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Research Article

Studies on the Efficacy of Leaves Extracts of *Centella asiatica* Against *Escherichia coli, Samonella typhi* and *Staphylococcus aureus*

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Abstract

Centella asiatica is a traditional herb which is reported to have many biological properties that account for the ethnomedicinal properties of the plant. Studies were carried out to determine the antimicrobial activity of aqueous, 70% ethanol and absolute ethanol leaf extracts of *Centella asiatica* by agar well diffusion assay. The tested bacterial strains were *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Salmonella typhi* ATCC 19430. The zone of inhibitions produced by the three extracts against the selected bacteria strains were recorded together with the standard drug, Ciprofloxacin (15µg) used as positive control with water and dimethyl sulfoxide (DMSO) used as negative control. The study showed that all the selected bacteria were susceptible to the aqueous, 70% ethanol and absolute ethanol leaf extract of *C. asiatica* with the aqueous leaf extract exhibiting the highest antimicrobial activities. The 70% ethanol extract recorded the lowest MIC of 1.56mg/ml for *S. typhi* whereas that of the aqueous and organic extracts were 6.25mg/ml. The result obtained confirms the antibacterial activity of the leaf extracts of *C. asiatica* revealed by other researchers.

Keywords: *Centella asiatica; E. coli* ATCC 25922; *Staphylococcus aureus* ATCC 25923; *Salmonella typhi* ATCC 19430; Antimicrobial Activity of Leaf Extracts

Introduction

The use of plants for the management and treatment of different infectious diseases of humans has been in existence since ancient times [17]. The therapeutic properties of these plants are mainly due to the presence of secondary metabolites such as alkaloids, cardiac glycosides, tannins, flavonoids, saponins, reducing compounds, minerals, and vitamins [30]. Terpenoids, give plants their odour and others like tannins are responsible for plant pigment. Again, secondary metabolites of plants have been described to be essential sources of various phytochemicals used directly or intermediately for the manufacture of pharmaceuticals [14]. Interestingly, several plant extracts known to possess antimicrobial properties are being used in traditional medicine [2]. *Centella asiatica* is among such medicinal plants that have been documented to possess antibacterial activities [16].

In recent times there is the emergence of antimicrobial resistance which has been a major health issue and still presents threat to health care system globally. The evolution and distribution of anti-

biotic resistance components in bacterial pathogens has also made diseases that were once easily treatable deadly [3]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [5]. For instance, among the family Enterobacteriaceae, *Escherichia coli* and *Salmonella typhi* have been the most commonly isolated species and are very well known to exhibit multidrug resistance [4], while *Staphylococcus aureus* had shown to be very resistant to a wide variety of antibiotics [19].

Urinary tract, gastrointestinal, and pyogenic infections are the common hospital-acquired infections caused by bacteria of the family Enterobacteriaceae [4]. Typhoid is a key example of this, with multidrug resistant strains of the bacterium *Salmonella typhi* becoming common in many developing countries. It is often resistant to the first-line antimicrobials commonly used to treat the disease, and is continuing to evolve as it spreads to new regions and populations, acquiring novel mutations providing resistance to newer antimicrobial agents, such as ciprofloxacin and azithromycin [31]. In view of this, there is the need to respond to these challenges that have emerged and having adverse effects on health care in general.

Since the last decade, there have been an increase in the investigation of plant extracts which have been inhibitory and effective against microorganisms. *Centella asiatica* has shown some activity in various *in vitro* experiments carried out by some literature. *Centella asiatica* is an ethnomedicinal plant used for several purposes. These include its usefulness in the form of cover crop in rubber and tea plantations, for tea and juice, in food products, a source of some micro and macro nutrients, proteins and vitamins like thiamine and ascorbic acid, vegetables for nutritional purposes and also cosmetics for skin maintenance. It is most used for medicinal purposes including inflammatory, cardiac, depurative, hypotensive, tonic, nervic, and stimulant [14]. Also, its pharmaceutical effect makes it useful in wound healing, mental and neurological disorders, atherosterosis, fungicidal, antibacterial, antioxidant and anticancer purposes [6].

Staphylococcus aureus is among the invasive Gram-positive microbes known as pyogenic cocci implicated in several diseases (boils, impetigo, toxic shock syndrome, food poisoning, and also a common cause of nosocomial infections) of human [19]. High rates of Methicillin resistant *S. aureus* (MRSA) imply that treatment for suspected severe *S. aureus* infections, such as common skin and wound infections must depend on second-line drugs in many countries, and thus standard prophylaxis with first-line drugs for orthopaedic and other surgical procedures will have limited effect in many settings [33].

In Ghana, some of the antimicrobial resistant studies conducted on bacterial species isolated from outpatients, established that most of the isolates (*Escherichia coli*, *Pseudomonas* spp., *Staphylococcus aureus*, *Streptococcus* spp. and *Salmonella typhi*) were multidrug-resistant [20]. A range of clinical bacterial isolates were resistant to important commonly used antimicrobials (ciprofloxacin, ceftriaxone, and amikacin) in the country [20]. There is the need to develop effective antimicrobial drugs against the threat to public health caused by antimicrobial resistant pathogens, which can cause serious and untreatable infections in humans.

Centella asiatica, is commonly known as Indian pennywort, Asiatic Penniwort or Gotukola, is a common perennial herbaceous creeper belonging to the family Apiaceae, which is distributed widely in the tropical and subtropical countries. The species is floating, emergent or terrestrial in grasslands, especially along riversides and also moist and shady areas [11]. The plant has been reported as one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, hence primarily known as a "Brain food" in India. The roots and leaves of the plant are used for medicinal purposes. Other ethnomedicinal uses of *C. asiatica* include anti- inflammatory, antiulcer, anticonvulsant antiviral, antibacterial, antifungal and many more [16]. The most characteristic chemical compounds found in this plant are called triterpenes which contain asiaticosides, an active ingredient responsible for the wound healing properties [28]. It contains large amount of pentacyclic triterpenoid saponins which is a major component of secondary metabolites providing active compounds which promotes cell rejuvenation and improves physical and mental health [14]. The leaves are tonic, rich in ascorbic acid; accelerate nervous activity and good for increasing memory [28]. The search for new effective antimicrobial compounds with improved activity to replace those that have become inactive is, therefore, necessary especially from nature.

In view of the increasing interest in the use of plants in the treatment of diseases, especially in developing countries where cost

of living is high and there is less scientific research on the use of known medicinal plants, antibacterial studies of *C. asiatica* will no doubt provide scientific basis to either support or debunk its uses in the treatment of microbial disease. Therefore, research into the antimicrobial activity of *C. asiatica* plant is essential because if found to be active, it can be used as an alternative to synthetic antimicrobial products. The objective of this research is to evaluate the potential of plant extracts of *C. asiatica* on standard microorganism strains that are of public health importance.

Materials and Methods

Collection of test plant *Centella asiatica*

The leaves of *Centella asiatica* were manually harvested from the Centre for Plant Medicine Research (CPMR) farms in the Eastern Region of Ghana for the extract preparation. The authentication process was carried out by the Plant Development Department, of Centre for Scientific Research into Plant Medicine (CPMR) where specimen voucher was made available at the herbarium of the Department.

Preparation of aqueous and ethanolic plant extracts

The harvested plant leaves were subjected to the appropriate preliminary processing including elimination of undesirable withered parts and contaminants through sorting, washing, and peeling. The fresh plant leaves were air-dried in a shade at room temperature [32] for 5 days and pulverized in a mortar. Forty grams portions of the pulverized plant material were cold macerated in 1,000 ml of 70% and absolute ethanol for 72 hours and filtrated through a Whatman No. 1 filter paper.

Another 40 g of the pulverized material was hot macerated in 1,000 ml of hot water followed by filtration. After filtration of the hot water solution, it was placed in an oven to dry. The ethanolic filtrates of the plant was concentrated using rotary evaporator at 50 ºC in round-bottomed flasks after which the residue was poured into a plastic container and dried in the oven.

Preparation of culture media

Nutrient Agar, Bacteriological Peptone and Mueller-Hinton agar were used. The media were prepared according to manufacturer's instructions.

Inocula preparation for agar well diffusion assay

The stock cultures of *Staphylococcus aureus ATCC 25923* and *Salmonella typhi ATCC 19430* were sub-cultured onto fresh Nutrient agar plates and stored in a refrigerator. Three to four well-isolated colonies of the same morphological type of each organism were suspended in test tubes containing 4 ml of sterilized bacteriological Peptone and incubated at 37 ºC for 2-6 hours to attain the turbidity of 0.5 McFarland standard. The turbidity of the actively growing broth cultures was adjusted with sterile Bacteriological Peptone to obtain turbidity optically comparable to that of 0.5 McFarland Standard (approximately 1-2 x 108 CFU/ml for E. coli ATCC 25922).

Antimicrobial activity assay

The agar well diffusion method [12] was used to investigate the antimicrobial properties of the crude extracts. Within 15 minutes after adjusting the turbidity of the inoculum suspension, 25 µl of the suspension was dispensed onto the centre of the dried surface of a Mueller-Hinton agar plates and labelled, respectively. The plates were inoculated by streaking with a sterile swab over the entire sterile agar surface with bacteria. The procedure was repeated by streaking two more times, rotating the plate each time to ensure an even distribution of inoculums. A sterilized cock borer of diameter 4 mm was used to punch seven holes in the media and 85 µl of 200 mg/ml of the plant extracts was dispensed into the respective labelled holes. A standard drug (15 µg/ml of ciprofloxacin) was used as positive control test, while 5% v/v dimethyl sulfoxide (DMSO) and sterile distilled water were used as negative control test or ethanolic and aqueous extracts, respectively. Duplicates of each plate were made, and the procedure repeated for the other organisms. The plates were kept in the refrigerator for 4 hours for complete diffusion of the extract and incubated at 37 ºC for 24hrs. After the incubation period, the diameter of each zone of inhibition was measured in millimeters (mm) with a standard ruler. Measured zone of inhibition for extracts less than 7 mm was considered as no antimicrobial activity observed and greater than 7 mm as sensitive or active (considered having some amount of antimicrobial activity).

Inocula preparation for Minimum Inhibitory Concentration (MIC) assay

The organisms to be tested were sub-cultured onto a nutrient plate and incubated at 37 ºC for 18- 24 hours to obtain a pure growth. Using a straight wire or loop, at least four (4) individual colonies from the pure culture was transferred into Bacteriological peptone. Colonies were emulsified in the peptone to give an equivalent turbidity of 0.5 McFarland Standard (equivalent to a growth

of 1-2 X 108 CFU/ml for E. coli ATCC 25922) (C LSI, 2012). Within 15 minutes after adjusting the turbidity of the inoculum, 1ml of the suspension was transferred into 10 ml blank bacteriological peptone tube (i.e. adjusted McFarland Standard) for the MIC assay.

Determination of minimum inhibitory concentration (MIC)

To determine the MIC of plant extract using the microplate dilution method as described by [8] was used. A one hundred microlitre of ethanol/aqueous extract was added to 100 µl of sterile distilled water in the first well in the 96-well microplate and mixed well with a micropipette; 100 µl of this dilution was added to bacteriological peptone in the next well in the column and the process was repeated. This yielded a two-fold serial dilution in the original extract. The process was repeated for the other plant extracts in other columns of the microplate. A solution of a reference antibiotic, 15 µg/ml of ciprofloxacin was also serially diluted in another column of the microplate as a positive control. A 100 µl of an actively growing test organism (adjusted McFarland Standard) was then added to each of the dilutions. The microplate was sealed and incubated overnight at 37ºC. After incubation, 40 µl of 0.2 mg/ml INT (p-Iodonitrotetrzolium voilet) dissolved in a sterile distilled water was added to each of the wells after incubation. Microplates were examined after additional 30 to 120 minutes incubation. Bacterial growth was indicated by a red colour of INT reduced to the formazan. The lowest concentration at which a decrease in the red colour is apparent compared to the next dilution was taken as the MIC value. Minimum inhibitory concentration was deduced from the lowest concentration at which no growth took place.

The minimum bactericidal concentration (MBC)

The MBC values were deduced from wells with lowest concentrations at which no growth was recorded after culture for 24 hours of incubation described by [18]. A small sample from each of the wells was transferred to fresh nutrient agar plates and was incubated overnight at 37 ºC. The plates were examined for the presence or absence of living organisms. Plates with no microbial growth were regarded as the minimum bactericidal concentrations.

Phytochemical screening

The phytochemical components of aqueous, 70% ethanol and absolute ethanol extracts of the leaves of *C. asiatica* were determined. The bioactive components assayed for are saponins, 17 reducing sugars, polyuronides, cyanogenic glycoside, alkaloid, triterpenes, phytosterols, flavonoids, anthocyanosides and phenolics.

Statistical analysis

The antimicrobial activity was determined by measuring the diameter of zone of inhibition and the mean of two replicates were recorded. Statistical analyses were performed using Anova of Stata11 software including the comparison of the inhibition zone between the three extracts. Inhibition zones are expressed as mean ± Standard error.

Results

Phytochemical constituents of aqueous and 70% ethanol and absolute ethanol leaf extracts of *Centella asiatica*

Phytochemical studies of leaves extract of *C. asiatica* revealed the presence of saponins, alkaloids, flavonoids, polysterols and reducing sugars. The Aqueous extract gave positive results for the presence of sapronins and lkaloids, while the organic extracts showed the presence of saponins, reducing sugars, alkaloids, flavonoids and polysterols.

Bioactive Component	Aqueous extract	70% Ethanol extract	Absolute Ethanol extract
Saponins	+	+	+
R. Sugars	-	+	+
Phenolic compounds	-	-	-
Polyuranides	-	-	-
Cyanogenicglycosides	-	-	-
Alkaloids	+	+	-
Flavonoids	-	+	-
Anthracenoside	-	-	-
Triterpenes	-	-	-
Phytosterols	-	+	-

Table 1: Phytochemical constituents of aqueous and 70% ethanol and absolute ethanol leaf extracts of *Centella asiatica.*

+ = Presence of the bioactive component; - = Absence of the bioactive component.

Antimicrobial activities of aqueous leaves extract of *Centella asiatica* on test organisms

The effect of the aqueous leaves extracts of *Centella asiatica* on the test organisms is reported in table 2. The table shows that, all

the bacteria tested in the study were susceptible to the aqueous extract at concentrations of 200, 100, 50 and 25mg/ml. However, at a concentration of 12.5mg/ml, none of the bacteria species was susceptible it can be hypothesized that at lower concentrations might have been too weak to cause significant effect on the test bacteria. Generally, *Salmonella typhi* was the most susceptible with average zones of inhibition (24.5mm, 22.5mm and 18mm at 100, 50 and 25mg/ml) respectively, although the zone of inhibition (12.0 ± 1.0) mm was reduced to about half at a concentration of 200mg/ml. *Escherichia coli* was recorded as the second most susceptible organism with average zones of inhibition 23, 22, 21 and 19.5mm at 200, 100. 50 and 25mg/ml respectively. The other bacteria species (*Staphylococcus aureus, Paratyphi A* and *Paratyphi B*) recorded lower zones of inhibition as compared to *Escherichia coli* and *Salmonella typhi*. *Paratyphi B*. The implication is that these bacteria were not susceptible to the leaves extract at a concentration of 25mg/ml. For example, at a concentration of 200mg/l, *S. aureus* recorded 12.0 ± 0mm zone of inhibition whereas no inhibition was recorded for *Paratyphi* A. Interestingly, the effect of the extracts on *Paratyphi B* was similar to the observations obtained for *E. coli* and *S. typhi*. Ciprofloxacin, an antibiotic which was used as positive control was very effective in the inhibition of all test bacteria species and recorded average zones of inhibition of 26, 30, 18.5, 24 and 30mm for *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Paratyphi A, Paratyphi B* respectively. Expectedly, there were no inhibition zones for water which was used as negative control.

Test Organism	Diameter (mm) of zone of inhibition in indicated concentrations (mg/ml)						
	12. 5	25	50	100	200	Cipro 15µg/ml	Water
E. coli ATCC 25922	0 ± 0.0	19.5 ± 0.0	21 ± 0.5	22 ± 0.0	23 ± 0.0	26 ± 0.0	0 ± 0.0
S. typhi ATCC19430	0 ± 0.0	18 ± 0.0	22.5 ± 0.5	24.5 ± 0.5	12 ± 1.0	30 ± 0.0	0 ± 0.0
S. aureus ATCC25923	0 ± 0.0	8 ± 0.0	10.5 ± 0.5	12 ± 0.0	12 ± 0.0	18.5 ± 0.5	0 ± 0.0
Paratyphii A	0 ± 0.0	14 ± 2.0	16.5 ± 1.5	18 ± 2.0	0 ± 0.0	24 ± 0.0	0 ± 0.0
Paratyphii B	0 ± 0.0	0 ± 0.0	17 ± 1.0	18 ± 0.0	19.5 ± 0.5	30 ± 0.0	0 ± 0.0

Table 2: Influence of aqueous extract of leaves of *Centella asiatica* on zones of inhibition of test bacteria at indicated concentrations.

Antimicrobial activities of 70% ethanol extract of leaves of *Centella asiatica* on test organisms

The data obtained are presented in table 2: results indicated that, all the test microorganisms were susceptible to 70% ethanol extract at the following concentrations 200, 100 and 50mg/ml except for *Paratyphi B* which was not susceptible in any of the concentrations. At 25 and 12.5mg/ml, none of the test bacteria was susceptible, it is conjectured that such concentration might have been too small to cause and inhibition effect. *Salmonella typhi* appeared to be highly susceptible with average inhibition zones of 16mm, 14.5mm and 12mm at 200,100 and 50mg/ml respectively. *Paratyphi A* was the second most susceptible organism with average zones of inhibition of 15 and 13mm at concentrations of 200 and 100mg/ml respectively. However, concentration of 50mg/ml did not show any activity against *Paratyphi A. Staphylococcus aureus* was the next susceptible test organism with average inhibition zones of 14, 10.5 and 9mm at 200, 100 and 50mg/ml respectively. *Escherichia coli* had the least susceptibility with an average inhibition zone of 12mm at 200mg/ml whereas no inhibition zone was recorded for the other concentrations. A standard antibiotic Ciprofloxacin which served as positive control was effective against all the test bacteria species except *Paratyphi* A whereas DMSO served a negative control, was ineffective against all test bacteria.

Antimicrobial activities of absolute ethanol extract of leaves of *Centella asiatica* on test organisms

The data obtained are presented in table 4: results showed that, all the test microorganisms were significantly ($p < 0.05$) susceptible to the absolute ethanol leaves extract in 200, 100 and 50mg/ml except for *Paratyphi B* which was not susceptible in any of the concentrations. Concentrations of 25 and 12.5mg/ml, did inhibit growth of the test bacteria in media which was amended with 70% ethanol leaves extract. Data revealed that *Paratyphi A* appeared to be highly susceptible with average inhibition zones of 16 mm, 13 mm and 11 mm at concentrations of 200, 100 and 50mg/ml respectively. *Staphylococcus aureus* was the second most susceptible organism

Test organisms	Diameter (mm) of zone of inhibition at indicated concentrations (mg/ml)						
	12.5	25	50	100	200	Cipro 15µg/ml	DMSO (5%)
E.coli ATCC2592 2	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	12 ± 0.0	27 ± 0.0	0 ± 0.0
S.typhii ATCC19430	0 ± 0.0	0 ± 0.0	12 ± 0.0	14.5 ± 0.5	16 ± 0.0	24 ± 0.0	0 ± 0.0
S.aureus ATCC25923	0 ± 0.0	0 ± 0.0	9 ± 0.0	10.5 ± 0.5	14 ± 0.0	19.5 ± 0.5	0 ± 0.0
Paratyphii A	0 ± 0.0	0 ± 0.0	0 ± 0.0	13 ± 3.0	15 ± 2.0	22.5 ± 0.5	0 ± 0.0
Paratyphii B	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

Table 3: The effect of 70% ethanol leaves extract of *Centella asiatica* against zone of inhibition of four bacteria genera at indicated concentrations.

with average zones of inhibition (12,10.5 and 8.5) mm at the same concentrations, respectively. This was followed by *Samonella typhi* whereas *Escherichia coli* was the least susceptible which recorded an average zone of inhibition of 11mm in only 200mg/ml concentration. As observed in previous data tables 1 and 2, Ciprofloxacin served as a positive control was effective against all test bacteria. Dimethyl sulfoxide (DMSO) which was a negative control did not show any activity against the test organisms.

Test organisms	Diameter (mm) of zone of inhibition in indicated concentrations (mg/ml)						
	12.5	25	50	100	200	Cipro 15µg/m l	DMSO (5%)
E. coli ATCC259 22	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	11 ± 0.0	22.5 ± 0.5	0 ± 0.0
S. typhii ATCC194 30	0 ± 0.0	0 ± 0.0	9 ± 0.0	10 ± 0.0	11.5 ± 0.5	27 ± 0.0	0 ± 0.0
S. aureus ATCC259 22	0 ± 0.0	0 ± 0.0	8.5 ± 0.5	10.5 ± 0.5	12 ± 0.0	19.5 ± 0.5	0 ± 0.0
Paratyphii A	0 ± 0.0	0 ± 0.0	11 ± 1.0	13 ± 0.0	16 ± 0.0	23 ± 0.0	0 ± 0.0
Paratyphii B	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	23.5 ± 0.0	0 ± 0.0

Table 4: Influence of absolute ethanol leaves extract of *Centella asiatica* against 4 genera of bacteria at indicated concentrations.

Determination of minimum inhibitory concentration of various leaves extract of *Centella asiatica* against *Escherichia coli*

Table 5a, the (+) indicates colour change of the extract from green to red which indicates the growth of microbes while the (-) indicates no colour of the extract which is interpreted as the absence of microbial growth (Plate 1). The first microplate well of a concentration to show no colour change was recorded as the M.I.C value. Data from table 5b, showed that the least MIC value for aqueous leaves extract recorded for *Salmonella typhi* was 6.25mg/ml. It was followed by 12.5mg/ml which showed activity against *E. coli*. whilst *S. aureus* recorded a MIC of 25mg/ml. On the contrary *Paratyphi A* obtained the highest MIC value of 50mg/ml. The implications are that the lower concentration of the aqueous leaves extract is more effective on *S. typhi* as compared to the other microorganisms (*S. aureus, E. coli* and *Paratyphi A*). However, these other microorganisms are susceptible at higher concentrations of the aqueous leaves extract of *C. asiatica*. Ciprofloxacin which served as a positive control recorded the highest MIC value of 3.125mg/l. This shows that ciprofloxacin was very effective in inhibiting growth of all bacteria tested in this present study.

Determination of minimum inhibitory concentrations of 70% ethanol leaves extract of *C. asiatica* on test microorganisms

Data obtained from table 6b shows the MIC values against the test bacteria. The 70% ethanol leaves extract was more effective against *Salmonella typhi* and recorded MIC value of 1.56mg/ml, it was followed by *E.coli* (12.5mg/ml) and *S. aureus* (12.5mg/ml) whereas *Paratyphi A* had the highest MIC value 50mg/ml indicating that it is the least susceptible to the extract. Similar observation was recorded here where lower concentrations of the extract was more effective on a specific bacterium. That is the extract was more effective on *S. typhi* as compared to the other microorganisms (*S. aureus, E. coli* and *Paratyphi A*). However, these other microorganisms were also susceptible to 70% ethanol extract at higher concentrations. As it was observed previously, ciprofloxacin which served as a positive control recorded 0.39 mg/ml as the least MIC value which demonstrates its effectiveness against the test organisms.

Minimum inhibitory concentrations of absolute ethanol leaves extract of *C. asiatica* on test organisms

Tables 7b and 8 show minimum inhibitory concentrations of absolute ethanol leaves extract of *C. siatica*. The lowest MIC value

Concentration (mg/ml)	Aqueous Extract		70% ethanol extract		Absolute extract	Ciprofloxacin	
	Inoculum	Control	Inoculum	Control	Inoculum	Control	Inoculum
Control	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-
6.25	-	+	-	+	-	+	-
3.125	-	+	-	+	-	+	-
1.56	-	+	-	+	-	+	-
0.78	-	+	-	+	-	+	-
0.39	-	+	-	+	-	+	-

Table 5a: Minimum Inhibitory Concentration of different extract of *Centella asiatica* leaves against *Escherichia coli.*

Test organisms	Extract M.I.C (Mg/Ml)	Ciprofloxacin M.I.C (Mg/Ml)
E. coli ATCC25922	12.5 ± 0.00	3.125 ± 0.00
S. typhii ATCC19430	6.25 ± 0.00	0.39 ± 0.00
S. aureus ATCC25923	25 ± 0.00	0 ± 0.00
Paratyphii A	50 ± 0.00	0 ± 0.00

Table 5b: Minimum Inhibitory Concentration of aqueous extract of *Centella asiatica* leaves against test organisms.

Aqueous Extract			70% ethanol extract		Absolute extract		Ciprofloxacin	
Conc. (mg/ml)	Control	Inoculum	Control	Inoculum	Control	Inoculum	Control	Inoculum
50	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-
3.125	-	+	+	-	-	+	-	-
1.56	-	+	+	-	-	+	-	-
0.78	-	+	+	+	-	+	-	-
0.39	-	+	+	+	-	+	-	-

Table 6a: Minimum inhibitory concentration of various leaves extracts of *Centella asiatica* against *S. typhi.*

Positive (+) = color change (green to red) indicating growth of microorganisms, and Negative (-) = no color change indicating absence of growth of microbes.

Test organisms	Extract M.I.C (mg/ml)	Ciprofloxacin M.I.C (mg/ml)
E. coli ATCC25922	12.5 ± 0.00	3.125 ± 0.00
S. typhii ATCC19430	1.56 ± 0.00	0.39 ± 0.00
S. aureus ATCC25923	12.5 ± 0.00	0 ± 0.00
Paratyphii A	50 ± 0.00	0 ± 0.00

Table 6b: Minimum Inhibitory Concentration of 70% ethanol extract of *Centella asiatica* leaves against test organisms.

of 6.25mg/ml was recorded on *Salmonella typhi* and *S. aureus* followed by *E. coli* (12.5mg/ml) whereas *Paratyphi* A recorded the highest MIC value of 50mg/ml. This shows that a lower concentration of the absolute ethanol extract showed higher antimicrobial activity on *S. typhi* and *S. aureus* as compared to the two other microorganisms. However, the remaining organisms were susceptible only at higher concentrations of the extract. Here also ciprofloxacin which served was a positive control recorded 0.39 mg.ml as the least MIC value showing its effectiveness against all the test microorganisms.

	Aqueous extract		70% ethanol extract		Absolute extract		Ciprofloxacin	
Conc. (mg/ml)	Control	Inoculum	Control	Inoculum	Control	Inoculum	Control	Inoculum
50	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
12.5	-	+	-	-	-	-	-	-
6.25	-	+	-	+	-	-	-	-
3.125	-	+	+	+	-	+	-	-
1.56	-	+	+	+	-	+	-	-
0.78	-	+	+	+	-	+	-	-
0.39	-	+	+	+	-	+	-	-

Table 7a: Minimum Inhibitory Concentration of the various extract of *Centella asiatica* leaves against *S. aureus.* Positive (+) = color change (green to red) indicating growth of microorganisms, and Negative (-) = no color change indicating absence of growth of microbes.

Test organisms	Extract M.I.C (mg/ml)	Ciprofloxacin M.I.C (mg/ml)
E. coli ATCC25922	12.5 ± 0.00	3.125 ± 0.00
S. typhii ATCC19430	6.25 ± 0.00	0.39 ± 0.00
S. aureus ATCC25923	6.25 ± 0.00	0 ± 0.00
Paratyphii A	50 ± 0.00	0 ± 0.00

Table 7b: Minimum Inhibitory Concentration of absolute ethanol extract of *Centella asiatica* leaves against test organisms.

Determination of minimum bactericidal/bacteriostatic concentrations of aqueous, 70% and absolute extract of leaves of *C. asiatica* against *E. coli, S. typhi, S. aureus* and *Paratyphi A*

Table 9a shows growth of *E. coli* was recorded in all the various extracts of *C. asiatica*. It implies that the various leaves extracts *C. asiatica* did not kill the test bacterium *E. coli* but rather inhibited its growth. The various extracts of *C. asiatica* were bacteriostatic instead of bactericidal. The control of the various extracts was not inoculated with the *E. coli* in other to compare it with the extracts containing the microorganism and therefore showed no growth after incubation.

Same results were obtained when *S. typhi* was used as a test microorganism (Table 9b). Growth of *S. typhi* was recorded in all the extracts inoculated with the test bacterium. The results show that the various extracts of *C. asiatica* leaves did not kill *S. typhi* but rather inhibited its growth which makes the various extracts of *C. asiatica* bacteriostatic to *S. typhi* as observed in *E. coli*.

	Aqueous Extract		70% ethanol extract		Absolute extract		Ciprofloxacin	
Conc. (mg/ml)	Control	Inoculum	Control	Inoculum	Control	Inoculum	Control	Inoculum
50	-	-	-	-	-	-	-	-
25	-	+	-	+	-	+	-	-
12.5	-	+	-	+	-	+	-	-
6.25	-	+	-	+	-	+	-	-
3.125	-	+	-	+	-	+	-	-
1.56	-	+	-	+	-	+	-	-
0.78	-	+	-	+	-	+	-	-
0.39	-	+	-	+	-	+	-	-

Table 8: Minimum inhibitory concentration of the various extract of *Centella asiatica* leaves against *Paratyphi* . Positive (+) = color change (green to red) indicating growth of microorganisms, and Negative (-) = no color change indicating absence of growth of microbes

Table 9c represents the effect of the various extracts on *S. aureus*. It was observed that growth was recorded in all the concentrations of the aqueous extract except for the concentrations of 50mg/ml and 25mg/ml. That is at concentrations of 25mg/ml and 50mg/ml of aqueous extract of *C. asiatica* leaves were bactericidal on *S. aureus*. Growth of *S. aureus* was also observed in all concentrations of 70% ethanol extract except the concentrations of (12.5, 25 and 50) mg/ml. Similarly, 70% ethanol extract at specific concentrations were bactericidal to *S. aureus*. There was growth of *S. aureus* in all concentrations of the absolute ethanol extract except at (6.25, 12.5, 25 and 50) mg/ml making these concentrations bactericidal on *S. aureus*. The remaining concentrations which showed growth of microorganisms in the other various extracts were interpreted as bacteriostatic to *S. aureus* (Table 9c). Table 9d represent data obtained on *Paratyphi* A (*S. paratyphi*), data was similar to Tables 9a and 9b, where growth of *S. typhi* and *E. coli* in all the extracts inoculated with these same microorganisms. This shows that the various extracts of *C. asiatica* leaves did not kill the *S. paratyphi* but rather inhibited its growth which makes the various extracts bacteriostatic to *S. paratyphi* (Table 9d).

	Aqueous extract		70% ethanol extract		Absolute extract		Ciprofloxacin	
Conc. (mg/ml)	Control	Inoculum	Control	Inoculum	Control	Inoculum	Control	Inoculum
50	-	+	-	+	-	+	-	-
25	-	+	-	+	-	+	-	-
12.5	-	+	-	+	-	+	-	-
6.25	-	+	-	+	-	+	-	-
3.125	-	+	-	+	-	+	-	-
1.56	-	+	-	+	-	+	-	-
0.78	-	+	-	+	-	+	-	-
0.39	-	+	-	+	-	+	-	-

Table 9a: Minimum bactericidal/bacteriostatic concentration of the various extract of *Centella asiatica* leaves against *Escherichia coli.* Positive (+) = color change (green to red) indicating growth of microorganisms, and Negative (-) = no color change indicating absence of growth of microbes.

	Aqueous extract		70% ethanol extract		Absolute extract		Ciprofloxacin	
Conc. (mg/ml)	Control	Inoculum	Control	Inoculum	Control	Inoculum	Control	Inoculum
50	-	+	-	+	-	+	-	-
25	-	+	-	+	-	+	-	-
12.5	-	+	-	+	-	+	-	-
6.25	-	+	-	+	-	+	-	-
3.125	-	+	-	+	-	+	-	-
1.56	-	+	-	+	-	+	-	-
0.78	-	+	-	+	-	+	-	-
0.39	-	+	-	+	-	+	-	-

Table 9b: Minimum bactericidal/bacteriostatic concentration of the various extract of *asiatica* leaves against *Salmonella typhi.* Positive (+) = color change (green to red) indicating growth of microorganisms, and Negative (-) = no color change indicating absence of growth of microbes.

	Aqueous Extract		70% ethanol extract		Absolute extract		Ciprofloxacin	
Conc. (mg/ml)	Control	Inoculum	Control	Inoculum	Control	Inoculum	Control	Inoculum
50	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
12.5	-	+	-	-	-	-	-	-
6.25	-	+	-	+	-	-	-	-
3.125	-	+	-	+	-	+	-	-
1.56	-	+	-	+	-	+	-	-
0.78	-	+	-	+	-	+	-	-
0.39	-	+	-	+	-	+	-	-

Table 9c: Minimum bactericidal/bacteriostatic concentration of various extract of *Centella asiatica* leaves against *Staphylococcus aureus.* Positive (+) = color change (green to red) indicating growth of microorganisms, and Negative (-) = no color change indicating absence of growth of microbes.

Discussion

Nature has been a source of medicinal treatments for thousands of years, with the use of plants as prototypes for drug development and for the extraction of active compounds. Plant-based systems therefore continue to play an essential role in primary health care around the world, with natural compounds been used in treatment of common diseases such as malaria, cholera, pneumonia, tuberculosis, asthma, skin infections, wound to mention but few due to their anti-inflammatory, antimicrobial, and cell-stimulating properties [1,21,22]. *Centella asiatica* is used as a traditional herbal medicine

	Aqueous extract		70% ethanol extract		Absolute extract		Ciprofloxacin	
Conc. (mg/ml)	Control	Inoculum	Control	Inoculum	Control	Inoculum	Control	Inoculum
50	-	+	-	+	-	+	-	-
25	-	+	-	+	-	+	-	-
12.5	-	+	-	+	-	+	-	-
6.25	-	+	-	+	-	+	-	-
3.125	-	+	-	+	-	+	-	-
1.56	-	+	-	+	-	+	-	-
0.78	-	+	-	+	-	+	-	-
0.39	-	+	-	+	-	+	-	-

Table 9d: Minimum bactericidal and bacteriostatic concentration of various extract of *Centella asiatica* leaves against *Paratyphi A* (*S. typhi*).
Positive (+) = color change (green to red) indicating growth of microorganisms, and Negative (-) = no color change indicating absence of growth of microbes.

in Asiatic countries for hundreds of years to improve wound healing, and it is becoming popular in the West Brinkhaus., *et al*. (as cited in [6]).

This study investigated the effectiveness of *C. asiatica* against selected bacteria (*Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*). Findings demonstrated that aqueous, 70% ethanol and absolute ethanol leaves extract of *C. asiatica* at specific concentration were active against the test bacteria species due to the presence of bioactive compounds such as saponins, R-sugars. alkaloids, flavonoids and phytosterols. Saponins were presence in all the three extracts whereas alkaloids were found in aqueous and 70% ethanol extracts. On the contrary, R-sugars were detected in 70% and absolute ethanol extract whilst flavonoids and phytosterols were present in 70% ethanol only (Table 1). Generally, all the extracts the aqueous, 70% ethanol and absolute ethanol extracts of leaves of *C. asiatica* exhibited significantly varying antimicrobial activities and were effective in inhibiting the growth of all test bacteria at the concentrations between 25 mg/ml to 200 mg/ml (Tables 2 – 9d) [1] reported the primary active constituents of *C. asiatica* are saponins (triterpenoids) and their sugar esters, which include asiaticoside, Asiatic acid, and madecassic Brinkhaus., *et al*. Gohil., *et al*. (as cited in [1]). These components are preclinically effective on systemic scleroderma, abnormal scar formation, and keloids [13]. Asiaticoside is the main active ingredient of *C. asiatica* and exhibits significant wound healing activity in normal and delayed- healing models Shukla, Rasik, Jain., *et al*. ([1]). Several studies also demonstrated the effect of *C. asiatica* in wound healing when topically applied ([9,10,24]. Its mechanism of action has been attributed to increase cellular proliferation and collagen synthesis at the wound site, increase in angiogenesis, and it has an effect on keratinization, which aids in thickening skin in areas of infection Pazyar., *et al*. Sunilkumar, Parameshwaraiah, and Shivakumar, Incandela., *et al*. Rosen, Blumenthal, and McCallum, Poizot and Dumez (as cited in [1]).

Similar observations were made by [15], when aqueous-ethanol extracts of *C. asiatica* were found to inhibit the growth of selected microbes such as *E. coli, S. typhi* and *S. aureus*. Later, studies by, showed the activity of *C. asiatica* leaf extracts against some strains of bacteria including *E. coli* and *S. aureus* implying that the leaves extract could have a broad spectrum of antimicrobial activities [25] findings indicated that *Centella asiatica* is one of the important plants which exhibit antibacterial activity against wide variety of bacteria such as *Cornebacterium diptheriae*, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* [26] reported the antimicrobial activity of *C. asiatica* on three local isolates of bacteria (*Bacillus subtilis* (ATCC- 6633), Staphylococcus au-

reus (ATCC6538), *E. coli* (ATCC-14169), *Pseudomonas aeruginosa* (ATCC-27853), and a fungus *C. albicans* (ATCC-10231). The extract showed some degree of antimicrobial activity against these species. This current study used the three extracts to assess the antimicrobial sensitivity of the test bacteria to determine the minimum inhibitory concentration for each test bacterium species (Tables 5a - 8). The extracts were also used to determine zone of inhibition for each test microorganism. Data obtained suggested that the zone of inhibition (mm) increases as concentration of the extracts was increased. Data in this study suggested that the highest zones of inhibition was recorded in the aqueous extract as compared to the ethanolic extracts which is in contrary to other reports by [6,23,27]; where their findings connote that ethanol, leaves extract of *C. asiatica* was significantly effective against bacteria species than aqueous leaves extract. These varying results may be attributed to impact of environmental conditions on the physiology of *C. asiatica* at different part of the world, or because of different extraction methods used by these researchers affected the phytochemical profile of *C. asiatica* [29] reported that when ethanol was used as solvent the extract contained alkaloids, saponin, tannin, flavonoids, phlobatannins, anthraquinones and cardiac glycoside but most of these phytochemicals were absent except saponin in this study. It is supposed that these phytochemicals may be necessary for the inhibitory effect of *C. asiatica*.

A fortuitous condition was created by the 70% ethanol extract and absolute ethanol extracts were tested on the microorganisms where 70% ethanol extract showed more inhibitory effects than the absolute ethanol extract. This may be as a result of the enhanced solubility of the active components in organic solvents [7]. *Salmonella typhi* was highly susceptible to aqueous leaf extract recording zones of inhibition of 24.5mm, 22.5mm and 18mm at 100, 50 and 25mg/ml respectively with the exception of that in 200mg/ml same results was obtained for 70% ethanol extract. On the other hand, *Paratyphi A* was more susceptible to absolute ethanol extract recording zones of inhibition values of zones of 16mm, 13mm and 11mm at 200, 100 and 50mg/ml respectively. The data reported in this present study indicates that the absolute ethanol extract was the most potent among the three extracts tested the results confirmed reports by [6]. in a similar study. There was a positive correlation between the concentrations of 50 and 100mg/ml (r = 0.9962) among the three leaves extract of *C. asiatica.*

Except for *Paratyphi* B, all the extracts showed a significant antimicrobial activity against the isolates. The following MIC values were recorded in this study 6.25mg/ml was recorded for *Salmonella typhi* for aqueous leaves extract, 70% ethanol extract 1.56mg/ml was recorded for *Salmonella typhi*, absolute ethanol extract 6.25mg/ml *Salmonella typhi* and *S. aureus* respectively. Future studies were carried out to determine the bactericidal activity of the test extracts, it was observed growth was attained by all isolates grown in the aqueous medium except *S. aureus* at concentrations of 25 and 50mg/ml. Result obtained for 70% ethanol extract, was akin to that of aqueous leaves extracts where there growth was not recorded at concentrations of 12.5, 25 and 50mg/ml with absolute alcohol recording same as above where *S. aureus* did not grow at the concentrations of 6.25, 12.5, 25 and 50mg/ml. However, the positive control, ciprofloxacin was very effective and exhibited bactericidal activity to all the test bacteria isolates.

Conclusion

The leaves extract of *Centella asiatica* exhibited antibacterial activity against the three isolates of bacteria *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* that cause wound infection, urinary tract infection, gastrointestinal tract infection and typhoid. There is potential application of natural extracts and their isolated active compounds from leaves extract of *C. asiatica* for the development of formulation.

Bibliography

1. Ataide A J., *et al*. "Natural actives for wound healing: A review". *Phytotherapy Research* 32.9 (2018): 1664-1674.

2. Assob JCN., *et al*. *Journal of the International Society for Complementary Medicine Research* 11 (2011): 70.

3. Brown E D and Wright G D. "Antibacterial drug discovery in the resistance era". *Nature* 529.7586 (2016): 336-343.

4. Chaudhary U and Aggarwal R. "Extended spectrum-lactamases (ESBL)-An emerging threat to clinical therapeutics". *Indian Journal of Medical Microbiology* 22.2 (2004): 75.

5. Cohen M L. "Epidemiology of drug resistance: implications for a post— antimicrobial era". *Science* 257.5073 (1992): 1050-1055.

6. Dash BK., *et al*. "Antibacterial and antifungal activities of several extracts of Centella asiatica L. against some human patho-

genic microbes". *Life Sciences and Medicine Research* (2011): 1-5.

7. De Boer HJ., *et al*. "Antifungal and Antibacterial activity of some herbal remedies from Tanzania". *Journal of Ethnopharmacology* 96 (2005): 461 469.

8. Eloff JN. "A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria". *Planta Medica* 64 (1998): 711-713.

9. Gohil KJ., *et al*. "Pharmacological review on Centella asiatica: A potential herbal cure-all". *Indian Journal of Pharmaceutical Sciences* 72.5 (2010): 546-556.

10. Gul Satar N., *et al*. "Comparison of the effects of bitter melon (Momordica charantia) and gotu kola (Centella asiatica) extracts on healing of open wounds in rabbits". *Kafkas Univ Vet Fak Derg,* 19 (2013): 161-166.

11. Gupta AK. "Centella asiatica". The IUCN Red List of Threatened Species. IUCN T168725A19645149 (2013).

12. Holder IA and Boyce S T. "Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture". *Burns* 20.5 (1994): 426-429.

13. Hong S S., *et al*. "Advanced formulation and pharmacological activity of hydrogel of the titrated extract of C. asiatics". *Archives of Pharmacal Research* 28.4 (2005): 502-508.

14. Joshi K and Chaturvedi P. "Therapeutic efficiency of Centella asiatica (L.) Urb. An underutilized green leafy vegetable: an overview". *International Journal of Pharma and Bio Sciences* 4.1 (2013): 135-149.

15. Kedzia B., *et al*. "Studies on the biological properties of extracts from C. asiatica (L) Urban herb". *Kerba Polonica* 53 (2007): 1.

16. Mala A and Tulika T. "Therapeutic efficacy of Centella asiatica (L.) and Momordica charantia: As traditional medicinal plant". *Journal of Plant Sciences* 3 (2015): 1-9.

17. Mustapha AA. "Ethno-medico-botanical uses of Securidaca longepedunculata fresen (family-polygalaceae) from Keffi local government, Nasarawa state, Nigeria". *Journal of Natural Remedies* 13.2 (2013): 133-137.

18. Nester EW., *et al*. "Microbiology: A Human Perspective". New York: McGraw-Hill Journal (2004).

19. Nwinyi OC., *et al*. "Evaluation of antibacterial activity of Pisidium guajava and Gongronema latifolium". *Journal of Medicinal Plants Research* 2.8 (2008): 189-192.

20. Opintan JA., et al. "Laboratory-based nationwide surveillance of antimicrobial resistance in Ghana". *Infection and Drug Resistance* 8 (2015): 379.

21. Pazyar N., *et al*. "Skin wound healing and phytomedicine: a review". *Skin Pharmacology and Physiology* 27.6 (2014): 303-310.

22. Pereira R F and Bartolo P J. "Traditional therapies for skin wound healing". *Advances in Wound Care* 5.5 (2016): 208-229.

23. Rekha SR., *et al*. "Antibacterial efficacy and minimum inhibitory concentrations of medicinal plants against wound pathogens". *Biomedical and Pharmacology Journal* 11.1 (2018): 237-246.

24. Sawatdee S., *et al*. "Evaluation of the topical spray containing Centella asiatica extract and its efficacy on excision wounds in rats". *Acta Pharmaceutica* 66.2 (2016): 233-244.

25. Seevaratnam V., *et al*. "Functional properties of Centella asiatica (L.): a review". *International Journal of Pharmacy and Pharmaceutical Sciences* 4.5 (2012): 8-14.

26. Sultan R A., *et al*. "Biological activities assessment of Centella asiatica (Linn.)". *Journal of Herbs, Spices and Medicinal Plants* 20.3 (2014): 319-327.

27. Taemchuay D., *et al*. "Antibacterial activity of crude extracts of Centella asiatica against Staphylococcus aureus in bovine mastitis". *Kasetsart Veterinarians* 19.3 (2009): 119-128.

28. Tiwari S., *et al*. "Centella asiatica: A concise drug review with probable clinical uses". *Journal of Stress Physiology and Biochemistry* 7.11 (2011): 38-44.

29. Udoh DI., *et al*. "Inhibitory Effect of Varying Concentrations of Leaves' Extracts of Centella Asiatica (Gotu Kola) on Some Microorganisms of Medical Importance". *International Journal*

of Chemical, Environmental and Pharmaceutical Research 3.2 (2012): 142-148.

30. Vinoth S., *et al*. "Evaluation of phytochemical, antimicrobial and GC-MS analysis of extracts of Indigofera trita LF spp. Subulata (Vahl ex poir)". *International Journal of Agricultural Research* 6.4 (2013): 358-367.

31. Wellcome Trust Sanger Institute. "Antibiotic resistance typhoid detected in countries around the world". *Science Daily* (2015).

32. World Health Organization. "Background document: the diagnosis, treatment, and prevention of typhoid fever" (2003).

33. World Health Organisation. "Antimicrobial resistance: Global report on surveillance". WHO Press. 20 Avenue Appia (2014).