Original Research Article

Antimicrobial Activity of Herbal Plants used for Treatment of Gastroenteritis Infection in Tharaka Nithi County, Kenya

Abstract

Herbals have been used for decades to treat gastrointestinal infections across the world including Tharaka-Nithi County in Kenya. Crude extracts from herbals have long been prescribed by traditional healers for treatment of typhoid, cholera and shigellosis. However, the effectiveness of extracts obtained using different extraction solvents such as methanol, acetone and hexane has not fully been evaluated. This study aimed at evaluating the effect of solvents (Ethanol, hexane and methanol) on the yield of crude extract from plants (*Erythrinnaabyssinica, Aspiliapluriseta, Vangueriainfausta,Ficus sycomorus, Carissa edulis*), assess the effect of the solvents on extracted active metabolites and determine the effect of these solvents on extract's bioactivity against *Escherichia coli, Vibrio cholerae, Shigella flexneri,*and *Salmonella typhi*. Crude extracts were obtained by soaking dried ground plant parts in individual solvents then concentrated by rotary evaporator. The phytochemical screening to detect plant metabolites was done qualitatively. Bioassays to analyze the efficacy of the plant crude extracts against the microbes was carried out in 4×5×3×3 factorial experiment laid out in completely randomized design. Determination of bioactivity of herbal extracts was carried out using minimal inhibition concentration and minimum bactericidal concentration methods. Data obtained on the bioactivity assay (Count on the bacteria colony forming units) was analyzed using Kruskal Wallis test at $\alpha = 0.05$ and medians were compared by Wilcoxon rank sum test in Scientific analysis System version 9.4. Methanol solvent produced higher quantities of crude extracts for all the herbal samples used with *Vangueriainfausta* producing the highest extract (5.06g). Most phytochemicals were present in Methanolic extracts compared to hexane and acetone extracts. There was a significant ($p< 0.05$) difference in the bioactivity of different herbal plants against bacterial pathogens at different concentrations of crude extract. The efficacy of plant extract increased progressively from 100ppm to 1000ppm concentration. Methanol is recommended for use in extraction of medicinal plant extracts as it leads to an improved potency as compared to hexane and acetone.

Keywords:Herbal-plants, antimicrobial-activity, cholera, typhoid, shigellosis, Tharaka-Nithi County

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Comment [MOU2]: Ecoli is a normal commensal of GIT

I. INTRODUCTION

Medicine is fundamental to healthcare in management and prevention of illnesses given that healthcare is a basic human need to reduce mortality and disease burdens [1] [2]. Access to safe, effective and affordable medicine is among the components of the United Nations' Sustainable Development Goals [3]. Yet, the occurrence of low-quality medicines continues to be a pervasive challenge due to increased drug resistance [4]. Comparative research on alternative antimicrobial agents with greater efficacy is needed to counteract resistant pathogens that are continually selected by current therapeutic regimen [5]. Traditional medication from plant extracts have been in use for years to cure many ailments, thus, herbal are alternative and relatively safer sources of treatment [6]. Traditional medicinal herbs contain bioactive compounds which have few side effects, have low propensity to develop resistance, are less toxic and are associated with improved efficacy [7]. Recent advances in research involving traditional herbal medicine have resulted in identification of several bioactive compounds with antimalarial, anti-cancer and antibiotic properties [8]. Important antimicrobial agents of plant include alkaloids, phenylpropanoids i.e., flavonoids and terpenoids that include saponins [9] [10].

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Kenya has a wide range of flora with over 7,000 plant species [11]. Up to 70% of the rural populace use home remedies from plant parts as the first source of medicine to treat infections [12] [13]. Different plant parts that include flowers, leaves and fruits are used as sources of home remedies [14]. The current study evaluated the antimicrobial activity of *Carissa edulis* roots,*Aspiliapluriseta* leaves, *Vangueriainfausta* leaves and *Erythrina abysinnica* stem that are traditionally used in treatment of gastrointestinal infections in Tharaka-Nithi County. The health benefits of plants mentioned above are attributed to their richness in phytochemical compounds with physiological effects against gastroenteritis bacteria [15]. However, the phytochemicals present in these crude extracts including those used by herbalists in Tharaka-Nithi County is not fully determined.

Solvents that include water, acetone, hexane, methanol and ethanol, are used to extract bioactive compounds from plant materials [16] [8]. Studies has indicated that solvent used in extraction significantly affects extract's yield, quality, extraction velocity, and bioactivity which is attributed to differences in polarity [16] [17] [18]. Use of ethanol has become popular because beside being safe for infusing food items, it does not corrode container used in addition to being easily recoverable with consistent outcome in bioassays involving crude extract [19]. Some of the active metabolites that have been extracted using ethanol as solvent are; alkaloids, glycosides, flavonoids, terpenoids, steroids, tannins, saponins, and reducing sugars [20] [19]. Efficiency of alcohol has been attributed to its polarity that resulted in extraction of more active ingredients [21] [20]. As reported by Abarca-Vargas *et al.*[22] and Hikmawanti *et al.*[20], ethanol is considered a protic organic solvent that has a polarity index value of 5.2 and with the dielectric constant of 24.55. Methanol as an extraction solvent has resulted in amino acid, carbohydrate, flavonoids, phenolic compounds, phytosterol, proteins, saponins and tannin [23] [24]. Hexanoic plant crude extracts have been reported not to contain Phenol, Saponins and Carbohydrates [25].The levels of phenolics and flavonoids is determined by the solvent and has been reported (butanol > methanol > ethyl acetate > hexane > chloroform [24]).

Understanding of the medicinal herbs' aspects such as phytochemical, pharmacological and standardization is necessary the for constitution of dosage in an informed manner [11]. It is therefore necessary to evaluate different solvent concentrations that will produce the most efficacious extract against the test pathogens. In this respect, this study evaluated effect of different organic solvents (Methanol, hexane, and acetone) on the efficacy of crude extract from five plant species (*Carissa edulis* roots, *Aspiliapluriseta* leaves, *Ficus sycomorus* stems, *Erythrina abyssinicca* barks and *Vangueriainfausta* leaves) against human pathogenic bacteria (*Vibrio cholerae*,*Salmonella typhi* and *Shigella flexneri*) associated with gastrointestinal infections.

Comment [MOU4]: Why shigella flexneri was selected over other species?

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II. MATERIALS AND METHODS

2.1 Study Area

The plants used in this study were collected in Tharaka-Nithi County in Kenya which is located at a latitude coordinate of 0º9´25.03´´S and longitude coordinates of 37º58´41.48´´E. Tharaka-Nithi County has four sub counties; Maara, Tharaka North, Tharaka South and Meru South, covering a total area of 2,662.1 km^2. The human population in Tharaka-Nithi County is estimated at 365,330 [26]

2.2 Experimental Design

Bioassays to analyze the efficacy of the plant crude extracts against the microbes was carried out in 3×3×3×5 factorialexperiment laid out in completely randomized design (CRD) having three factors, with factor A being the microbial pathogens (At three levels-*Vibrio cholerae*, *Salmonella typhi* and*Shigella* species). Factor B was the concentrations of crude plant extracts at three levels (1000ppm, 500ppm, 100ppm). Factor C was solvent at three levels (Hexane, acetone and methanol).Lastly factor D wasplant extract at 5 levels (*Erythrinnaabyssinica, Aspiliapluriseta, Vangueriainfausta, Ficus sycomonus,Carissa edulis*).

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2.3 Collection of plant samples, Extraction of crude extracts and Phytochemical Analysis

The commonly used herbs were collected with assistance from traditional herbal dispensers in Tharaka North based on the information gathered through questionnaires on the herbs [27]. Information gathered included the part of the plant used, method of extraction and dose. The plant samples were wrapped in aluminum foils and put in Ziplock bags and transported to Chuka university chemistry laboratories for extraction of the metabolites. In the laboratory, the samples were cleaned using running water and rinsed by use of distilled water. They were then chopped in to small sizes using a sterilized panga after which they were left on the bench so as to dry for 21 days. The dried samples were then blended in to soft powder. Extraction of crude extract was performed using methanol, acetone and hexane solvents. 100 grams of each ground sample was weighed and dissolved in 350 ml of methanol, acetone and hexane. They were then allowed to stand for 48 hours so that extraction can take place. Thereafter, filtration was done by use of filter papers number 1. The extracts of the three solvents were then concentrated by use of Rotary evaporator at 40 degrees Celsius and were left to dry in the fume chamber. The concentrated extracts were then weighed using a beam balance and wrapped in aluminum foils, placed in stoppered containers and then transported to KEMRI CDC Kisumu for antimicrobial analysis.

Comment [vk8]: Explain what is panga

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Each plant sample was analyzed for presence of tannins, flavonoids, alkaloids, cardiac glycosides, phenolics, saponins, steroids and terpenoids. Qualitative Test for tannins was done according to Ejikeme *et al.* [28]. Five grams of individual plant powder sample was dissolved in five ml of methanol and then filtered. To the filtrate, 3ml of ferric chloride was added. The occurrence of brownish-green or a blue-black color indicated positive test. Test for flavonoids was done following procedure used by Hossain *et al*.[29] where 2% of the sodium hydroxide was dispensed to each plant sample and followed by addition of dilute hydrochloric acid. The presence of a golden yellow precipitate showed a positive test. Test for saponins was done according to Ejikeme *et al.* [28] where distilled water and each sample filtrate was vigorously shaken till a stable persistent froth was formed. To the mixture, drops of olive oil was added. Formation of an emulsion upon addition of olive oil showed a positive test. Salkowski test as explained by Yadav and Agarwala[30] was used to test for terpenoids presence where three milliliters of each plant extract were dissolved in three milliliters of chloroform. Three milliliters of concentrated sulphuric acid were added where a grayish colour indicated a positive test.

Wagner test procedure used by Ghosh *et al*.[31] was adopted to test for the presence of alkaloids where two milliliters of plant extract was dissolved in two milliliters of ethanol. Two drops of 1M Hydrochloric acid were added to each test tube and boiled for a minute. Three drops of Mayors reagent were added. Formation of a reddish -brown precipitate indicated a positive test. Ferric chloride test as described by Kumar *et al.*[32] was used to test for the presence of phenolics where one milliliter of ferric chloride solution was added to three milliliters of each plant extract sample. Formation of a green colour indicated a positive test for phenolics. Steroids were tested by adding two milliliters of acetic anhydride were added to two milliliters of each plant extract sample followed by

addition of two milliliters of sulphuric acid. Formation of a blue or green colour indicated a positive test for steroids. Kellar-Kiliani test as described by Parekh and Chanda 2007 was used to test for glycosides where two milliliters of glacial acetic acid were added to five milliliters of each extract. One milliliter of ferric chloride was then added followed by addition of one milliliter of concentrated sulphuric acid. A green blue coloration of the solution indicated a positive test for glycosides.

2.4 Antimicrobial activity of the Plant Crude Extracts against test bacteria organisms

a. Preparation of the Crude extract, Culture Media and MicrobialCulture Inoculant

Different concentrations of each plant extracts were made dissolving 0.1grams of each crude plant extract to1 ml 0.2% DMSO making a concentration of 1 g/ml [33]. A 1000 ppm solution was prepared by 1ml of the stock solution into 1000ml of distilled water from which 500ppm and 100ppm concentrations was prepared. The TSB was prepared using a procedure described in the literature [34] where 15 grams of TSB powder was suspended in 500ml of distilled water and then mixed thoroughly. It was slightly warmed to completely dissolve and then autoclaved at 121◦C for 15 minutes prior to cooling at room temperature.

b. Minimum Inhibitory Concentration and Minimum bactericidal Concentration of the Extracts

Pure colonies of type cultures of the microorganisms were obtained from KEMRI CDC Kisumu and was sub cultured on Tryptic Soy Broth as per the Clinical Laboratory Standards of US [35]. *Shigella* species which is a member of Enterobacteriaceae family was incubated at a temperature of 35 ºC in air for 18 hours while *Vibrio cholerae* and *Salmonella typhi* which are anaerobes was incubated at 10% carbon (IV) oxide, 10% hydrogen and 80% nitrogen. Standardization of bacterial colony cells was done using 0.5 McFarland standard. The McFarland standard was prepared from 0.5ml of 0.048M $BaCl_2$ (1.17% w/v) added to 99.5ml of 0.18M sulphuric acid (1% v/v) and stirred [36]. The optical density of the turbidity was measured using a spectrophotometer [37] to obtain the final inoculum concentration of bacterial colony forming units (cfu)/ml. The optical density of the colonies was evaluated using a spectrophotometer and adjusted to equal the 0.5 McFarland standard (10^8 CFU/ml) by adding sterile distilled water.

Inoculations was done by adding 20 µL of each bacterial suspensions to individual 100 µL of TSB in a microtiter plate where each of the treatment was replicated three times. The TSB + DMSO was used while TSB alone as negative control. At the end of the incubation period, A 100 µl of the bacteria under different treatments were cultured by spread plate method on the nutrient agar and was incubated for up to 18 hours. At the end of the re-incubation session, each of the plates was divided into four equal parts using a maker pen and counting of the bacteria CFUs was done for every treatment. The extract concentration at which the CFUs counted was < 10 colonies was assumed to be MBC [38].

2.5 Data analysis

Data obtained on the bacteria colony forming units (Counts) was analyzed using Kruskal Wallis procedure method α = 0.05 in Statistical Analysis System (SAS) version 9.4.The medians from the

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Comment [MOU16]: Standardization of the bacterial broth. Delete cells

Comment [MOU17]: delete 'of bacterial colony'. Add 'in colony forming units'

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You have not mentioned about inoculating bacteria under different treatments. This part is not clear. Add a picture of the culture plate

results were compared by ranking (Wilcoxon rank sum test) in SAS. Analysis only compared plant extracts and excluded results for positive and negative control set ups.

III. RESULTS

3.1 Phytochemical Screening of Extracts from Herbal Plants to Treat Gastrointestinal Infections in Tharaka Nithi County

Methanol as solvent produced higher quantities of crude extract for all the herbal samples used. *Vangueriainfausta* produced the highest crude extract with methanol (5.06g) while *Aspiliapluriseta* produced lower yield (0.73g). *Carissa edulis* produced the highest crude extract with acetone (3.7g) while *Aspiliapluriseta* produced lower yield (0.53g). *Carissa edulis* produced the highest crude extract with hexane (1.81g) while *Erythrina infausta* produced lower yield (0.06g). The masses of crude extracts obtained are presented in (Table 1).

Table 1: Mass of crude extracts obtained using methanol, acetone and hexane solvents

Plant sample	Hexane	Acetone	Methanol
Carissa edulis	1.81g	3.7g	4.02g
Aspiliapluriseta	0.45g	0.6g	0.73g
Physalis peruviana	1.25g	2.4g	3.74g
Vangueriainfausta	1.32g	1.13g	5.06g
Erythrina abysinnicca	0.06g	1.61g	1.76g
Ficus sycomorus	0.5g	1.22g	1.6g

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Several phytochemical compounds were identified from the herbs (Table 2). In methanolic extracts all the seven herbs contained tannins, saponins and phenolic compounds. Flavonoids were only absent in *Ficus sycomorus*, terpenoids were absent only in *Aspiliapluriseta* and *Vangueriainfausta* leaves. Alkaloids were present in *Carissa edulis,Vangueriainfausta* leaves and barks. Steroids were present in *Ficus sycomorus*, *Carissa edulis,Erythrina abyssinica* barks only while *Vangueriainfausta* leaves were the only containing glycosides.

Table 2: Phytochemicals present in crude extracts of some medicinal plants used for treatment of gastrointestinal infections

Sample	Tnns[a]	Flv[b]	Spnn[c]	Tpn[d]	Alk[e]	Phen[f]	Ster[g]	Glyc[h]
				methanolic extracts				
Fs[1]	+	-	+	+	-	+	+	-
Ce[2]	+	+	+	+	+	+	+	_
Ap[3]	+	-	+	-	-	+	-	_
Ea[4]	+	+	+	+	-	+	+	-
Vil[5]	+	+	+	_	+	+	_	+
				Hexanoic extracts				
Fs[1]	+	_	+	_	_	+	_	_
Ce[2]	+	+	+	+	_	_	+	_
Ap[3]	+	_	_	_	_	+	_	_
Ea[4]	+	+	+	_	_	+	+	_
Vil[5]	+	+	+	_	_	_	_	+
				Acetonic extracts				
Fs[1]	+	_	+	_	_	_	_	_
Ce[2]	+	+	+	_	_	_	+	_
Ap[3]	+	_	_	_	_	_	_	_

Ea[4]	+	+	+	–	–	+	–	–
Vil[5]	+	+	–	–	+	–	–	+

[1]*F. sycomorus,* [2]*C. edulis,* [3]*A. pluriseta,* [4]*E. abysinnica*(barks). [5]*V. infausta*(leaves). [a]Tannins, [b]Flavonoids, [c]Saponins, [d] Terpenoids, [e] Alkaloids, [f]Phenolics, [g] Steroids, [h]Glycosides

3.2 Effect of Solvents Choice and Plant on Efficacy of Plant's Bacterial Colony Forming Units

The solvent choice significantly($p < 0.05$) affected the bacteria colony forming units formed by *E. coli, Vibrio cholerae* and *Shigella flexneri* subjected to *Erythrina abysinnica* extracts. The highest bacteria cfu (*Median* = 217 cfu) and reduced bacteria cfu (*Median* = 0 cfu) were observed in *E. coli* hexanoic and Methanoic extract respectively when bacteria were subjected to *Erythrina abysinnica* extracts (Table 3).The solvent choice significantly($p < 0.05$) affected the bacteria colony forming units formed by *E. coli, Salmonella typhi, Vibrio cholerae and Shigella flexneri* subjected to *Aspiliaplurisetae*xtracts. The highest bacteria cfu (*Median* = 276cfu) and reduced bacteria cfu (*Median* = 1 cfu) were observed in *E. coli* and *Vibrio cholerae* in hexanoic and acetonic extracts respectively when bacteria were subjected to *Aspiliaplurisetae*xtracts (Table 3).The solvent choice significantly($p < 0.05$) affected the bacteria colony forming units formed by *E. coli, Salmonella typhi, Vibrio cholerae and Shigella flexneri* subjected to *Vangueriainfaustae*xtracts. The highest bacteria cfu (*Median* = 278cfu) and a reduced bacteria cfu (*Median* = 17cfu) were observed in *Shigella flexneri*and *Salmonella typhi* in hexanoic and acetonic extract respectively when bacteria were subjected to *Vangueriainfaustae*xtracts (Table 3).The solvent choice significantly($p < 0.05$) affected the bacteria colony forming units formed by *E. coli, Salmonella typhi, Vibrio cholerae and Shigella flexneri* subjected to *Ficus sycomorous*extracts. The highest (*Median* = 140cfu) was observed in *Salmonella typhi*in hexanoic extract while a reduced cfu (*Median* = 10cfu) were observed in *E. coli in* both hexanoic and acetonic extract when bacteria were subjected to *Ficus sycomorus* extracts (Table 3).The solvent choice significantly($p < 0.05$) affected the bacteria colony forming units formed by *E. coli, Salmonella typhi, Vibrio cholerae and Shigella flexneri* subjected to *carissa edulis* extracts. The highest bacteria cfu was observed in *Salmonella typhi* and *Shigella flexneri*(*Median* = 198cfu) in hexanoic extract. Likewise, a reduced cfu (*Median* = 65cfu) was observed in *E. coli* in hexanoic extract when bacteria were subjected to *carissa edulis* extracts (Table 3).

Table 3: Effect of different solvents on efficacy plant's extracts against bacteria growth

Plant Extract	Test Bacteria	Hex[6]	Ace[7]	Met[8]	Kruskal-Wallis statistics			
					N	*H*	df	*P* value
Eryth[1]	*E*[k]	217[a]	200[a]	0[b]	9	19.376	2	<.0001
	S[t]	187	177	167	9	2.084	2	0.352
	V[l]	211[a]	6[c]	154[b]	9	19.323	2	<.0001
	S[t]	199[a]	56[b]	146[a]	9	19.142	2	<.0001
Aspi[2]	*E*[k]	276[a]	232[a]	3[b]	9	17.801	2	0.0001
	S[t]	178[a]	165[a]	146[b]	9	15.737	2	0.0004
	V[l]	200[a]	1[c]	7[b]	9	20.544	2	<.0001
	S[t]	222[a]	166[b]	28[b]	9	13.302	2	0.0013
Vang[3]	*E*[k]	250[c]	243[b]	187[a]	9	19.441	2	<.0001
	S[t]	256[a]	234[b]	17[c]	9	20.538	2	<.0001
	V[l]	265[a]	213[b]	187[b]	9	19.413	2	<.0001
	S[t]	278[a]	220[b]	187[b]	9	20.711	2	<.0001
Ficus[4]	*E*[k]	55[a]	10[b]	10[b]	9	12.622	2	0.0018
	S[t]	140	123	111	9	6.091	2	0.05
	V[l]	87	109	30	9	2.309	2	0.3153

Comment [MOU23]: Minimum bactericidal concentration for Eryth was found only with Methanol and acetone extract with CFU of 0 and 6 respectively for Ecoli and Vibrio cholerae.
Delete the whole paragraph and only write about the MBC for each plant product. Again table is self explanatory. The whole para is very confusing.

Comment [MOU24]: Minimum bactericidal concentration for Eryth was found only with Methanol and acetone extract with CFU of 0 and 6 respectively for Ecoli and Vibrio cholerae.

	S[t]	79[b]	111[a]	27[b]	9	12.169	2	0.0023
	E[k]	65[c]	155[a]	143[b]	9	16.414	2	0.0002
Cari[5]	*S*[t]	198[a]	168[b]	148[c]	9	18.618	2	<.0001
	V[l]	30[b]	176[a]	148[a]	9	6.909	2	0.0316
	S[t]	198[a]	156[b]	140[b]	9	12.384	2	0.002

[a]*Median* values followed by the same letters in rows are not significantly different at α = 0.05. [1]*Erythrinnaabyssinica,* [2]*Aspiliapluriseta,* [3] *Vangueriainfausta,* [4]*Ficus sycomonus,*[5]*Carissa edulis,* [k] *E. coli,* [t] *S. typhi,* [l] *V. cholerae,* [f] *S. flexineri,* [6] Hexane extract, [7]Acetonoic extract, [8]Methanoic extract

There was a significant ($p < 0.05$) effect of exposing test bacteria to various plant extracts at different concentration on the bacteria colony forming units formed (Table 4). Exposing *E. coli* to 1000ppm plant extracts resulted in significantly reduced bacteria cfu counts in methanoic extracts of *Erythrina abysinnica* (*Median* = 0 cfu), *Aspiliapluriseta* (*Median*= 3cfu) and *Ficus sycomorus* (*Median* = 0 cfu) while remaining significantly higher in hexanoic extract of *Aspiliapluriseta* (*Median* = 276 cfu) and *Vangueriainfausta*(*Median* = 250 cfu). Exposing *S. typhi* to 1000ppmof plant extracts*resulted in* significantly lower bacteria cfu counts in methanolic extracts of *Ficus sycomorus* (*Median* = 13cfu) *andVangueriainfausta(Median* = 17cfu) while remaining significantly higher in hexanoic(Median = 256cfu*) and acetonic* (*Median* = 256 cfu*) extracts ofVangueriainfausta* respectively. Exposing *V. cholerae* to 1000ppm of plant extracts resulted in significantly lower bacteria cfu counts in acetonic extracts of *Erythrina abysinnica*(*Median* = 0cfu) and *Aspiliapluriseta(Median* = 1cfu) while significantly higher was observed in hexanoic(Median = 265cfu)extractof *Vangueriainfausta*. Exposing *Shigella flexneri* to 1000ppm of plant extracts resulted in significantly lower bacteria cfu counts in acetonic extracts of *Erythrinaabysinnica*(*Median* = 2cfu) and methanolic extract of *Ficus sycomorus(Median* = 1cfu) and significantly higher cfu was observed in hexanoic(*Median* = 278 cfu)extractof *Vangueriainfausta*(Table 4).

Exposing *E. coli*to500ppm plant extracts resulted in significantly reduced bacteria cfucounts in methanolic extracts of *Erythrina abysinnica* (*Median* = 0 cfu)and *Aspiliapluriseta* (*Median*= 3 cfu) but remained significantly higher in hexanoic extract of *Vangueriainfausta(Median*= 258 cfu). Exposureof*S. typhi* to1000ppmof plant extracts resulted in significantly reduced bacteria cfu counts in methanolic extracts of *Vangueriainfausta(Median* = 62 cfu) but remaining significantly higher in hexanoic (*Median* = 266 cfu) extracts of*Vangueriainfausta*. Exposing *V. cholerae* to 500ppm of plant extracts resulted in significantly reduced cfu counts in acetonic extracts of *Ficus sycomorus(Median* = 4cfu) and*Aspiliapluriseta(Median* = 5cfu) while bacteria cfu counts were significantly higher in hexanoic(*Median* = 287cfu)extract of *Vangueriainfausta*. Exposing *Shigella flexneri* to 500ppm of plant extracts resulted in reduced bacteria cfu counts in methanolic extracts of *Carissa edulis* (*Median* = 27cfu) and higher cfu was observed in acetonic(*Median* = 265 cfu)extractof *Aspiliapluriseta*(Table 4).

When*E. coli*was exposed to 100ppm plant extracts, significantly reduced bacteria cfucounts occurred in methanolic extracts of *Aspiliapluriseta* (Median = 0 cfu*)* but remained significantly higher in hexanoic extract of *Vangueriainfausta(Median*= 296 cfu). Exposure of *S. typhi* to 100ppm of plant extracts resulted in significantly reduced cfu counts in acetonic extracts of *Erythrinnaabyssinica*(*Median* = 22cfu) but remaining significantly higher (*Median* = 284cfu*)* in

hexanoic extracts of *Vangueriainfausta*. Exposing *V. cholerae* to 100ppm of plant extracts resulted in significantly reduced cfu counts in acetonic extracts of *Aspiliapluriseta(Median* = 9cfu) while significantly higher in hexanoic *(Median* = 265cfu)extract of *Vangueriainfausta*. Exposing *Shigella flexneri* to 100ppm of plant extracts resulted in reduced cfu counts in acetonic extracts of *Vangueriainfausta* (*Median* = 22cfu) and higher bacteria cfu was observed in hexanoic(*Median* = 288cfu)extractof *Vangueriainfausta*(Table 4).

Table 4: Effect of different solvents and extract concentrations on efficacy of plant's extracts against bacteria growth

Plant Extract	Sol	N	Plant extracts at 1000ppm					Kruskal-Wallis statistics		
			Eryth[1]	Aspi[2]	Vang[3]	Fic[4]	Cari[5]	*H*	Df	*p*-value
E[k]	Hex[6]	3	217 [b]	276 [a]	250 [a]	57 [c]	21 [c]	12.433	4	0.014
	Ace[7]	3	154 [b]	232 [a]	243 [a]	11 [c]	165 [b]	11.750	4	0.019
	Met[8]	3	0 [c]	3 [c]	187 [a]	3 [c]	80 [b]	11.835	4	0.018
S[t]	Hex[6]	3	163 [b]	178 [b]	256 [a]	67 [c]	189 [b]	11.145	4	0.025
	Ace[7]	3	22 [d]	165 [b]	234 [a]	122 [c]	168 [b]	12.833	4	0.012
	Met[8]	3	167 [a]	146 [a]	17 [c]	13 [b]	145 [a]	11.212	4	0.024
V[l]	Hex[6]	3	144 [c]	200 [b]	265 [a]	57 [d]	8	13.524	4	0.009
	Ace[7]	3	0 [d]	1 [d]	213 [a]	122 [c]	176 [b]	13.099	4	0.011
	Met[8]	3	164 [b]	7 [c]	220 [a]	7 [c]	38 [c]	12.077	4	0.016
S[f]	Hex[6]	3	144 [c]	222 [b]	278 [a]	28 [d]	200 [b]	13.257	4	0.010
	Ace[7]	3	2 [d]	166 [b]	202 [a]	110 [c]	20 [d]	12.9	4	0.011
	Met[8]	3	109 [b]	28 [d]	187 [a]	4 [d]	89 [c]	12.923	4	0.012
Plant extracts at 500ppm										
E[k]	Hex[6]	3	215 [b]	234 [a]	258 [a]	47 [c]	65 [c]	11.479	4	0.022
	Ace[7]	3	200 [a]	188 [a]	222 [a]	11 [d]	167 [c]	10.444	4	0.034
	Met[8]	3	0 [c]	0[c]	198 [a]	10 [c]	143 [b]	13.68	4	0.008
S[t]	Hex[6]	3	187 [b]	233 [a]	266 [a]	140 [c]	203 [b]	11.979	4	0.017
	Ace[7]	3	177 [a]	200 [a]	195 [a]	133 [b]	177 [a]	10.627	4	0.031
	Met[8]	3	133 [a]	117 [a]	62 [c]	111 [b]	136 [a]	10.961	4	0.027
V[l]	Hex[6]	3	234 [b]	225 [b]	287 [a]	89 [c]	30 [d]	11.979	4	0.017
	Ace[7]	3	6 [b]	5 [b]	189 [a]	4 [b]	176 [a]	10.627	4	0.031
	Met[8]	3	123 [b]	132 [a]	152 [a]	89 [c]	148 [a]	10.961	4	0.027
S[f]	Hex[6]	3	238[b]	223 [b]	278 [a]	80 [d]	185[c]	10.252	4	0.036
	Ace[7]	3	41[c]	265 [a]	201 [b]	132 [c]	172 [b]	12.533	4	0.013
	Met[8]	3	146	154	142	27	130	8	4	0.092
Plant extracts at 100ppm										
E[k]	Hex[6]	3	258 [a]	276 [a]	296 [a]	64 [b]	74 [b]	11.487	4	0.022
	Ace[7]	3	217 [b]	283 [a]	208 [b]	6 [d]	99 [c]	12.833	4	0.012
	Met[8]	3	3[d]	0 [d]	87 [b]	38 [c]	159 [a]	13.597	4	0.008
S[t]	Hex[6]	3	220 [b]	266[a]	284 [a]	160 [c]	189 [b]	12.022	4	0.017
	Ace[7]	3	22 [d]	165 [b]	234 [a]	122 [c]	168 [b]	12.833	4	0.012
	Met[8]	3	204 [b]	267 [a]	202 [b]	115 [d]	166 [c]	12.433	4	0.014
V[l]	Hex[6]	3	211 [b]	265 [a]	89 [c]	188 [b]	8 [d]	13.233	4	0.010
	Ace[7]	3	76 [d]	9 [e]	243 [a]	113[c]	178 [b]	13.548	4	0.008
	Met[8]	3	193	154	162	27	171	8.956	4	0.062
S[f]	Hex[6]	3	267 [a]	279 [a]	288 [a]	103 [c]	198 [b]	11.215	4	0.024
	Ace[7]	3	84[c]	76 [c]	22 [d]	110 [b]	156 [a]	12.9	4	0.012
	Met[8]	3	189 [a]	167 [a]	181 [a]	120 [b]	165 [a]	12.714	4	0.013

[a]*Median* values followed by the same letters in rows are not significantly different at α = 0.05. [1]*Erythrinnaabyssinica,* [2]*Aspiliapluriseta,* [3] *Vangueriainfausta,* [4]*Ficus sycomonus,*[5]*Carissa edulis,* [k] *E. coli,* [t] *S. typhi,* [l] *V. cholerae,* [f] *S. flexineri,* [6] Hexane extract, [7]Acetonoic extract, [8]Methanoic extract

Comment [MOU25]: There are less colonies with 500 ppm plant extract compared to 1000 ppm. How can you explain that? Similarly in other samples also lower number of colonies in lower concentration of plant extracts compared to higher concentration.

IV. DISCUSSION

4.1 Phytochemical Extraction Using Different Solvent and Analysis of Crude Extracts

A higher yield is observed in methanol then acetone and then in hexane extracts. These observations are comparable to other results obtained in related studies such as those of Do *et al*(2014) on the extraction yield of*Limophilaaromatica* and that of Kuppusamy *et al.* [39] on *Commelinanudiflora.* Further, these results are supported by studies which have demonstrated that the efficiency of extraction process is dependent on type of the solvent used [40] [41]. Variation on the amount of extract obtained by different solvent is attributed to difference in their polarity [42]. This could be that the medicinal herbs contain a higher number of polar compounds. High polar compounds are soluble in solvents that have high polarity like in the case of methanol [42]. Liu *et al.*[43] describes methanol, a polar solvent as a solvent with improved efficiency of solvation as a result of interaction of hydrogen bonds of the polar sites in the metabolites and the solvent than the non-polar.

The phytochemical analysis results showed the occurrence of secondary metabolites such as phenols, saponins, tannins, flavonoids, terpenoids and glycosides which are known for medicinal attributes as well as physiological activities of the herb [44]. These phytochemicals may be produced in response to inversion of the plant by the microbes. The phytochemicals have been observed to have antimicrobial properties against numerous micro-organisms [45] [46]. Extracts from *Carissa edulis* had compounds such as terpenoids, tannins, steroids flavonoids, saponins, alkaloids, phenolics and absence of glycosides. The current results are in line other related studies such as those of Nawaz [47] who reported occurrence of such compounds in stem bark of *Ficus sycomorus*. The results are further in agreement with a study carried out by Madivoli *et al.*[48].

Extracts from *Ficus sycomorus* had tannins, saponins, terpenoids, phenolics, steroids while flavonoids, alkaloids and glycosides were absent. The occurrence of these metabolites was similar to a study by Nawaz *et al.* [47] who observed tannins, flavonoids, saponins and phenolics. This was also in agreement with a study carried out in Sudan by Osama and Awdelkarim[49]. However, in their study flavonoids and alkaloids were present, this difference could be linked to associated to the difference in climatic conditions or even the soil type in which this herb grow from where the samples were collected.

Aspiliapluriseta extracts had tannins, saponins and phenolics while flavonoids, terpenoids, alkaloids, steroids and glycosides were absent. This was similar to a study carried out by Njeru and Muema[50] in Mbeere community, Embu County which is used in treatment of tuberculosis. The results of this study differed with those carried out around Maseno University, Kenya by Musyimi *et al.* [51] who found the presence of alkaloids, steroids and glycosides which were absent in this study. In both studies methanol as a solvent was used for extraction, therefore the difference could be attributed to different rainfall amount or even the soil type in these different regions. *Erythrina abysinnica* extracts had tannins, flavonoids, saponins, terpenoids, phenolics, steroids while alkaloids and glycosides were absent. These results are supported by a similar study conducted in Tharaka-Nithi county on utilization of herbal medicine among children under five by Nzuki[52] who identified presence of tannins, terpenoids, alkaloids, phenolics and flavonoids.

Comment [MOU26]: Your table says Hexanoic extracts are more than acetone.

Comment [MOU27]: ?Explain inversion of plant

Comment [MOU28]: In line with

Comment [MOU29]: Which among all these extracts have anti gastrointestinal effects?

Comment [MOU30]: Did they all use the same method of extraction as yours?

Comment [vk31]: Did they all use the same method of extraction as yours?

Comment [MOU32]: Reframe the sentence

Vangueriainfausta leaf extract had tannins, flavonoids, saponins, alkaloids, phenolics and glycosides while terpenoids and steroids were absent. These results agree with report of Obakiro *et al.*[53] who observed the presence of flavonoids, alkaloids, phenolics and saponins. These results also are in line with results from a study carried in Zimbabwe by Manyarara *et al.* [54] where the extract is used in treatment of candidiasis. Saponins are present in almost all medicinal herbs which grounds for their curative potential. Biological role of phytochemicals in plants is solely to protect them against invasion by injurious pathogens and organisms. That notwithstanding, certain saponins have been reported to be toxic to endotherms but such toxicity is lower towards mammals. Since they are toxic to quite a number of organisms, saponins find their use in pharmacological, antibiotic and fungicidal applications [55]. Flavonoids which are based on a 15-carbon skeleton is comprised of two benzene ring connected through a heterocyclic pyrane ring. The basic flavonoid structure is the aglycone. These flavonoids have many biochemical properties including antioxidant property which is governed by function group arrangement. The substitution, configuration and the total number of hydroxyl groups determines the antioxidant property mechanisms such as the ability to chelate metal ions and in scavenging of radical [56] [57]. The mechanism of antioxidant action involves upregulation or protection of antioxidant defences, suppression of injurious reactive oxygen species which is formed by either inhibition of the enzymes or through chelation of the trace elements that are involved in free radical generation and also by scavenging reactive oxygen species [46].

Studies by Meng *et al.* [58]as well as that by Kumar and Pandey[59] show that flavonoids are present in herbal medicines and are reported to be effective as cardio protective agents, antibacterial, antioxidants and an important part of pharmaceutical and medical applications. In plants, the living and non-living factors bring about production of ROS leading to oxidative stress [59]. The production of flavonoids is improved as a result of this oxidative stress and they exhibit the ability to hinder ROS generation, ability to quench ROS immediately they are formed [59]. Further, they have the potential of absorbing the energetic solar wavelengths. The antioxidant capability as well as the ability of absorbing ultra violet wavelengths depend on how different rings of flavonoids are substituted [59]. According to Cazorolli *et al.* [60], flavonoids inhibit enzymes-based activities including peroxidase and those of xanthine oxidase which take part in generation of free radical thus reducing the oxidative damage brought about by the macromolecules.

Comment [MOU33]: expand

The phenolic compounds are among the highest and common plant phytochemicals [61]. Phenolic compounds have anti-inflammation properties, anti-aging properties, and have the advantage of improvement of endothelial function and causing the inhibition of the cell proliferation and angiogenesis activities [62]. Phenolic compounds accounts for the antioxidant properties exhibited by most plants used as herbal medicine [63]. Phenolic compounds in plants comprises of chemicals like flavonoids, tocopherols and phenolic acids [63] and are available in almost all plant parts. Phenolics have roles such as scavenging of free radicals, have anticarcinogenic effect as well as antioxidant properties. Phenolics are useful in prevention of human chronic diseases like those associated with bacterial as well as parasitic infections [64].

Comment [MOU34]: anti-inflammatory

Steroids have been reported to be endowed with antibacterial phytochemicals [65]. Further, the steroids are considered to be essential phytochemicals as they are associated with sex hormones [30]. Despite having cytotoxic properties, steroids have medical significance [30]. Reports indicate that alkaloids obtained from plants are used as therapy for anaesthetics, antioxidants, antidiabetic, antimicrobial and muscle relaxants. In plants, alkaloids offer protection against injurious singlet reactive oxygen which may be produced in plant tissues and excited by light source [66]. According to report by Ayoola *et al.*[67], alkaloids possess anti-bacterial properties that are exploitable for treatment of bacterial related diseases. Additionally, alkaloids have anti-plasmodicas well as the analgesic significance [67]. Tannins comprises of complex derivatives of the acid (polyhydroxy benzoic) an organic or non-nitrogenous compound endowed with the ability of precipitating proteins. They are capable of dissolving in water to form colloidal solutions. Further, tannins are soluble in acetone alcohol but are however slightly soluble in organic solvents including chloroform and ethyl acetate. Presence of groups such as carboxylic as well as the free phenolic gives tannins solutions acidic characteristics [68]. Tannins are medicinally used as antidiarrheal, as haemostatic and as antihemmorrhoidal metabolites. Due to their anti-inflammatory activities, are useful in the treatment of enteritis, bowel disorders and gastritis. Further, tannins may be used to inhibit viral infections [69], bacterial infection as well as to treat parasitic infections [70]. According to Awuchi[71] glycosides are molecules that have their sugar attached to a different functional group by way of glycosidic bond. Reports by Njoku and Obi [72] indicate that glycosides lower blood pressure.

Comment [MOU35]: Anti spasmodic

4.2 Effect of Solvents Choice and Plant on Efficacy of Plant's Bacterial Colony Forming Units

Ficus sycomorus extracts gave the reduced colonies growth of tested bacteria that included *Escherichia coli* (5 cfu). *Vibrio cholerae* 7.08 cfu and *Shigella flexneri*(13.28 cfu). Reduced colony growth of*Salmonella typhi* (28.34 cfu) was observed with crude extract of *Erythrina abysinnica*. Slightly higher colonies of *Shigella flexneri*and *Vibrio cholera* were observed in the crude extract from *Vangueriainfausta*while higher colony units of *Escherichia coli* were observed with the extracts from *Carissa edulis*. According to Korir *et al*. [73]. *Erythrina abyssinica* extracts are active against gram positive bacteria as well as on the gram-negative bacteria but have lower efficacy against *E. coli.*

Comment [MOU36]: How do you tell its reduced colonies. Is there a standard below which its considered as non significant?

Differences in response of different bacteria species towards extracts of some plant species have been reported in other work [74]. Specifically, evaluation of antimicrobial activity of Ficus sp extracts inhibit different bacteria species at different levels [75]. Variability of plant extracts in reducing the growth of bacterial colony units in this study could be attributed to plants species with different strength of phytochemicals. This argument is supported by related work of Odunbaku *et al.*[76] and Chilufya *et al*. [77] on the minimal inhibition of sycomorous. According to Mostafa *et al.*[78]inhibition of different plant extracts against microorganisms may vary depending on plant's chemical constituents and volatility of such chemicals.

Environmental and climatical variations have been cited to influence the concentrations of plant's phytochemicals thus affecting plant's antibacterial activity [77]. Antimicrobial constituents in plant extracts include compounds such as alkaloids, flavonoids, terpenoid and phenolics [79]. These antimicrobial contents have the potential to disrupt microbial cells limiting cell functions by inducing

Comment [MOU37]: climatic

cell death or inhibit biosynthesis of important enzymes and amino acids [80] [79]. Additionally, bioactivity of extracts from the plants could further be attributed to the hydrophobicity attributes enabling extracts to react with cell membrane and mitochondria and altering cells permeability [81] [82]. Variation in colony forming units observed is an indication that different bacteria species differ in their susceptibility to different plant extracts.

4.3 Effect of Varied Concentrations of Crude Plant Extracts on Bacteria Growth

Reduction of bacteria colony forming units in all test bacteria evaluated reduced progressively with increasingly higher concentration of extracts. Minimum inhibition concentration in this study was at 0.5 ppm for all the micro-organisms studied and for all the plants extracts used. The minimum inhibition concentration of *Vangueriainfausta* stem bark and crude extracts of *Physalis peruviana* leaves may be higher than 1000ppm and may be the reason why there was no inhition in all dillutions of the tested gastroenteritis pathogens. In the case of *Aspiliapluriseta,* according to reports, methanolic extracts exhibit broad-spectrum bio-activity against microbes (*Escherichia coli,Staphylococcus aureus*) at the concentration of 6.26 – 25 µg/mL [50]. Musa *et al.*[83]indicated a minimal inhibitory concentration of the extracts from *F. sycomorus* bark ethanolic extracts on *S. typhi* and *E. coli* range of 6.25-1.56 mg/ml which were higher than the observed minimal inhibition concentration in this study. The concentration in this study remains lower than 50mg/ml and 300mg/ml documented earlier by Odunbaku *et al.*[76]. Variation in phytochemical activity may have been attributed to variability of environment and climate which have effect on plant phytochemical constituents. According to Li *et al.*[84] phytochemical constituents of same plant species growing under different environmental conditions can be different.

The methanolic extracts from *Erythrina abysinnica, Aspiliapluriseta, Ficus sycomorus* performed better against*Escherichia coli*. Better performance of methanoic extract against bacteria species observed in this study are supported by other studies [85] [75] [86]. Manimozhi *et al.*[85] reported that MeOH extract of *Ficus species* exhibited better activity against all bacteria tested that included *E. coli*, *B. subtilis*, and *S. aureus*. Variability of the extracts obtained by different solvent may be attributed to their differences in polarity status. Accordingly, it has been observed that Polarity of solvents has influence on the extraction efficiency of plants phytochemicals [16]. Hence, active phytochemical constituents that may not extract efficiently into acetone and hexane extract, methanol extraction is likely to provide more consistent with wider antimicrobial activity.

Comment [MOU38]: inhibition

Comment [MOU39]: dilution

Comment [MOU40]: Do they inhibit Ecoli also which is a normal commensal flora of gastrointestinal tract? Mention its effects on normal flora of GIT and any side effect, if there are any.

Comment [MOU41]: Add- results with

Comment [vk42]: results with

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