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Phytochemical study and evaluation of the antimicrobial activities of three plants in the treatment of typhoid fever in Togo

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Typhoid fever, an infectious disease impacting millions worldwide, has led to the utilization of a traditional medicine recipe in Togo consisting of *Carica papaya, Cocos nucifera,* and *Persea americana*. This study aimed to assess the phytochemical properties and antimicrobial activities of the traditional medicine recipe against typhoid fever. Qualitative tests, employing characterization reactions, were employed to identify phenolic compounds, alkaloids, terpenes and sterols, anthocyanins, reducing sugars, and cardiac glycosides in the hydroethanolic extract. The total polyphenol content was determined using the *Folin-Ciocalteu* reagent reduction method, while the Butanol-HCl method was employed to ascertain the proanthocyanidol content. The results indicated that the hydroalcoholic extract of the recipe is rich in phenolic compounds (0.86±0.002 mgAGE/g). Various plant organs exhibited phenolic compound contents ranging from 0.31±0.006 to 0.52±0.005 mgAGE/g. The condensed tannin contents varied between 0.21%±0.001 and 0.35%±0.003 mgCE/g, while the antiradical activity ranged from 0.069±0.007 to 0.074±0.000 mgAAE/g. The recipe demonstrated higher activity against hospital strains, with a minimum inhibitory concentration (MIC) ranging between 6.25 and 12.5 mg/ml. The MIC of plant organs varied from 25 to 100 mg/ml.

Key words: *Carica papaya, Cocos nucifera, Persea americana*, typhoid fever, Togo.

INTRODUCTION

Typhoid fever, a widespread infectious disease affecting millions globally, is primarily prevalent in regions with precarious hygienic conditions, including developing countries in Asia, Africa, and Latin America (Legba et al., 2020). In 2018, the World Health Organization (WHO) estimated an annual occurrence of 11 to 20 million cases

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of typhoid in developing countries, resulting in 128,000 to 161,000 deaths. Togo, in 2016, recorded 8,279 cases of typhoid fever with 294 hospitalizations, as reported by the Health Information Division of the Ministry of Health (Ministry of Health and Social Protection, 2017). The conventional treatment for typhoid involves antibiotic therapy following stool or blood culture examinations. However, bacterial resistance to these drugs has been on the rise due to their indiscriminate use. Traditional medicine emerges as an alternative treatment, as the local use of natural plants is well-established in Asia, Latin America, and Africa (Bibitha et al., 2002).

The abundance of secondary metabolites in medicinal plants presents potential alternatives for the treatment of various pathologies. In developing countries like Togo, where resources are limited, medicinal plants serve as a crucial source of medicines for the majority of the population (Kpodar et al., 2016). Consequently, local communities often rely on plants to address various diseases. While scientific studies have highlighted the growing importance of traditional medicine in treating various pathologies in Togo (Gbekley et al., 2018; Hoekou et al., 2017; Kpabi et al., 2020), few studies have explored the treatment of typhoid fever with a plant recipe. Therefore, this study aims to contribute to the evaluation of the phytochemical constituents of the recipe and to explore its antiradical and antimicrobial potentials through detailed analysis.

MATERIALS AND METHODS

Plant material

The plant material consists of the leaves of *Persea americana*, the roots of *Carica papaya* and the roots of *Cocos nucifera.*

Microbial strains

Antimicrobial tests were performed with 12 microbial strains of the genus *Salmonella* including a reference strain *Salmonella typhimurium* ATCC 14028 provided by University Hospital Center campus, University Hospital Center Sylvanus Olympio, Precilabo and the National Institute of Hygiene (INH). The clinical strains were *Salmonella typhi* 0325 (S1), *Salmonella typhi* 018 (S2), *Salmonella typhi* 0235 (S3), *Salmonella spp* 032 (S4), *Salmonella typhi* 0560 (S5), *Salmonella spp* 0518 (S6), *Salmonella spp* pl (S7), *Salmonella spp* OMB (S8), Salmonella spp 0718 (S9), *Salmonella spp* 0667 (S10). *Salmonella spp* 0101 (S11).

Culture media and reagents

To carry out extractions, phytochemical, antimicrobial and anti-free radical tests, the following reagents were used: Nutrient Agar, Muller Hinton Medium, Sterile Swabs, Microplates, Distilled Water, Ethanol, Hydrochloric Acid, Merck's Folin-Ciocalteu Reagent, Butanol, Gallic Acid, Ascorbic Acid, Sodium Carbonate, Ammoniacal Iron Sulfate, sulfuric acid, sodium phosphate, ammonium molybdate, sulfuric acid, sodium hydroxide, petroleum ether, petroleum ether, acetic anhydride, chloroform, magnesium shavings, Dragendorff's reagent, Meyer's reagent.

Sample collection

Fresh leaves of *P. americana*, roots of *C. papaya* and *C. nucifera* where collected between 15 April and 28 June 2022 in Djagblé, Togoville and Tsévié (Togo). The plants where identified and confirmed at the Herbarium of Department of Plant Biology, Faculty of Sciences, University of Lomé (FDS-UL).

Preparation of extracts

The plant materials (fresh leaves and roots) where dried in the laboratory (LAMICODA) at room temperature (25°C) and pulverized into a fine powder for extraction by a Moulinex brand of Binatone. Several extracts were prepared from the powder obtained. The procedure was carried out using ethanol-water (70:30 v/v) and organic solvent such as petroleum ether.

Fat extraction

Fat of the powders from the different plant organs was extracted with petroleum ether. Thus to four hundred grams of the powders of each extract we added two liters of petroleum ether for twenty-four hours.

Hydroalcoholic extraction

After fat extraction, we carried out the Hydroalcoholic extraction by maceration in a hydroalcoholic solution (70% alcohol at 95 and 30% distilled water). So we took 2g of each powder to which are added 3 liters of hydroalcoholic solution.

According to the indications of the traditional therapist, the recipe is composed of 35% *C. papaya*, 35% *P. americana* and *30% C. nucifera.* After 48 h of contact in the dark, the mixture is decanted then filtered on filter paper (wattman n°1). The filtrates obtained are then evaporated under vacuum at 50ºC. at 125 revolutions per minute using a Heidolph type rotavapor. The concentrated hydroalcoholic extract thus obtained was freeze-drying at low temperature and stored in a cool place away from light, in a dry and sterile bottle for carrying out the various tests.

Characterization of phenolic compounds

In a tube containing 2 ml of extract, we added a few drops of iron perchloride (Fe_2Cl_3). The presence of phenols is shown by brown, green or dark green precipitate.

Characterization of alkaloids

In a tube containing 2 ml of extract, we added a few drops of Dragendorff's reagent. The formation of a red or orange-red precipitate indicates the presence of alkaloids (Trease and Evans, 1987).

Characterization of flavonoids

By adding a few drops of 1/10 NaOH solution to 1 ml of extract, the formation of a red-orange color becoming colorless on addition of dilute hydrochloric acid indicates the presence of flavonoids (Tiwari et al., 2011).

Characterization of tannins

Lead acetate test

By adding 3 ml of previously prepared extract to 1 ml of 10% lead acetate, the formation of a blue, blue-black, whitish or brownish precipitate indicates the presence of tannins in the tested extract.

Test with ferric chloride reagent

One militer of each extract is mixed with two ml of water and one or two drops of one percent ferric chloride reagent. The formation of a blue, blue-black or black color confirms the presence of gallic tannins, while the appearance of a green or dark green color indicates the presence of catechic tannins in the extract studied (Duraisamy et al., 2020).

Characterization of triterpenes and sterols

An etheric extract is prepared from 1 g of plant powder in 20 ml of ether for 24 h. The sterols and tri-terpenes are revealed by adding 1 ml of $CHCl_3$ to the residue of 10 ml of the evaporated macerate. The solution obtained is divided into two test tubes, and then one to two ml of concentrated H_2SO_4 is added to one of the tubes, the other will serve as a control. The formation of a brownish-red or purple ring at the contact area reveals their presence.

Characterization of anthocyanins

They revealed by adding to 5 ml of 5% infused 5 ml of sulfuric acid (H_2SO_4) at 10% to 5 ml of ammonium hydroxide (NH_4OH) at 50%. A red coloration in an acid medium and purplish blue in a basic medium testifies to the presence of anthocyanins (Du and Francis, 1973).

Search for reducing sugars

The demonstration of reducing compounds was made by Fehling's liquor. Fehling's liquor solution (Reagent A: copper sulphate and reagent B: hydroxide of potassium) is added to the test tubes containing an aqueous solution of each extract and the tube is heated using a Bunsen burner flame until the appearance of a brick red precipitate which testifies to the presence of reducing compounds in the sample analyzed (Tiwari et al., 2011).

Characterization of cardiac glycosides

0.5 g of each extract is diluted in 5 ml of distilled water, 2 ml of glacial acetic acid containing a drop of ferric chloride solution is added. To this mixture is added 1 ml of concentrated sulfuric acid. A brown ring at the interface will indicate the presence of a deoxy sugar characteristic of cardenolide. A purple ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually expand into this color (Harborne, 1998).

Evaluation of the total polyphenol content

The assay of total polyphenols was done according to the method described by (Singleton et al., 1999) taken up by (Karou, 2006). It evaluates all the reducing phenolic compounds of the Folin-Ciocalteu reagent (FCR) consisting of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMO_{12}O_{40}$).

This is an oxidation-reduction reaction in a basic medium during which the OH function of the phenols is oxidized while the RCF is reduced to a mixture of blue oxides of tungsten (W_8O_{23}) and molybdenum (MO_8O_{23}) with the formation of a phenolate ion.

Evaluation of the proanthocyanidol content

The proanthocyanidol content was evaluated by the Butanol-HCl method, described by Porter et al. (1985). The method is based on the oxidative depolymerization reaction of condensed tannins in an acid medium (catalyst). The reaction leads to the release of anthocyanidins (colored molecules) corresponding to the cleaved monomers which absorb at 540 nm.

Evaluation of antimicrobial activity

Antimicrobial tests were performed using the 96-well plate microdilution method (Anani et al., 2015). Isolation in pure culture of the strains was carried out on Nutrient Agar. From a culture of 18-24 h on nutrient agar, we prepared a suspension in physiological saline with a turbidity equivalent to the Mc Farland standard 0.5 (≈107 CFU/ml).

2.5 g of each extract was dissolved in 25 ml of sterile distilled water to obtain a solution of concentration 100 mg/ml.

Sterilization of extracts is made by filtration on a 0.45 nm millipore membrane.

Sterility tests

The authors plated 100 µl of each extract on MH. Extracts that did not show growth are considered pure.

Microbicidal activity of extracts

Minimal Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined using the microdilution technique in 96-well plates with Muller Hinton broth (MHB) (Wayne, 2003). To achieve this, stock solutions of the total extracts, sterilized by millipore membrane filtration with a diameter of 0.45 µm, were prepared at a concentration of 100 mg/ml in an ethanol/water mixture (10/90). One hundred microliters (100 µl) of MHB were dispensed into the wells of the microplate, and successive dilutions ranging from 100 to 6.25 mg/ml of extract were prepared with MHB. Microbial turbidity suspensions corresponding to Mac Farland 0.5 were created using microorganisms from a 24-h culture at 37ºC, following a Gram control. Subsequently, 100 µl of this microbial suspension was brought into contact with the extracts and their dilutions in the wells. Positive control wells (microbial suspension without extract) and negative controls (MHB + extract) were also included. Gentamicin served as a reference antibiotic. Finally, the plates were mixed and incubated at 37ºC for 24 h. The MBC was determined by taking 100 µl of suspension from wells without visible growth and seeding it on nutrient agar. Incubation was carried out at 37ºC for 24 to 48 h, after which the colonies were counted.

Study of the synergistic, antagonistic, additive effect of the extracts

The effect of combining the different extracts was assessed using the checkerboard method (Toudji et al., 2018). The dilutions are

Table 1. Qualitative phytochemistry of extracts.

Extract	Phenols	Alkaloids	Flavonoids	Tanins		Terpenes and stérols	Anthocyanins	Reducing sugars	Cardiac glycosides
				Ferric chloride	Lead acetate				
E1	+	+	+	-	-	+	-	-	+
E2	++	+	+	+	+	+	+	+	+
E3	+	+	+	+	+	+	-	+	-
E4	++	+++	++	++	++	+++	+	++	+++

E1: hydroalcoholic extract of *C. papaya*, E2: hydroalcoholic extract of Cocos nucifera, E3: hydroalcoholic extract of *P. americana*, E4: hydroalcoholic extract of the recipe, - Absent, + present, ++ scarce, +++ Abundant.

always of order 2 for each extract. In each well, 50µl of each dilution of the two extracts to be combined, to which is added 100 µl of the bacterial inoculum. The different combinations are assessed by calculating the fractional inhibitory concentration index (ICIF):

if ICIF<1: there is Synergy; 1<ICIF<2: No interaction; ICIF>2: Antagonism; ICIF= 1: additivity

Antibiogram of the germs studied

A bacterial suspension was prepared in sterile distilled water from a pure 24-hour culture on nutrient agar. This suspension was compared to the McFarland 0.5 standard solution, equivalent to 108 CFU/ml. The obtained suspensions were swabbed onto Mueller Hinton agar, with the thickness determined following the method of Bonnet et al. (2013). Antibiotic discs were then placed on Petri dishes, and after 10 to 15 minutes, the plates were incubated at 37°C for 24 h. The diameters of the zones of inhibition were measured and compared with sensitivity standards. The antibiotics tested included Ampicillin (Ampi), Ceftriazone (ceftri), Ceftazidime (cefta), Erythromycin (Eryt), Lincomycin (Linco), Pefloxacin (Peflo), Ofloxacin (Oflo), and Ciprofloxacin (Cipro).

Bactericidal kinetics

For this test, the strains studied were tested by a single concentration of MIC of the hydroalcoholic extract. Thus, 100 µl of each microbial suspension (108 CFU/ml) of MHB was brought into contact with 100 µl of extract (100 mg/ml) at initial time (t = 0). Samples of 100 µl were plated on nutrient agar at t = 0 and after incubation times of 15, 30, 45 min and 24 to 48 h for certain germs. The dishes were incubated at 37°C and the colonies were counted in 24 h. Control microbial suspensions without extract were made.

Statistical analysis

Experiments were carried out in triplicate, and mean values are shown with standard deviation (mean ± SD).

RESULTS AND DISCUSSION

Qualitative and quantitative analysis of extracts

Characterization reactions revealed the presence of phenols, alkaloids, flavonoids, terpenes and sterols as well as tannins, reducing sugars and cardiac glycosides in significant quantities in the recipe as well as in the plant organs taken in isolation. Table 1 shows the qualitative phytochemistry of extracts.

The results of quantitative analysis showed that the recipe had a higher total phenol content (0.86±0.002 mgAGE/g) than the plant organs. The hydroalcoholic extract of *C. papaya* showed the lowest value (0.31±0.006 mgAGE/g). The different proanthocyanidol contents for the different plant organs and recipe ranged from 0.21%±0.001 to 0.35%±0.003 catechin equivalents per gram. Table 2 shows the quantitative analysis of extracts.

Studies have shown the presence of phenolic compounds in different plant organs (Asghar et al., 2016; da Silva et al., 2013; Kingne et al., 2018). Phenolic compounds are the main characteristic molecules of the plant kingdom. Their role is to defend plants against pathogens. They ensure human and animal nutrition and health. They are capable of scavenging free radicals and inhibiting lipid peroxidation by reducing hydroxyl, superoxide and pyroxyl radicals. They are also capable of scavenging metal ions, as they have chelating properties. Polyphenols have significant antioxidant activity, higher, for example, than that of vitamin (Delattre et al., 2005; Haile and Kang, 2019; Stagos, 2019; da Silva et al., 2013) demonstrated the antioxidant properties of tannins. We have therefore determined the anti-free radical activity of plant organs and the recipe.

Anti-free radical activity

Table 3 shows the various results obtained, which are around 0.07 mgAAE/g. The differences

Table 2. Quantitative analysis of extracts.

Compounds extracts	Total polyphenols (mgAGE/g)	Proanthocyanidols (mgCE/g)
E1	0.31±0.006	0.21±0.001%
E2	0.53±0.005	0.38±0.005%
E3	0.52±0.005	0.22±0.001%
E4	0.86±0.002	0.35±0.003%

mgAGE/g: milligram equivalent gallic acid per gram extract, mgCE/g: milligram catechin per gram extract.

Table 3. Anti-free radical activity of extracts.

Extract	Anti-free radical activity (mgAAE/g)
E1	0.071±0.004
E2	0.069±0.007
E3	0.071±0.003
E4	0.074±0.000

mgAAE/g: milligram ascorbic acid per gram extract.

Table 4. Antimicrobial activity of extracts.

Extracts strains	*C. papaya*			*P. americana*			*C. nucifera*			Recette		
	MIC	BMC	Aa	MIC	BMC	Aa	MIC	BMC	Aa	MIC	BMC	Aa
ATCC	50	50	1	50	100	2	50	50	1	12.5	12.5	1
S1	25	25	1	50	50	1	25	25	1	12.5	25	2
S2	50	50	1	100	100	1	50	100	2	12.5	12.5	1
S3	25	25	1	25	50	2	50	100	2	6.25	12.5	2
S4	50	50	1	50	50	1	50	50	1	12.5	25	2
S5	50	50	1	50	100	2	50	50	1	6.25	12.5	2
S6	100	100	1	100	100	1	100	100	1	12.5	12.5	1
S7	50	50	1	25	50	2	25	50	2	6.25	6.25	1
S8	25	50	50	50	50	1	50	100	2	12.5	25	2
S9	25	50	2	25	50	2	12.5	50	4	6.25	6.25	1
S10	25	25	1	12.5	50	4	25	50	2	6.25	6.25	1
S11	25	50	2	25	50	2	25	50	2	6.25	25	2

The checkerboard method enabled us to observe an additive effect following the combination of extracts.

obtained are not significant either at plant organ or recipe level.

The antiradical activity of the 3 plant organs was evaluated using the DPPH method, and several authors have demonstrated the antioxidant properties of the plants used in our work. (Zhang et al., 2022) obtained an effective concentration of C. *papaya* roots extracts at 50% of 14.07 mmol. $l^{-1.}$ *C. nucifera* presented an antioxidant power of 9.8 µg/ml with the DPPH method (da Silva et al., 2013). The EC50 of *P. americana* was evaluated at 50 µg/ml (Kingne et al., 2018). Free radicals are unpaired molecules derived from oxygen and nitrogen. Their production is useful because they can help immune system cells attack bacterial cells, tumor cells and virus-infected cells. The formation of oxygen-derived free radicals (the superoxide radical: O2- and the hydroxyl radical OH-) is a normal process in aerobic respiration systems. The body has a system for eliminating these free radicals. Oxidative stress occurs when there is an imbalance between the production of free radicals and their elimination (Karou, 2006).

Antimicrobial activity and study of the synergistic, antagonistic and additive effects of extracts

Table 4 show that the various plant organs have an activity on the bacteria studied. MICs ranged from 6.25 to 100 mg/ml and BMCs from 6.25 to 100 mg/ml. Antimicrobial activity (Aa) is bactericidal (BMC/MIC= 1) and bacteriostatic (BMC/MIC >1).

The hydroalcoholic extracts of *P. americana* and *C.*

Table 5. ICIF determination (1).

Association strains	*Cp/Pa CMI*	CIF_{cp}	CIF_{Pa}	*ICIF*	*Action*	*Cp/Cn CMI*	*CIFcp*	CIF_{pa}	*ICIF*	*Action*
ATCC	25	0.5	0.5	1	Ad	50	1	1	2	-
S1	50	0.5	1	1,5	-	25	1	1	2	-
S2	25	0.5	0.5	1	Ad	25	0.5	0.5	1	Ad
S3	50	2	2	4	-	25	1	0.5	1,5	-
S4	25	0.5	0.5	1	Ad	25	0.5	0.5	1	Ad
S5	12.5	0.25	0.25	0,5	S	25	0.5	0.5	1	Ad
S6	50	0.5	0.5	1	Ad	50	0.5	0.5	1	Ad
S7	12,5	0.5	0.5	1	Ad	12.5	0.5	0.5	1	Ad
S8	25	0.5	0.5	1	Ad	25	0.5	0.5	1	Ad
S9	12.5	0.5	0.5	1	Ad	25	1	2	3	-
S10	25	1	2	3	-	12.5	0.5	0.5	1	Ad
S11	12.5	0.5	0.5	1	Ad	25	1	1	2	-

Cp= *C. papaya*, Cn= *Cocos nucifera*, Pa= *Persea Americana*, Ad= additivity; S= synergy; - =no interaction.

nucifera leaves have an MIC of between 12.5 and 100 mg/ml, compared with the hydroalcoholic extracts of *C. papaya* roots 25 and 100 mg/ml. Recipe, on the other hand, showed MICs of between 6.25 and 12.5 mg/ml on most of the strains studied. All strains are sensitive to Gentamicin 40mg/m. Tables 5 and 6 show the ICIF determination of 1 and 2.

The mixture of various plant organs used in traditional medicine to treat typhoid fever demonstrates inhibition of in vitro growth against different Salmonella strains. When compared to the individual plant organs, the recipe exhibits a more potent action on the studied Salmonella strains. The obtained Minimum Inhibitory Concentrations (MICs) in our study ranged from 6.25-12.5 mg/ml for the recipe and 12.5-100 mg/ml for the isolated plant organs.

Previous research by Ngwanguong et al. (2023) demonstrated the anti-salmonella activity of *C. papaya* leaves with MICs ranging from 64 to 512 µg/ml. Lima et al. (2015) also showed the action of *C. nucifera* on *S. typhi*. In a study on various *P. americana* leaf extracts, Idris et al. (2009) found petroleum ether and ethyl acetate extracts to be active on *S. typhimurium,* with MICs of 50 and 40 mg/ml, respectively. The observed differences in MIC values may be attributed to variations in methodology and the type of solvent used for extraction.

The efficacy of the recipe against different Salmonella strains is attributed to the additive activity of all the plant organs and the presence of tannins and other polyphenolic substances in the recipe. Numerous studies have substantiated the antibacterial activity of tannins (Elegami et al., 2002; Scalbert, 1991; Tomás-Barberán et al., 1990).

Antibiotic susceptibility profile of strains

The antibiotic susceptibility profile shows that the clinical strains tested are almost all susceptible to the quinolones tested, and almost all resistant to erythromycin and ampicillin. Table 8 shows the antibiogram of strains studied.

No bacterial strain exhibited sensitivity to all antibiotics, and this observation can be attributed to a variety of factors related both to antibiotics and bacteria. The effectiveness of an antibiotic hinges on its ability to penetrate the bacterial membrane without undergoing modification. Antibiotics function by inhibiting bacterial wall synthesis, nucleic acid synthesis or function, protein synthesis, or by affecting the plasma membrane. Microorganisms possess natural or acquired resistance mechanisms to counteract the action of antibiotics.

Gram-negative bacilli, for instance, are naturally resistant to water-soluble antibiotics due to their ability to modify the binding points of antibiotics, rendering them resistant (Ouadja et al., 2021). The outer membrane of the bacterial wall, containing lipids, presents a challenge for antibiotics to penetrate. The observed sensitivity of strains to Ceftriaxone can be explained by the fact that this antibiotic belongs to a new generation, thereby exposing strains less frequently to opportunities for developing resistance. Consequently, the use of this recipe holds significance in addressing resistance issues observed in microbial strains.

Microbial growth inhibition kinetics

At a concentration twice their minimum inhibitory concentration (MIC), the authors observed remarkable activity within half an hour against the different strains studied. After one hour, only a few colonies of strains 3, 5, 9, and the reference strain were observed. Following 24 h, no microorganisms were detected in the medium. Table 9 shows the inhibition of bacterial growth.

The kinetics of bacterial growth inhibition measure the duration of exposure of a strain to a specific dose of

Table 6. ICIF determination (2).

Association strains	*Cn/Pa CMI*	*CIFcn*	*CIF_{Pa}*	*ICIF*	*Action*
ATCC	25	0.5	0.5	1	Ad
S1	25	1	0.5	1.5	-
S2	50	1	0.5	1.5	-
S3	12.5	0.25	0.5	0.75	S
S4	25	0.5	0.5	1	Ad
S5	25	0.5	0.5	1	Ad
S6	50	0.5	0.5	1	Ad
S7	12.5	0.5	0.5	1	Ad
S8	25	0.5	0.5	1	Ad
S9	12.5	1	0.5	1.5	-
S10	12.5	0.5	1	1.5	-
S11	12.5	0.5	0.5	1	Ad

Table 7. Antibiogram of strains studied.

ATB strains	Ampi	Ceftri	Cefta	Netil	Eryt	Linco	Peflo	Oflo	Cirpo
ATCC	R	S	R	S	R	R	S	S	S
S1	R	S	S	R	R	S	S	S	S
S2	R	S	S	S	S	R	S	S	S
S3	R	R	S	S	R	S	S	S	S
S4	R	S	R	S	R	S	S	S	R
S5	S	S	R	S	S	R	R	S	S
S6	R	S	S	R	S	S	R	R	S
S7	R	S	S	S	R	S	R	S	S
S8	R	R	S	S	R	S	S	R	S
S9	R	S	S	R	R	S	R	S	R
S10	R	S	S	R	R	S	S	S	S
S11	R	S	R	R	S	S	S	S	S

S: Sensitive, R: Resistant, ATB = Antibiotic.

Table 8. Inhibition of bacterial growth.

Time (hour)	0	1/2	1	24
ATCC	+ Ind	+ 550	+08	-
S1	+ Ind	+ 320	-	-
S2	+ Ind	+ 360	-	-
S3	+ Ind	+ 750	+11	-
S4	+ Ind	+ 330	-	-
S5	+ Ind	+ 645	+07	-
S6	+ Ind	+ 520	-	-
S7	+ Ind	+ 480	-	-
S8	+ Ind	+ 585	-	-
S9	+ Ind	+ 548	+05	-
S10	+ Ind	+ 450	-	-
S11	+ Ind	+ 505	-	-

+ : Presence, Ind: Uncountable, - : Absence.

phytomedicine. The absence of microorganisms in the medium after 24 h serves as a positive therapeutic indicator, providing valuable information for the management of patients suffering from typhoid fever.

Conclusion

The traditional medicine recipe composed of *C. papaya, C. nucifera, and P. americana* is employed for the treatment of typhoid fever. Our study assessed the in vitro efficacy of the recipe against various strains of Salmonella, as well as the effectiveness of individual plant organs. The findings revealed that the traditionally prepared recipe contains diverse phytochemical compounds, notably phenolic compounds and tannins, which influence its antiradical and antibacterial activities against the tested Salmonella spp. strains. The hydroalcoholic extract of the recipe inhibits the growth of different Salmonella spp. strains through the combined action of soluble constituents from various plant organs.

While the results obtained provide valuable insights, further exploration through bioguided fractionation and evaluation of pharmacological properties remains essential. These results substantiate the traditional use of the recipe for typhoid fever treatment and could serve as a foundation for developing new drugs targeting typhoid fever.

CONFLICT OF INTERESTS

The authors have declared any conflict of interests.

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