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In vitro anti-radical and anti-*salmonella* activities of *sarcocephalus latifolius*, *lannea barteri*, *uvaria chamae*, *parkia biglobosa* and *khaya senegalensis*

Abstract

This study aims to analyze the chemical compounds and evaluate the anti-radical and anti-*Salmonella* activities of extracts from *Sarcocephalus latifolius*, *Lannea barteri*, *Uvaria chamae*, *Parkia biglobosa* and *Khaya senegalensis* using respectively standards phytochemical methods, phosphomolybdates reduction method and micro dilution method in liquid medium associated with plating on agar medium. Qualitative phytochemical tests revealed the presence of alkaloids, flavonoids and tannins in all extracts. Quantitative phytochemical analysis showed that the stem bark of *Khaya senegalensis*, *Parkia biglobosa* and *Lannea barteri* displayed high anti-radical properties with respectively 9.12±0.17mgEAA/g, 9.30±0.15mgEAA/g and 8.28±0.23mg EAA/g values of antioxidants. Antimicrobial tests on *Salmonella* strains yielded MIC that varied with plants extracts: 6.25 to 12.5mg/ml for *Sarcocephalus latifolius* and *Lannea barteri*, 25mg/ml for *Uvaria chamae*, 50mg/ml for *Khaya Senegalensis* and *Parkia biglobosa*. These observations could be use to design new drugs that will provide better therapeutic options for the treatment of Salmonellosis and oxidative-related diseases.

Keywords: plant extract, phytochemical analysis, anti-radical and anti-*salmonella* activities

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Abbreviations: *S. latifolius, sarcocephalus latifolius; L. barteri*, *lannea barteri; U. chamae*: *uvaria chamae; P. biglobosa*, *parkia biglobosa*; *K. senegalensis*, *khaya senegalensi;* MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; *S. typhi*, *salmonella typhi; S. enteric, salmonella enteric; S. paratyphi*, *salmonella paratyphi; S. typhimirium*, *salmonella typhimirium;* FCR, folin ciocalteu reagent; ND, not determined; EGA, equivalent gallic acid; EC, equivalent catechin; EAA, equivalent ascorbic acid; CERMETRA-TOGO, centre d'etude et de recherche en médecine traditionnelle apliquée du togo

Introduction

Infectious diseases account for 45% of deaths in low-income countries. Bacterial infections account for 70% of the mortality caused by microorganisms.[1] The genus *Salmonella*, more specifically the serotype *typhi* or less frequently the serotypes *paratyphi* A, B or C are responsible of typhoid fever and paratyphoid fever. Tens of millions of cases are recorded every year in the world, with over one hundred thousand deaths a year, the majority of them in developing countries.[2] The increased resistance of microorganism's strains to the molecules presents on the market and the new WHO recommendations that require each country to introduce natural treatment methods into their health system are pushing many universities and pharmaceutical laboratories to explore medicinal plants, which are less expensive and have fewer side effects.[3] It is in this perspective that the anti-radical and the anti-*Salmonella* potential of the extracts from five medicinal plants used in the Togolese pharmacopoeia to treat fevers and gastroenteritis, has been done. These include *Sarcocephalus latifolius*, *Lannea barteri*, *Uvaria chamae*, *Parkia biglobosa* and *Khaya senegalensis*.

Sarcocephalus latifolius is a shrub of family *Rubiaceae* that usually grows in humid areas of tropical Africa. Its roots are used for several applications in traditional medicine.[4] Its anti-radical properties have been reported by Antia et al.[5] Its roots extracts also have antibacterial activities against *Salmonella* and other bacteria.[6] *Lannea barteri* is a tree of family *Anacardiaceae* whose preparation is used in the treatment of gastritis, fever among others.[7] Njinga et al.[8] showed the anti-*Salmonella* activity of its leaves extract. *Uvaria chamae* belong to the family *Annonaceae*. It is a plant from the Togolese Pharmacopoeia that is used in the treatment of gastrointestinal pain, yellowing, wound healing, fever and constipation.[9] A phytochemical study conducted by Kone et al.[10] showed that the aqueous extracts of the roots of the plant have anti-radical properties. Ogbulie et al.[11] in 2007 reported its bactericidal effect against bacteria including *Salmonella typhi*. *Parkia biglobosa* is a tree of family *Mimosaceae*. It bears fruit in the form of long pods suspended in clusters, containing numerous black seeds coated with yellow pulp. Its trunk bark has been successfully used in the treatment of many diseases including fevers, diarrhea, and severe stomach aches.[12] The anti-radical properties of trunk bark extract have been reported by Komelafe et al.[13] The same extract is active against a large number of microorganisms including *Salmonella typhi* and *Shigella dysenteriae*.[14] *Khaya senegalensis* is a tree of family *Meliaceae* that is used in the African pharmacopoeia to treat dermatitis, malaria, fever, jaundice and stomachaches.[15] Atawodi et al.[16] reported the anti-radical properties of the plant trunk bark

extract. The anti-*Salmonella* action of the same extracts was shown in a previous study conducted by Abdallah et al.[17]

Material and methods

Framework of study

Extractions, phytochemical and microbiological tests were carried out in "Laboratoire de Microbiologie et de Contrôle de qualité des Denrées Alimentaires (LAMICODA)", of the University of Lomé.

Plant material

It is made up of different organs of medicinal plants harvested in the maritime region of Togo, in the localities of "Aképé", "Noépé" and "Badja" on 02-10- 2016. These are: the trunk bark of *Khaya senegalensis*, *Parkia biglobossa* and *Lannea barteri*, root bark of *Sarcocephalus latifolius* and roots of *Uvaria chamae*. The plants were authenticated in the Botanical Laboratory of the University of Lomé. The herbarium numbers are respectively TG01797, TG15084, TG09467, TG07536 and TG01950.

Preparation of plants extracts

The plant material is cut into small blocks and then dried out of the sun at the Laboratory temperature between 16 and 24 degrees Celsius before being pulverized. 500ml of the ethanol-water mixture (70% -30%) is added to 100g of vegetable powder previously weighed in a beaker. The whole is stirred for 24 hours and filtered on Wattman paper. The various filtrates obtained are evaporated at 40°C with a rotary evaporator. A solution of 100mg/ml of the extracts was prepared by dissolving 5g of dry extract in 50 ml of distilled water. This extract solution was filtered through 0.45µm millipore membrane. The sterilized extract was used for antimicrobial test.

Test organism

Four strains of *Salmonella* were tested; two clinical strains (*Salmonella typhi* and *Salmonella enterica*) isolated in the Bacteriology Laboratory of "Institut National d'Hygiène (INH)" of Lomé in October 2017, and two reference strains (*Salmonella typhimirium* ATCC 14028 and *Salmonella paratyphi* ATCC 9150). These strains were isolated and then stored at 2-8°C on the nutrient agar. The two clinical strains tested are multirésistante. *Salmonella enterica* is resistant to more molecules than *Salmonella typhi*. *Salmonella enterica* is resistant to ampicillin, ticarcilin, cefalotin, cefoxitin, amikacin, gentamicin, tobramicin and nalidixic acid. *Salmonella typhi* is resistant to cefalotin, cefoxitin, amikacin, gentamicin and tobramicin.

Bacteriological tests

The authentication and antibiogram of the clinical strains were carried out on the VITEK 2 bioMérieux device, with the identification and antibiogram cards of the bioMérieux brand. The technique used for antibacterial tests is the microdilution in liquid medium combined with plating on agar medium.[18,19] The strains of *Salmonella* are transplanted into the nutrient agar and incubated at 37°C for 24hours (overnight). The following day, a control Gram was performed on the 24hours colonies and a microbial suspension was prepared by triturating a colony in Müller Hinton broth so as to obtain turbidity comparable to 0.5Mac Farland. 100µl of the suspension obtained is spread on a nutrient agar in order to appreciate its microbial load.

The 0.5Mac Farland standard was prepared with a mixture of 0.05ml of a 1% barium chloride solution and 9.95ml of a 1% sulfuric acid solution. The mixture is homogenized and stored in the dark at room temperature.[20]

Sterile microplates are placed under a hood with laminar flow. The wells of the first column are left empty, and 100µl of Müller Hinton broth are dispensed into the other wells. Then, 200µl of the extracts of concentration 100mg/ml is introduced into the empty well of the first column and successive geometrical dilutions of reason 2 have been made successively using 100µl taken from the 200µl of undiluted extracts. 100µl of the microbial suspension is then added at each well.

Negative controls are carried out by putting 100µl of Muller Hinton broth only in one well and 100µl of extracts supplemented with 100µl of Mueller Hinton broth on the other hand. The MIC is determined by reading the plates. The MIC corresponds to the lowest concentration of the extract of the well which did not exhibit a culture visible to the naked eye. The determination of the MBC follows the colony count on the Petri dishes. The MBC corresponds to the concentration of the extract of the well which inhibits 99.99% of the starting inoculums.

Qualitative phytochemical screening

Qualitative phytochemical tests have identified alkaloids, flavonoids, tannins and saponosides according to standard procedures as described by Harbone.[21] The alkaloids give turbidity or precipitate with the Dragendorf reagent. Flavonoids are colored red or orange in the presence of the Shibata reagent. The tannins are colored in black or blue-black by iron perchloride. After vigorous agitation of 15min, the persistence of foam of 1cm for at least 5min reveals the presence of saponosides.

Quantitative phytochemical screening

The total polyphenols assay used the Folin Ciocalteu reagent (FCR) as described by Singleton et al.[22] The principle is based on the oxidizing properties of the OH function of the phenols and on the reducing properties of the FCR. Reduction of the FCR reduces its colorimetric properties which are then quantified by spectrophotometry. The standard used is gallic acid. The Butanol/HCl method was used to assay proanthocyanidins. It is a colorimetric reaction that takes place in two stages. In a first step, the polymers of flavan-3-ol are hydrolyzed in a butanol-HCl medium. The hydrolysis is complete and releases monomers of catechin and epicatechin type. The second step consists in oxidizing these monomers under the action of Fe^{III} to give the cyanidin whose red coloring is a function of the concentration of proanthocyanidines.[23] The standard used is Catechin. The method used for the *anti-radical* tests is the phosphomolybdates reduction. The principle is based on the reduction of Mo^{VI} molybdate to Mo^{V} molybdate by the antioxidant compounds with the formation of a Mo^{V} phosphomolybdate green complex which has a maximum absorption at 695nm.[24]

Statistical analysis

A pre-test was carried out before the first manipulation. The tests were repeated twice. In each case, averages and errors were calculated for the concentrations measured in the two sets of experiments. These averages were statistically compared using one-factor ANOVA at $P<0.05$.

Results and discussion

In other to study the anti-*Salmonella* activities of extracts from *Sarcocephalus latifolius*, *Lannea barteri*, *Uvaria chamae*, *Parkia biglobosa* and *Khaya senegalensis* a microdilution technique was performed.[18,19] Table 1 shows the MIC and the MBC of plants extracts on *Salmonella* strains. The anti-*Salmonella* investigation revealed MIC from 6.25 to 12.5mg/ml with *Sarcocephalus latifolius*, 12.5mg/ml with *Lannea barteri* and 25mg/ml with *Uvaria chamae*. The anti-*Salmonella* activities obtained depend on plants extracts studied. But in general, all the different strains of *Salmonella* tested had the same behavior with the same extract. The hydroethanolic extracts of the root bark of *Sarcocephalus latifolius* had a bactericidal effect on strains at MIC of 6.25mg/ml on *Salmonella typhi*, *Salmonella typhimirium*, *Salmonella paratyphi*, and 12.5mg/ml on *Salmonella enterica*. This was consistent with the works of Deeni et al.[6] and Aguora et al.[5] on the antibacterial properties of the roots of *Sarcocephalus latifolius*. They found respectively MIC of 5mg/ml with the hydromethanolic extracts and 10mg/ml with aqueous extracts on *Salmonella* strains.

Table 1 MIC and MBC of Plants Extracts

	S. latifolius		***L. barteri***		***U. chamae***		***P. biglobosa***		***K. senegalensis***	
	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml
S.Typhi	6.25	12.5	12.5	25	25	50	50	50	50	>50
S. enterica	12.5	12.5	12.5	12.5	25	50	50	50	50	>50
S. paratyphi ATCC 9150	6.25	12.5	12.5	25	25	50	50	50	50	>50
S. typhimirium ATCC 14028	6.25	12.5	12.5	25	25	50	50	>50	ND	ND

ND, not determined

Hydroethanolic extracts of the trunk bark of *Lannea barteri* had a bactericidal effect on the tested strains at a MIC of 12.5mg/ml. Njinga et al.[8] also reported the anti-*Salmonella* activity of the plant, but he tested the chloroform and ethyl acetate fractions of the leaves on *Salmonella typhi* ATCC 9184 and find respectively MIC of 2.5 and 5mg/ml. Antibacterial properties of extracts from the trunk bark of the same plant have been reported by several authors, but they did not test *Salmonella* strains. Donkor et al.[25] studied the bactericidal and bacteriostatic activity of the aqueous and ethanol extracts of the trunk bark of *Lannea barteri* on another three *Enterobacteriaceae*; they found MIC from 6.25mg/ml to 12.5mg/ml with the aqueous and the ethanolic extracts.

Uvaria chamae's MIC and MBC did not change from one strain to another (25mg/ml and 50mg/ml respectively). According to the value of the ratio MBC/MIC, the extract has a bactericidal effect on the strains tested.[26] Ogbulie et al.[11] also found bactericidal activity of ethanolic extracts from the roots of the same plant, but they used the disk diffusion technique on agar; and obtained an inhibition zone of 16 mm diameter with a concentration of 100µg/ml on *Salmonella typhi*. Antibacterial properties of root extracts of the same plant have been reported by Kone et al.,[10] but they tested a species of enterobacteria (*Shigella*) which has many characteristics in common with *Salmonella* and obtained MIC of 3.125mg/ml for the ethanol extract and 6.25mg/ml for the aqueous extract.

Results also showed a bactericidal effect of the hydroethanolic extracts of the trunk bark of *Parkia biglobosa* on the tested strains with a MIC of 50mg/ml. The bactericidal effect of the extracts of this plant has been previously proven by Millogo-Kone et al.[14] with a MIC lower than that obtained in this study. They found a MIC of 2.5mg/ml with the hydroalcoholic extracts of trunk bark on *Salmonella enterica* CIP 105 140. Bukar et al.[27] also obtained anti-*Salmonella* activities with extracts of the same plant; but this study focused on the leaves and pods. Hydroethanolic extracts of *Khaya senegalensis* trunk bark inhibited strains of *Salmonella typhi*, *Salmonella enterica* and *Salmonella paratyphi* at an initial concentration of 50mg/ml. The extract had no activity on *Salmonella typhimirium* at the initial concentration of 50mg/ml. There is no significant difference with the results obtained by Abdallah et al.,[17] who found a MIC of 50mg/ml for the aqueous extract and 25mg/ml for the ethanolic extract of the trunk bark of *Khaya Senegalensis* on a strain of the genus *Salmonella*. These values are lower than those obtained by Ugoh et al.[28] who also conducted antimicrobial tests of aqueous and ethanolic extracts of the trunk bark of *Khaya senegalensis* and found a MIC of 200mg/ml for both extracts on a strain of *Salmonella typhi*.

Table 2 Chemical components of plants extracts

	Alcaloïdes	**Flavonoïdes**	**Tanins**	**Saponosides**
K. senegalensis	+ +	+ +	**+ + +**	+
P. biglobosa	+ +	+ +	**+ + +**	+ +
L. barteri	+ +	+ +	**+ + +**	-
S. latifolius	+ + +	+	+	+ +
U. chamae	+ +	+	+ +	+ +

-, absence; +, Trace; ++, scanty; +++, Abundant

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The differences observed with the other authors could be explained by several factors, including the nature of the solvents of extractions and the antimicrobial tests used. In other to access to chemical components of this extracts, qualitative and quantitative evaluation were performed using standard phytochemical and phosphomolybdates reduction procedures.[21–24] Regarding the qualitative evaluation, results showed that all extracts studied contained alkaloids, flavonoids and tannins (Table 2). Saponosides has been revealed in extracts of the trunk bark of *Khaya senegalensis*, *Parkia biglobosa*, roots of *Sarcocephalus latifolius* and *Uvaria chamae*; but they were tested negative in the extracts of the trunk bark of *Lannea barteri*.

These results confirmed the report of several authors with extracts of *Khaya senegalensis*,[29] *Parkia biglobosa*,[30] *Lannea barteri*,[8] *Sarcocephalus latifolius*[5] and *Uvaria chamae*.[10] It also show that the tannins are more concentrated in the trunk bark extracts than the root extracts. The anti-*Salmonella* properties obtained with these extracts would probably be related to the isolated or conjugated action of one or some of these chemical compounds highlighted.

According to the quantitative evaluation, Table 3 shows the contents of total polyphenols, proanthocyanidins and antioxidants.

Table 3 Chemical contents of plants extracts

	Total Polyphenols (mg EGA/g)	Proanthocyanidines (mg EC/g)	Antioxydants (mg EAA/g)
K. senegalensis	18.53±0.03	62.85±0.67	9.12±0.17
P. biglobosa	33.24±0.14	57.14 ±0.08	9.30±0.15
L. barteri	18.03±0.14	67.14±0.23	8.28±0.23
S. latifolius	05.29±0.21	35.71±0.26	5.53±0.06
U. chamae	11.66±0.08	60.00±1.19	5.76±0.18

EGA, equivalent gallic acid; EC, equivalent catechin; EAA, equivalent ascorbic acid

The concentrations of total polyphenols are 18.53±0.03mg EGA/g for *Khaya senegalensis*, 33.24±0.14mg EAG/g for *Parkia biglobosa* and 18.03±0.14mg EAG/g for *Lannea barteri.* All extracts possess antioxidants. This shows that they displayed anti-radical potential. The stem bark of *Khaya senegalensis*, *Parkia biglobosa* and *Lannea barteri* displayed the high anti-radical properties with respectively 9.12±0.17mg EAA/g, 9.30±0.15mg EAA/g and 8.28±0.23mg EAA/g values of antioxidants. This confirms the anti-radical properties reported by many authors with the extracts of *Khaya senegalensis*,[16] *Parkia biglobosa*,[13] *Sarcocephalus Latifolius*[5] and *Uvaria chamae*.[10] These results also showed that total polyphenols and antioxidants concentrations are higher in trunk bark extracts than in root extracts. By comparing these results with those of qualitative phytochemical analysis, a hypothesis could be deduced that there would probably be a relationship between the levels of tannins, total polyphenols and antioxidants.

Conclusion

This study showed that the plants extracts tested contain alkaloids, flavonoids and tannins. The reduction of the phosphomolybdates by these various extracts proved that they are endowed with the anti-radical properties. The anti-*Salmonella* tests allowed classifying the extracts into three categories according to their activities. Extracts of the trunk bark of *Khaya senegalensis* and *Parkia biglobossa* had low activity on some *Salmonella* strains at MIC 50mg/ml. *Uvaria chamae* root extract gave an average activity on all strains at MIC 25mg/ml. The strongest activities are obtained with extracts of *Sarcocephalus latifolius* root bark and *Lannea barteri* trunk bark at MIC ranging from 6.25 to 12.5mg/ml. This study therefore supports the traditional use of these plants in Togo and different regions of the world, and may serve as a good source of new drugs against Salmonellosis and oxidative-related diseases.

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Conflict of interest

The authors declare there is no conflict of interest.

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