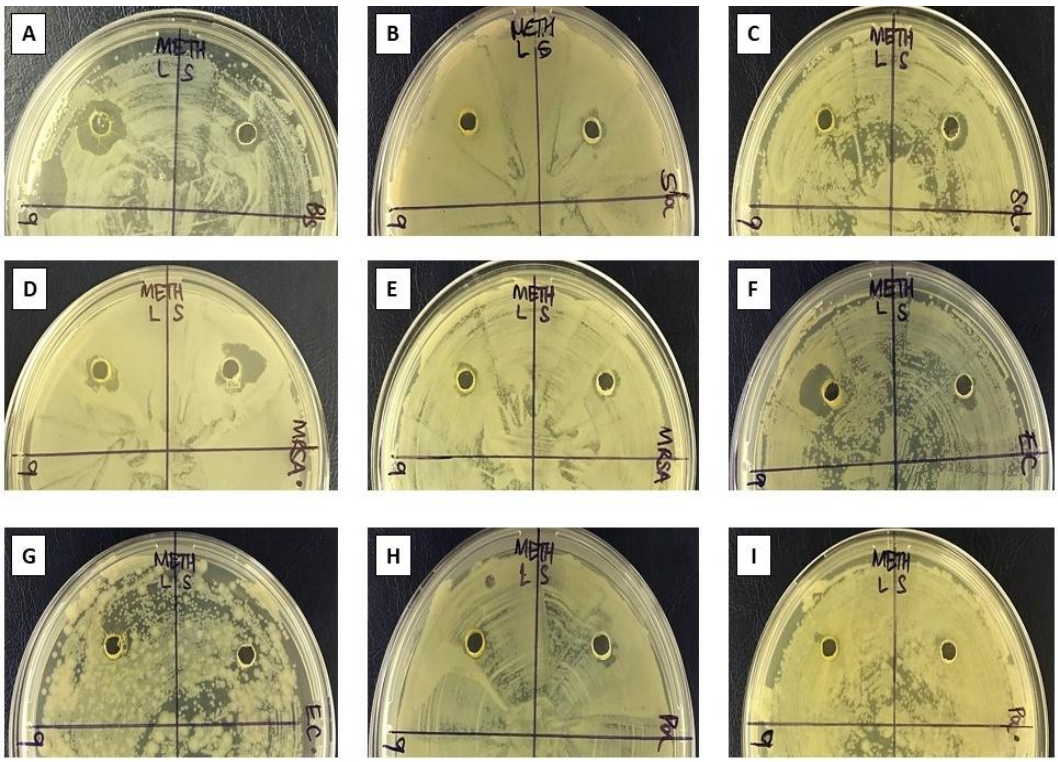

Figure 5. *Antibacterial activity against various pathogens using methanolic extracts of C. benghalensis leaf and stem. Gram-positive: (A) B. subtillus, (B) S. aureus, (C) S. aureus (ATCC 29213) and Methicillin-resistant S. aureus ((D) environmental and (E) clinical type); and Gram-negative: (F) E. coli, (G) E. coli (ATCC 25218), (H) P. aeruginosa and (I) P. aeruginosa (ATCC 25215)*

Overall, in this research, the extracts showed moderate to strong zones of inhibition against human pathogens. This could be attributed to the presence of phytochemicals with known antimicrobial potential such as 1-Butanol, 3-methyl-, formate, 9-Octadecen-1-ol, (Z)-, 9-Octadecenamide, (Z)-, 1,2,3,5-Cyclohexanetetrol, (1.α, 2.β, 3.α, 5.β), n-Nonadecanol-1, Phytol, Octadecanoic acid, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, 13-Docosenamide, (Z)-, Squalene, 1-Heptacosanol, Stigmasterol, α-Amyrin, Friedelan-3-one and Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-

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2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester. For example, phytosterols such as stigmasterol have the ability to inhibit cell surface proteins in bacterial cells as well as induce modifications to the bacterial cell membrane composition (Bakrim et al., 2022). The compound phytol has shown to induce bacterial cell death through oxidative stress by ROS accumulation, cell damage, cell division arrest and membrane depolarization (Lee et al., 2016). The type of solvents used in the extraction process influence the yield extracted as well as the content of the bioactive compounds which have significant effects of the biological activity of the extracts (Truong et al., 2019). The solvent of choice for the antibacterial activity assessment was methanol as this solvent enhances the extraction of non-water-soluble material as methanol has the ability to dissolve compounds that are both lipophilic and hydrophilic (Ibrahim and Kebede, 2020). This enables the extraction of more antibacterial compounds from the assorted plant material as compared to chloroform, hexane or aqueous crude extracts; for example, aqueous extracts tend to lose some of their antibacterial activity (Ibrahim and Kebede, 2020).

Plants have been known to provide a plethora of bioactive compounds, whether used in traditional medicines, marketed supplements or pharmaceutical drugs. The antimicrobial potential of medicinal plants has been exploited through the years. The antibacterial properties of *C. benghalensis* have been highlighted. These naturally occurring antimicrobial products could act alone or in combination with known antibiotics in order to enhance their activity against a wide range of microorganisms (Vaou et al., 2021). In terms of agriculture, *C. benghalensis* could be used to treat and improve the health of animals and reducing the use of synthetic antibiotics in animal feeds. The use of phytogenic feed additives such as medicinal plant extracts or powders are non-toxic, ecofriendly and inexpensive, and could be added to animal feeds to improve animal health and decrease mortality and morbidity rates (Ivanova et al., 2024). The increasing reliance on medicinal plants in industrialized societies have been traced from the traditionally used rural herbal medicines and the extraction and development of numerous drugs and chemotherapies (Sofowora et al., 2013). However, to avoid exploitation of medicinal plants, such as *C. benghalensis*, there needs to be conscious efforts made to properly identify, recognize and position of medicinal plants in health promotion, disease management and chronic disease management (Sofowora et al., 2013).

Conclusions

The present study conclusively shows that *C. benghalensis* is a valuable source of various bioactive compounds as revealed by phytochemical tests, TLC, EDX and GC-MS. Some important phytochemical compounds that were present in *C. benghalensis* leaves and stems were alkaloids, phenols, flavonoids, squalene, phytol and stigmasterol. These compounds are known to be pharmacologically important as they exhibit various biological activities. The inhibition of bacterial strains by the leaves and stem methanolic extract further authenticates the plant's candidacy in the pharmaceutical industry. For future research, the isolation and purifying bioactive compounds should be conducted and thereafter evaluating each of their antimicrobial capacity. Further pharmaceutical assays should also be conducted such as the antioxidant, anti-inflammatory and anticancer potential of *C. benghalensis* leaves and stems. Toxicity studies is also a key component that should be investigated on *C. benghalensis* as this is vital in ensuring the safety of the material in order to formulate new drugs.

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