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In Vitro Antimicrobial Efficacy of Individual and Combined Extracts Of Five Medicinal Plants, *Enantia chlorantha*, *Echinacea angustifolia*, *Acalypha indica*, *Alchemilla vulgaris*, and *Vernonia guineensis* on Strains of *Salmonella typhi*

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Original Research Article

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Abstract: Traditional medicine is the oldest form of health care system that has stood the test of time. The African continent holds an enormous resource in terms of floral biodiversity and its medicinal plants have remained a main reservoir of phytochemicals for pharmaceutical drug development. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as acute infectious diseases. In Cameroon, many plant species are used as traditional medicine to treat infectious diseases, and several interesting openings have originated for further inquiry following in vitro antimicrobial activity evaluation. Typhoid fever is a systemic infection transmitted through food and water contaminated with human feces. Its causative agent, Salmonella, is a primary cause of food poisoning worldwide. The aim of this study was to investigate the *in vitro* antimicrobial activity of methanol fractions of five mixtures of Cameroonian medicinal plants, *Enantia chlorantha*, *Echinacea angustifolia*, *Acalypha indica*, *Alchemilla vulgaris*, and *Vernonia guineensis* on strains of *Salmonella typhi*, thus bringing out the importance of considering Traditional medicine as an important aspect of health care delivery system in Africa. The general objective was to show the efficiency and potency profile with which *Enantia chlorantha* bark, *Echinacea angustifolia* plant, *A.indica* leaves, *Alchemilla vulgaris* plant, and *Vernonia guineensis* have on strains of *salmonella enterica* serovar typhi, first as individual extracts, then as a recipe mixture, thereby demonstrating the possible use of this mixture in the effective treatment of typhoid fever. The specific objectives was: 1) To do a phytochemical screening that will permit the identification of the plants phytochemical components, 2) To prepare individual methanol extracts of each of the plants used in the composition of the typhoid treatment mixture, and use each of these extracts to test for antimicrobial activity on *salmonella typhi* serotypes, and then test for any antimicrobial activity of the methanolic extract of the entire mixture of all possible combinations of all the plants and come up with possible results observed. Antibacterial assay shows us that while having antibacterial effect separately, the plants used in our recipe have a much more potent activity when associated together, and we can notice that there is an additive effect. With all the parameters put in place, we, rather than selecting either E24 or E25 as our most potent extract, decided to bring out, as our most potent extract, E30, containing the mixture of all the plant extracts (*E. angustifolia*, *A. vulgaris*, *A. indica* , *V. guineensis* and *A. affinis*), in its right proportion, with each plant having a role to play, and with an MBC/MIC value of 0.09.

Keywords: antibacterial; *Enantia chlorantha*; *Echinacea angustifolia*; *A.indica*; *Alchemilla vulgaris*; *Vernonia guineensis*; *Salmonella typhi*.

INTRODUCTION

Traditional medicine (TM), variously known as ethno-medicine, folk medicine, native healing, Complementary and Alternative Medicine (CAM) is the oldest form of health care system that has stood the test of time. Being ancient and culture-bound method of healing, humans have used this method of healing to cope and deal with various diseases that have threatened their existence and survival [1]. The African continent holds an enormous resource in terms of floral

biodiversity and its medicinal plants have remained a main reservoir of phytochemicals for pharmaceutical drug development [2- 4]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as acute infectious diseases as stipulated by Diallo *et al*. [5]. In Cameroon, many plant species are used as traditional medicine to treat infectious diseases, and several interesting openings have originated for further inquiry following in vitro antimicrobial activity evaluation [6].

Prior to the introduction of the cosmopolitan medicine, TM used to be the dominant medical system available to millions of people in Africa in both rural and urban communities. Indeed, it was the only source of medical care for a greater proportion of the population [7]. However, the arrival of the Europeans marked a significant turning point in the history of this age-long tradition and culture. It is explained that the introduction of Western medicine and culture gave rise to 'cultural-ideological clash' which had hitherto created an unequal power-relation that practically undermined and stigmatized the traditional health care system in Africa because of the over-riding power of the Western medicine [1]. Nevertheless most of the current drugs, especially antibiotics have considerable limitations in terms of antimicrobial spectrum, side effects, and their wide-spread overuse especially in the last decade mainly because of the increase in bacterial infections especially in poor countries, which has led to increasing clinical resistance of previously sensitive microorganisms and to the occurrence of uncommon infections [6, 8].

Since herbal medicines have widely been used and now form an integral part of the primary health care in many countries, they may constitute a reservoir of new antimicrobial substances to be discovered. Studies also claimed that some plants, which are already used as traditional medicine, possess antimicrobial properties against bacteria, fungi, and viruses [9, 10] and preparation from such plants considered to be effective against diseases of microbial etiology like small pox, tuberculosis, typhoid and diphtheria etc[11, 12].

Plant medicines are generally considered to be safer and less damaging to the human body than synthetic drugs. Furthermore, there is a current upsurge of interest in plants that is further supported by the fact that many important drugs in use today were derived from plants or starting molecules of plant origin: digoxin / digitoxin, the vinca alkaloids, reserpine and tubocurarine are some important examples [13]. In most cases, liquid dosage forms (e.g. decoction, infusion, teas, just to name a few) of plant remedies are used.

The aim of this study is to investigate the *in vitro* antimicrobial activity of methanol fractions of five mixtures of Cameroonian medicinal plants against salmonella enterica serovar typhi, , thus bringing out the importance of considering Traditional medicine as an important aspect of health care delivery system in Africa.

The general objective was to show the efficiency and potency profile with which *Enantia chlorantha* bark, *Echinacea angustifolia* plant, *A.indica* leaves, *Alchemilla vulgaris* plant, and *Vernonia guineensis* have on strains of *salmonella enterica* serovar typhi, first as individual extracts, then as a recipe mixture, thereby demonstrating the possible use of this mixture in the effective treatment of typhoid fever. The specific objectives was: 1) To do a phytochemical screening that will permit the identification of the plants phytochemical components, 2) To prepare individual methanol extracts of each of the plants used in the composition of the typhoid treatment mixture, and use each of these extracts to test for antimicrobial activity on *salmonella typhi* serotypes, and then test for any antimicrobial activity of the methanolic extract of the entire mixture of all possible combinations of all the plants and come up with possible results observed.

Typhoid fever is a systemic infection transmitted through food and water contaminated with human feces. Its causative agent, Salmonella, is a primary cause of food poisoning worldwide [14]. Certain pathogenic Salmonella serotypes adapted to humans, such as *S. typhi* and *S. para typhi* usually cause severe diseases in humans, such as enteric fever, commonly known as typhoid fever or enteric fever [14]. The case fatality rate is less than 1% with prompt and effective antimicrobial treatment, but may reach 41% in developing countries where access to care is limited [15].

Typhoid fever caused by *Salmonella enterica* serovar *Typhi* (S. typhi) remains a major health problem globally. It affects about 21.7 million people, with 217,000 deaths occurring worldwide on an annual basis [16].The global incidence of typhoid fever was estimated to be about 21 650 000 illnesses and 216 510 deaths during in 2000 and varies greatly between regions. Typhoid is endemic in many countries with poor sanitation and hygiene and limited access to safe water.

Over one-third of the population in developing countries lack access to essential medicines and it is claimed that in Africa, up to 80% of the population uses traditional medicine (TM) for primary health care. The provision of safe and effective TM/CAM therapies could thus become a critical tool to increase access to health care [17]. It is therefore not surprising that, in Africa, traditional healers and remedies made from plants play an important role in the health of millions of people.

MATERIALS AND METHODS

Material

Plant material requirements

- *A.vulgaris* whole plant,
- *A.indica* leaves
- *E.angustifolia* whole plant
- *A.affinis* bark
- *V.guineensis* root

Method

The various plant parts of the five different plants were each washed and dried, after which they were each ground to obtain the required powder forms: the powders of each obtained plant extracts were each labeled A-E following alphabetical order thus:

A.vulgaris whole plant powder- A
A.indica leaves powder - B
E. angustifolia whole plant - C
E.chlorantha bark - D
V.guineensis root - E

Next,100g of each of the plants A-E were each macerated in 1L of methanol for a period of 48 hours ,after which they were filtered, concentrated by use of a rotary evaporator at 90degrees and at the end, five extracts, A-E were obtained .

The percentage yield for each plant is calculated thus:

Percentage yield= mass of extract .100/mass of initial powder

Phytochemical screening

This is a qualitative means of putting into evidence the various chemical families that are present in each plant, which is usually effectuated by use of colored reactions. The color obtained during the process is usually as a result of a bond formation, or an unsaturation formation in a molecule. Appropriate reagents are sued for each test carried out, depending on the type of test to be carried out.

Various coloured reactions carried out

- ***Dragendorff Test***

Aim: Put into evidence the presence of alkaloids

- ***Coumarine Test***

Aim: Identification of coumarines

- ***Libermann-Buchard Test***

Aim: Put into evidence the presence of sterols and triterpenes.

- ***Shinoda Test***

Aim: Put into evidence the presence of flavonoids

- ***Phenol test***

Aim: Put into evidence the presence of phenols

- ***Fehling solution test***

Aim: Put into evidence the presence of reducted sugars

- ***Saponine test***

Aim: Put into evidence the presence of saponines

- ***Resine test***

Aim: Put into evidence the presence of resines

In vitro antimicrobial assay of selected plant extracts

Antimicrobial activity evaluation is aimed at determining antibacterial parameters (MIC and MBC) of the plant combination extracts. This antimicrobial activity will be carried out by the micro broth dilution in liquid medium method using micro plates. Each micro plate consists a series of 96 U-shaped wells, divided into twelve columns and 8 rows. Each well has a contenance of 200µL. The media used for the *in vitro* antimicrobial assay was EMB and MHB.

Preparation of plant extracts to be used

The five different plants to be used, A to E, are then mixed, and all the possible combinations of the plants are obtained. A total of 30 plant combinations mixed in equal proportions is obtained, and they are thus numbered E1 to E30.

Each plant combination was tested on salmonella strain, and the most active and effective combination mixtures selected and repeated several times, to obtain the best results.

Sterility control of plant extracts

A small quantity of the individual plant extracts dissolved in solvent was striated on Polyvitech culture medium and incubated at 37 degrees, to ensure that no prior contamination had occurred before the antimicrobial assay. Polyvitech was chosen as a culture media considering the fact that it is an enriched medium, and so will have a maximum number of bacteria grow on it.

On salmonella strain, and the most active and effective ones will be selected and taken into consideration, and then in turn will be repeated again and again, to ensure the obtention of good results.

Sub culturing of bacterial strains

The salmonella strain is sub cultured by striation method, then incubated at 37 degrees for a period of 18 to 24 hours in order to obtain a young culture, as well as isolated colonies. The isolated colonies were used for preparation of inoculum.

Table-1: Various combination mixtures for all the five plants made

EXTRACT NUMBER	COMPOSITION
E1	A
E2	B
E3	C
E4	D
E5	E
E6	A+B
E7	A+C
E8	A+D
E9	A+E
E10	B+C
E11	B+D
E12	B+E
E13	C+D
E14	C+E
E15	D+E
E16	A+B+C
E17	A+B+D
E18	A+B+E
E19	A+C+D
E20	A+C+E
E21	A+D+E
E22	B+C+D
E23	B+C+E
E24	B+D+E
E25	C+D+E
E26	A+B+C+D
E27	A+B+C+E
E28	A+C+D+E
E29	B+C+D+E
E30	A+B+C+D+E

Activity log: A=*A.vulgaris*, B=*A.indica*, C=*E.angustifolia*, D=*A.affinis*, E=*V.guineensis*

Preparation of inoculum

The inoculum is prepared from young colonies which are 18 to 24 hours old and Muller Hinton Broth. An isolated colony of *Salmonella typhi* is taken by use of a plastic anse and homogenized in 3ml of physiologically sterile water, and its turbidity measured by use of a densitometer. A value of 0.5 Mac corresponding to 1, 5.10-8 CFU/ml. Next 100µl of the prepared suspension is placed in a tube containing 10ml of MHB. The inoculum is observed to ensure that the turbidity (which was initially absent before the *Salmonella typhi* suspension was added) is turbid. Turbidity justifies that bacterial growth must have taken place.

Inoculation

In a 96- well microplate; 100µl of MHB was taken and introduced in each well of the plate. Next into a series of 12 numbered test tubes (C1 to C12), we introduced 1ml TSB contaminated by the germ to test. Then we added into the first row of wells 100µl of each of the combination plant extracts of well-known concentration according to the range of prepared concentrations, after which we carry out a two-fold of the extract till dilution till the ninth column of extracts is obtained. We then introduced 100µl ml of prepared inoculum previously prepared into each well. Column 10 is filled with just the plant extract and Muller Hinton Broth without any salmonella, noted EX, while column 11 is filled with *Salmonella typhi* inoculum without any extract, and will be used as the growth witness, noted TC. Column 12 is filled with just physiological water, and shall be used as sterility witness, noted as TS. Given the fact that a total of thirty extracts were to be tested and each microplate contains just 8 rows, a total of four microplates were used for the antibacterial assay. All the tubes were incubated at 37°C at 24 h. The assay was carried out three times. For combination mixtures involving two or more plants, equal volumes of each extract portion were taken and combined to yield 100 microlitres at the end. The table 2 following summarizes the different combinations.

Table-2: Procedure for two-fold dilutions of various plant combination extracts

	1	2	3	4	5	6	7	8	9	10	11	12
E1	2.5	1.25	0.625	0.3125	0.1562	0.078	0.03906	0.0195	.93.10°	Ex	TC	TS
E2	2.5	1.25	0.625	0.3125	0.1562	0.078	0.03906	0.0195	.93.10°	Ex	TC	TS
E3	2.5	1.25	0.625	0.3125	0.1562	0.078	0.03906	0.0195	.93.10°	Ex	TC	TS
E4	2.5	1.25	0.625	0.3125	0.1562	0.078	0.03906	0.0195	.93.10°	Ex	TC	TS
E5	2.5	1.25	0.625	0.3125	0.1562	0.078	0.03906	0.0195	.93.10°	Ex	TC	TS
E6	2 .5	1.25	0.625	0.3125	0.1562	0.078	0.03906	0.0195	.93.10°	Ex	TC	TS
E7	2 .5	1.25	0.625	0.3125	0.1562	0.078	0.03906	0.0195	.93.10°	Ex	TC	TS
E8	2.5	1.25	0.625	0.3125	0.1562	0.078	0.03906	0.0195	.93.10°	Ex	TC	TS

Determination of Minimum Inhibitory Concentration (MIC)

After incubation the MIC of each sample was determined by sowing 0,1ml of each concentration on mediums Hektoen and Eosin Methylene Blue (EMB). The MIC thus corresponds to the smallest concentration which inhibited the growth of the germs compared to the witness.

Determination of the Minimum bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the plant extract on the strains of bacteria was carried out according to provision supplied by Pandit *et al*. [18]. Briefly, 1 ml was taking from the mixture obtained in the determination of MIC tubes which did not show any growth and spread evenly on Hektoen or EMB media and incubated for 24 h. The least concentration of the extract with no visible growth after incubation was taken as the Minimum Bactericidal Concentration.

Determination of MBC/MIC

Calculation of MBC/MIC values will enable us to be able to determine either the bactericidal effect of our plant combinations (CMB/CMI< 4) or their bacteriostatic effects (MBC/MIC ≥ 4) [19]. Plant classification based on MIC is done as follows: – Strong inhibition: MIC less than 500 µg/ml; – moderate inhibition: MIC varying between 500 µg/ml and 1500 µg/ml; – weak inhibition: MIC greater than 1500 µg/ml [20].

RESULTS

Plant material harvesting yield

Table-3: Percentage of water loss on drying for each of the plants used

Plant	Plant part used	Fresh mass(g)	Dry mass(g)	Water loss (%)
A.vulgaris	Whole plant	2000	900	45
A.indica	Leaves	2500	600	24
E.angustifolia	Plant	1800	800	44.4
A.affinis	Bark	1900	1100	57.89
V.guineensis	Roots	2100	1000	47.6

Percentage water loss was greatest with *A. affinis*, which was closely followed by *V.guineensis*, *A.vulgaris*, and finally *E.angustifolia*

After grinding, each extract had a characteristic colour and taste, and specific quantities of each powder were obtained after grinding.

Table-4: Quantity of powder obtained for each extract, and aspect before extraction

Plant	Dry Mass(g)	Powder(g)	Appearance	Taste	Smell
A.vulgaris	900	600	Dark brown	Neutral	Mouldwood
A.indica	600	259	Bright green	Bitterish	Dattishsmell
E.angustifolia	800	400	Green	Neutral	Mould
A.affinis	1100	500	Dark Yellow	V bitter	Mould
V.guineensis	1000	650	Brown	Av.bitter	Bitter cola

Chemical extraction of plant parts used

Table-5: Percentage yield of each extract obtained after extraction.

Plant	Extract(g)	Yield (%)	Aspect	Colour
A.vulgaris	10	1.67	Paste like	Dark brown
A.indica	35	13.5	Paste-like	Dark green
E.angustifolia	15	3.75	Paste –like	Dark green
A.affinis	28	5.6	Sticky	Deep yellow
V.guineensis	7	1.076	Paste like	Light brown

Extraction of the various extracts was carried out using methanol, which happens to be the most polar solvent as compared to the other most commonly used solvents for extraction. From the table we can see that leaves had a percentage yield which was less than barks and roots.

Phytochemical screening

Table-6: Results of phytochemical screening;

Test	*A.vulgaris* *A*	*A.indica* *B*	*E.angustifolia* *C*	*A.affinis* *D*	*V.guineensis* *E*
Dragendorff	-	-	-	+	-
Coumarine	+	-	-	-	-
Libermann	-	-	+	-	-
Schinoda	+	+	-	+	+
Phenol	+	-	+	+	+
Fehling	-	-	-	-	-
Saponine				+	

Activity log: +=positive, -=negative

Phytochemical screening revealed a positive alkaloid and saponine tests for *A.affinis* only, while coumarine test was positive for *A.vulgaris* only. Phenol test was revealed positive for *A.vulgaris*, *E.angustifolia*, *A.affinis*, and *V.guineensis*. Fehling test came out negative for all plant extracts.

Coumarine test was positive in *A. vulgaris* only, while it was negative in the other four extracts. Liebermann's test revealed the presence of steroids in *E.angustifolia* only and not the others. Schinoda test revealed the presence of flavonoids in *A.vulgaris,A.indica* ,and *A.affinis:* Phenol test was positive for all except in *A.indica;* Fehling test came out negative for all five extracts.

Saponine test came out positive for *A.affinis* only. Finally, Resine test came out positive for *E.angustifolia* only.

Antimicrobial assay

Sterility control of plant extracts

Sterility control of the plant extracts revealed no prior contamination before the antimicrobial assay was carried out. No visible growth was observed in the extracts

Determination of Minimum Inhibitory Concentration (MIC): first assay

From the table and images obtained below, we can see that of the thirty extracts combinations tested, all of them each inhibited salmonella growth at the highest concentrations.Going further,we could notice that the more the mixtures were made, the more the activity on salmonella was more pronounced, implying one could notice an additive effect with various plant combinations.

We could on the figure 1 below note the colour changes of the wells; wells with clear colour changes portray wells where bacterial growth was visibly inhibited, while those with turbid aspects represent those wells where no visible bacterial well was observed.

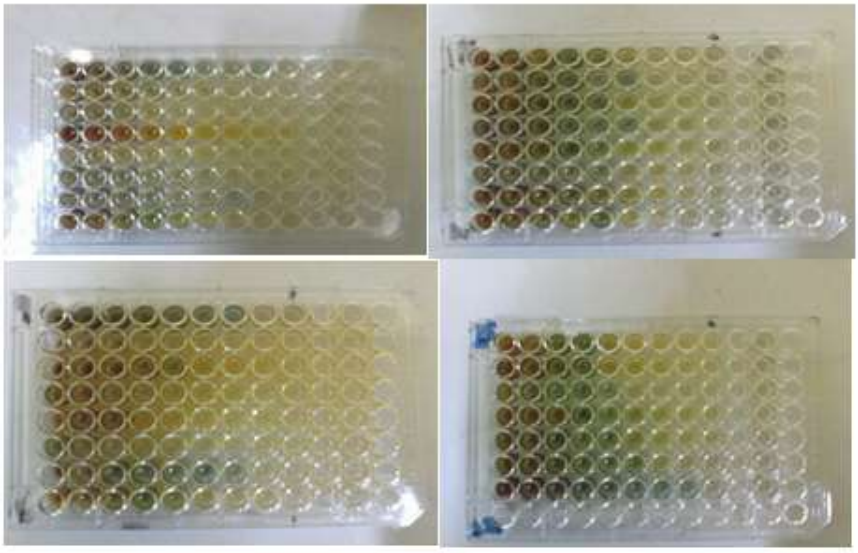

Fig-1: Four microplates used after 24-48 hours of inoculation

Table-7: Schematic representation of antibacterial assay results of the various plant combinations after 24-48 hours of incubation

E	1	2	3	4	5	6	7	8	9	10	11	12
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
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26												
27												
28												
29												
30												

Activity log: blue=bacteria inhibition, while red=bacterial growth, E=Extract combination+well number, corresponding to concentrations made.

After the results obtained above, the combination mixtures with the most potent activities were selected, and the assay was repeated three times, this time around, taking into consideration just the mixtures which inhibited the greatest. Five extracts were selected:

- E24 consisting B, D, E
- E25 consisting C, D, E
- E28 consisting A, C, D, E
- E29 consisting B, C, D, E
- E30 consisting A, B, C, D, E extracts

Determination of MIC: second and third assay

Determination of MIC: Second and third assay

The second and third assays were carried out in the same manner as the first assay, but with the most potent extracts this time.

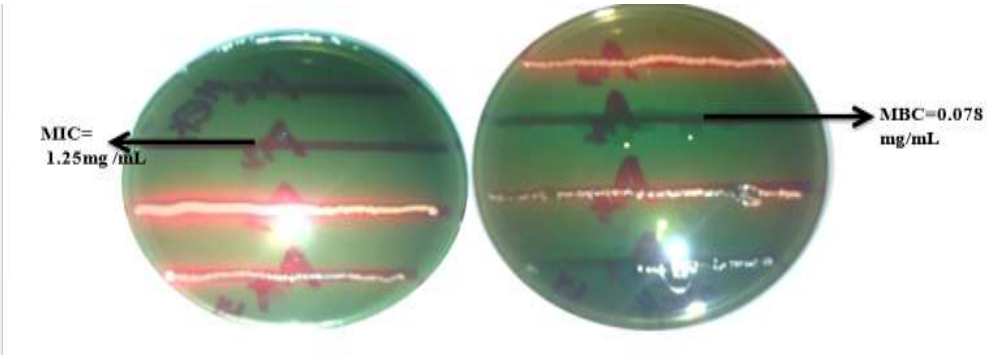

Fig-2: Determination of MIC and MBC of E24

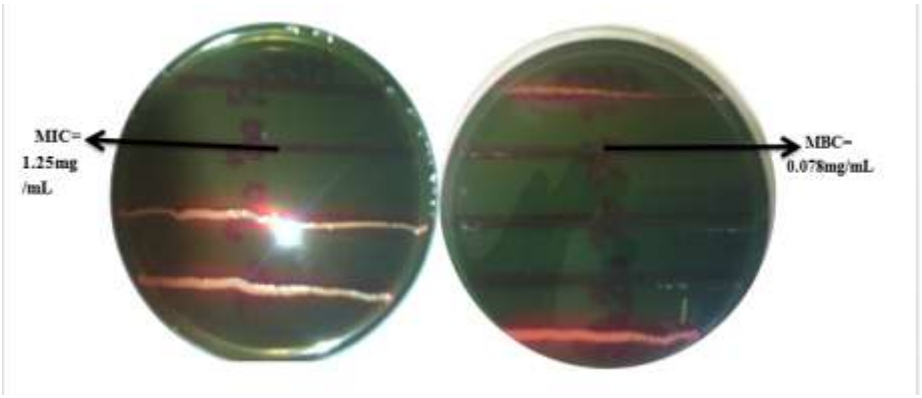

Fig-3: Determination of CMB of E25, consisting a mixture recipe of *E. angustifolia, A. affinis*, and *V. guineensis* after 24 hours

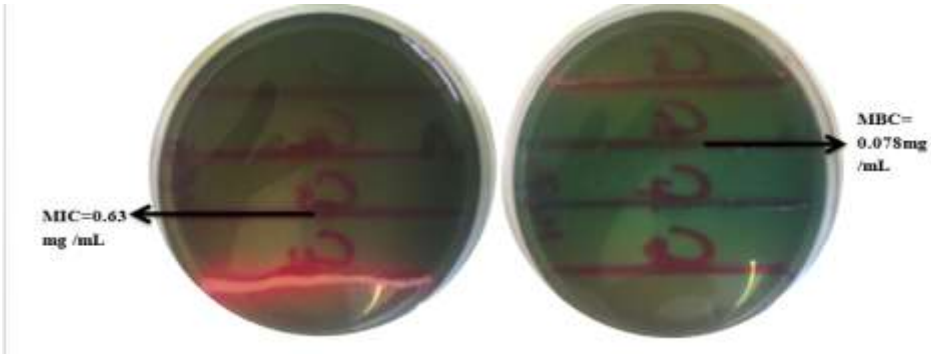

Fig-4: Determination of MIC and MBC of E28

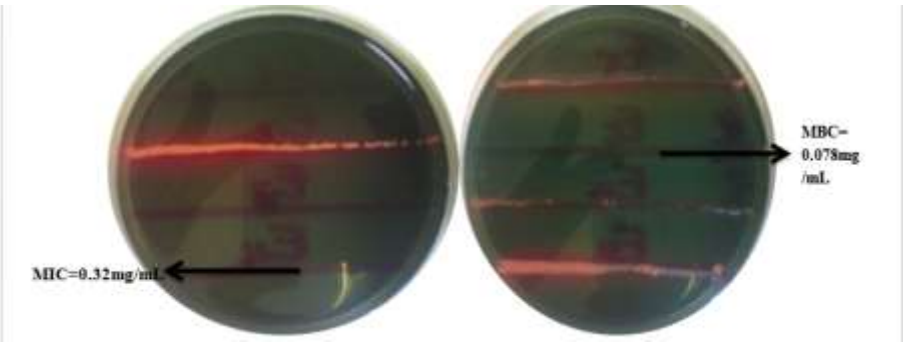

Fig-5: Determination of MIC and MBC of E29

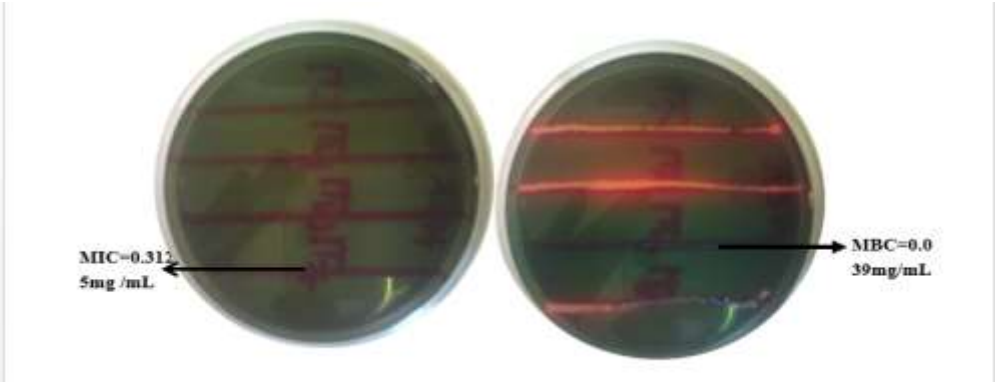

Fig-6: Determination of MIC and MBC of E30

From the images above, we can notice that our plant extracts have a remarkable activity. From the above images, we see that those regions where no visible bacterial growth is observed stands for the MIC, while those areas where there was very negligible bacterial growth (less than 0.01 mg/ml) is known as the MBC. We were then able to come up with certain values for MIC and MBC for some plant combinations, compared to some values of some reference antibiotics.

From the MIC and MBC values obtained above, we could come up with the following MBC/MIC table:

Table-8: Values obtained for MIC and MBC for the five most potent extract combinations

Extract number	MIC(mg)	MBC(mg)
E24	1.25	0.078
E25	1.25	0.078
E28	0.63	0.078
E29	0.32	0.078
E30	0.312	0.039
Ciprofloxacin Ceftriaxone Azithromycine Ampicilline	0.015µg 0.06µg 8µg 0.5µg	0.03µg 0.125µg 8µg 0.5µg

Table-9: MBC/MIC values for the five potent mixtures

Plant extract	MBC/MIC value
E24	0.06
E25	0.06
E28	0.12
E29	0.24
E30	0.09

DISCUSSION

Choice of solvent for extraction

Methanol, ethanol and acetone separately or mixed with water are commonly used to extract bioactive compounds from plant materials, depending on the intended use of the extract. In this study methanol was chosen as the extraction solvent because methanol formulations, though not very safe for human consumption, will permit the maximum obtention of crude extracts of phytochemicals from plant materials in the herbal medicine industry for therapeutic applications, and also, methanol extracts have been proven to produce the greatest yield as compared to extracts of other commonly used solvents, as well as produce the greatest antioxidant and antimicrobial activity [21].

RESULTS OF PHYTOCHEMICAL SCREENING OBTAINED

- Phytochemical screening revealed the presence of phenols in four out of five of the plants used in the recipe composition used in this study. The presence of phenols, as well as phenolic compounds is an indicative of antioxidant properties of the plant. *A. vulgaris; E. angustifolia*, *A. affinis*, and *V. guinneensi*s composition of phenols was equally affirmed in similar studies carried out by Focho *et al*.[27].
- Positive test for flavonoids and saponins obtained for *A. affinis* was equally obtained in our study, just like in a study carried out by Adebiyi *et al*. Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection [26], it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms.
- Shinoda positive test for *A. vulgaris*, *A. indica* and *V. guineensis* equally confirmed the results obtained by studies carried out by Ondrejovic *et al.* [22]; thus confirming the possession of some antibacterial properties by these plants.
- Coumarin test, which was positive just for *A.vulgaris*, confirmed the results obtained by Ondrejovic *et al.* [22]. Coumarins are phenolic substances made of fused benzene and α-pyrone rings. They are responsible for the characteristic odor of hay. As of 1996, at least 1,300 had been identified. Their fame has come mainly from their antithrombotic, anti-inflammatory, and vasodilatory activities. This thus explains the effective use of *A. vulgaris* in wound healing, as well as in bleeding, thus justifying its presence in this therapy combination. It is known that typhoid could possibly cause intestinal perforations and possible bleeding; so *A. vulgaris* has an important role to play in this recipe mixture.
- The positive alkaloid test for *A. affinis* justifies its possible place in this mixture therapy, for these heterocyclic nitrogen compounds are commonly found to have antimicrobial properties as well as microbiocidal effects (including against Giardia and Entamoeba species [23], though the major antidiarrheal effect is probably due to their effects on transit time in the small intestine.

Results of Antimicrobial assay obtained

Antibacterial assay shows us that the plants used in our recipe have a much more potent activity when associated together, as compared to when administered as individual extracts. We could then talk here of an additive effect.

The MIC of a bacterium to a certain antimicrobial agent gives a quantitative estimate of the susceptibility. MIC is defined as the lowest concentration of antimicrobial agent required to inhibit growth of the organism. The MIC informs you about the degree of resistance and might give you important information about the resistance mechanism and the resistance genes involved [24].

From the table and images obtained above, we can see that of the thirty extracts combinations tested, all of them each inhibited salmonella growth at the highest concentrations. Going further, we could notice that the more the mixtures were made, the more the activity on salmonella was more pronounced, implying one could notice an additive effect with various plant combinations.

All five plant extract mixtures did show significant bactericidal activity, given the fact that their MBC/MIC values obtained were all less than 4, most especially E24, E25 and E30 And these extracts are similar in that they each contained two plants in common: *A. affinis* and *V. guineensis*

The presence of *A. vulgaris* in the recipe mixture can be justified by the fact that it is known to possess wound healing properties. And knowing that in typhoid fever, there is usually perforation of small intestines (especially in chronic typhoid cases) and *Echinecea angustifolia* in this recipe mixture mostly plays the role of immune system booster.and *A.indica* is known to possess enough data to back up its antimicrobial properties [19, 25].

With all the parameters put in place, we, rather than selecting either E24 or E25 as our most potent extract, decided to bring out, as our most potent extract, E30, containing the mixture of all the plant extracts, in its right proportion, with each plant having a role to play, and with an MBC/MIC value of 0.09 (inferior to 4).

CONCLUSION

The objective of this study were to do the phytochemical screening, in order to identify the substances which could be responsible of the activities of the plants, and to evaluate antimicrobial activities for methanol extracts of a medicinal traditional recipe of *A, vulgaris, A. indica, E. angustifolia, A. affinis*, and *V. guineensis* plant materials.

From the results obtained in this study, the following main conclusions could be drawn:

- The positive alkaloid test for *A. affinis* justifies its possible place in this mixture therapy, for these heterocyclic nitrogen compounds are commonly found to have antimicrobial properties as well as microbiocidal effects (including against Giardia and Entamoeba species, though the major antidiarrheal effect is probably due to their effects on transit time in the small intestine.
- Phenol test was revealed positive for *A. vulgaris*, *E. angustifolia*, *A. affinis*, and *V. guineensis*. The presence of phenols, as well as phenolic compounds is an indicative of antioxidant properties of the plant.
- Coumarins which was present only in *A.vulgaris*, are phenolic substances made of fused benzene and α-pyrone rings. Their fame has come mainly from their antithrombotic, anti-inflammatory, and vasodilatory activities. This thus explains the effective use of *A. vulgaris* in wound healing, as well as in bleeding, thus justifying its presence in this therapy combination. It is known that typhoid could possibly cause intestinal perforations and possible bleeding; so *A. vulgaris* has an important role to play in this recipe mixture.
- The presence of steroids in *E. angustifolia* can justify an anti-inflammatory effect
- Flavonoids was present in *A.vulgaris*, *A.indica* ,and *A.affinis;* flavonoids are known to be synthesized by plants in response to microbial infection, then it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms.

Antibacterial assay shows us that while having antibacterial effect separately, the plants used in our recipe have a much more potent activity when associated together, and we can notice that there is an additive effect. With all the parameters put in place, we, rather than selecting either E24 or E25 as our most potent extract, decided to bring out, as our most potent extract, E30, containing the mixture of all the plant extracts (*E. angustifolia*, *A. vulgaris*, *A. indica* , *V. guineensis* and *A. affinis*), in its right proportion, with each plant having a role to play, and with an MBC/MIC value of 0.09 (inferior to 4).

- Methanol extract powders of the five plants showed renowned antibacterial effect, most especially bactericidal effects with MBC/MIC values ranging from 0.06 to 0.12

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