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In vitro studies of the antimicrobial properties and qualitative phytochemical analysis of selected Chadian medicinal plants

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Abstract The aim of this study was to evaluate the antimicrobial potential and perform the phytochemical screening of ethanolic (70%) leaf extracts of *Bauhinia rufescens* and *Ocimum basilicum* and root extracts of *Salvadora persica*, three plants used for the treatment of numerous diseases in Chad. The extraction of the plants was made in a ratio of 70% volume of ethanol against 30% volume of distilled water. The yields obtained in the extraction of the leaves of *Bauhinia rufescens*, *Ocimum basilicum*, and *Salvadora persica* roots were respectively 20, 17, and 8.8%. With the disc diffusion method, the results of the antimicrobial assessment revealed that *Bauhinia rufescens* leaf extracts were active against *Escherichia coli*, *Escherichia coli* ATCC 25922, *Salmonella typhi*, *Salmonella typhi* ATCC 35723, and *Shigella dysenteriae* with inhibition zones ranging between 20.500.2 and 100.14. *Ocimum basilicum* leaf extracts and *Salvarora persica* root extracts showed activities against *Escherichia coli*, *Escherichia coli* ATCC 25922, and *Salmonella typh*i with inhibition diameters ranging from 18.650.32 to 10.550.1 mm at 100 mg/mL. With the broth microdilution method, all the extracts of the three plants showed effectiveness against all the bacteria and fungi tested in this study with Minimum Inhibitory Concentrations ranging from 100 to 6.25 mg/mL and Minimum Bactericidal Concentrations also ranging from 100 to 6.25 mg/mL. A time-kill assay was also carried out in order to follow the activities of *Bauhinia rufescens*, the most active plant as function of time. After 45 min, the extracts completely inhibeted the growth of *Salmonella typhi* and after an h, bactericidal effects were observed against *Escherichia coli* and *Shigella dysenteriae.* The phytochemical screening on the hydro-ethanol extracts of three plants identified polyphenols, alkaloids, saponins, flavonoids and terpenes/steroids in all three plants. These chemical compounds may be endowed with antimicrobial properties.

Keywords Antimicrobial · *Bauhinia rufescens* · Chadian medicinal plants · Chemical compounds · *Ocimum basilicum* · *Salvadora persica*

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Introduction

Many plants have been used as medicines for thousands of years. Approximately 300,000 species of plant exist on the planet earth, 200,000 of which have been found to possess medicinal properties [1]. Medicinal plants are excellent reservoirs of therapeutic properties due to the existence of natural bioactive molecules called secondary metabolites [1]. These molecules are concentrated in different parts of the plant and in certain cases in specific organs of the plant. Many studies on medicinal plants have revealed the existence of secondary metabolites with biological properties such as polyphenols, alkaloids, terpenes etc. [2,3].

The development of bacterial resistance to antibiotics and the increasing toxicity of synthetic drugs have driven scientists to turn to the world of medicnal plants, in search of natural molecules that

are effective and free of any adverse effects. Besides, plant-based medicines have become more and more successful in some regions of the world in recent years, and have historically been an essential part of the health care system in others [4].

In this work, three medicinal plants from Chad were investigated for their potential antimicrobial properties: *Bauhinia rufescens*, *Ocimum basilicum*, and *Salvadora persica*.

Bauhinia rufescens, also called 'koulkoul' in Chadian Arabic, is a very branched bush belonging to the Fabaceae family. The plant has a pale grey bark, most often scaly. The persistent and biloba leaves are alternating. The inflorescences are in corymbe of approximately 5 cm long. The fruit is a spiral of dark brown color, containing 4 to 10 seeds. The root system of the plant is pivoting, with deep roots allowing access to the pad. This plant is a species of arid and semi-arid environments, especially the Sahel and Sahel-Sudan areas, found generally in the countries of West Africa, Central Africa and the Maghreb, more precisely in Niger, Burkina Faso, Algeria, Mauritania, Guinea, Sierra Leone, Mali, Côte d'Ivoire, Ghana, Benin, Nigeria, Cameroon, Chad, Sudan, and Ethiopia, on various dry soils generally sandy, lateral or clay [5]. In traditional medicine, the leaves in maceration have stomachic, anti-diarrheal, febrifuge, and anti-dysenteric properties. They are also used for the treatment of eye disorders and hypertension, in decoction. Also, it has been reported that the decoction of *Bauhinia rufescens* leaves is used in the treatment of diabetes, mycosis, and fibrosis [5]. For the treatment of leprosy, syphilis, and other venereal infections, the chopped, boiled roots are given as a drink [2]. The bark is a remedy for smallpox and some chest ailments. In Chad, the local population uses *Bauhinia rufescens* in the treatment of typhoid fever, malaria of diabetes and in the treatment of other common infections [5].

Ocimum basilicum or 'am-rihanna' in Chadian Arabic, is an annual aromatic plant, native to India and South Asia, where it has been grown for more than three thousand years. It is a herbaceous plant, woody, very branched, sometimes reaching 1 m in height. The opposite leaves are denticulated; the pectorals are oval or oboval, spotted at the base and acuminated at the top. The inflorescence, in terminal spiciform racem, is loose and formed by small groups of whitish flowers with two lips: the upper part is more developed and has four teeth at the top, while the lower part is short and rounded. There are numerous traditional medical uses for both the whole plant and its essential oil, especially in Africa and India. The leaves are used to treat a variety of conditions, including warts, worms, bronchitis, headaches, coughs, sunburns, diarrhea, and chronic dysentery when made into a decoction [5]. *Salvadora* persica, called locally 'Miswak' is a small tree or shrub that belongs to the Salvadoraceae family, with opposite leaves, with inflorescences in long, more or less branched clusters; tetrameter flowers, cupuliform, with yellowish-green short petals, with altering staminodes in the shape of short teeth; endocarpic, crustaceous, and single-seeded oval drupe. *Salvadora persica* is an indigenous Middle Eastern shrub. This little tree can be found throughout Africa, ranging from Senegal and Mauritania to Lake Chad, Nigeria, Mozambique, South Africa, India, Pakistan, Saudi Arabia, and Iran [5]. In Chad, the roots are used for dental cleanliness, while the infusion or decoction of the leaves is used to cure diarrhea and gastrointestinal diseases. Boils can be treated locally by applying a mixture of the powdered root and water. Syphilis was treated in Algeria, Egypt, and Libya with a mixture of powdered leaves, millet flour, and honey, which was eaten in small scoops every morning for 40 days. In Tanzania, oral candidiasis is treated locally three times a day using a paste made from ground bark powder and table oil [5].

These plants are commonly used for the treatment of a wide range of infectious diseases mainly caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Shigella dysenteriae Candida albicans*, particularly urinary tract infections, dysenteria, wound infections, enteric infections etc.

The qualitative phytochemical screening of the chemical contents of theses plants was also done.

Materials and Methods

Ethnobotanical survey

The ethnobotanical study was done from June to July 2021 in N'Djamena city in Center-West Chad. A semi-structured questionnaire was used to collect information such as plant name, pathology treated, components used, mode of administration, treatment outcomes, and source of knowledge. Survey respondents were mainly herbalists and were selected using purposive sampling method. They were selected in the city of N'Djamena on the basis of their reputation and ability to demonstrate a good knowledge of traditional herbal medicine.

Plant material and extracts preparation

Leaves of *Bauhinia rufescens* and *Ocimum basilicum* and roots of *Salvadora persica* were collected from some botanical gardens in N'Djamena in July 2021. The identification and authentication of plants were confirmed at the Department of plant biology of the University of N'Djamena. The plant parts were washed under running tap water, cut into small pieces and left to dry at room temperature (16 to 25 °C), then ground to fine powder using a mechanical blender. 1000 mL of 70% ethanol was added to 100 g of plant powder previously weighed in a beaker. The whole was put to agitation for 24 h, then filtered on Wattman paper N1. The different filtrates were evaporated at 40 °C using a rotary evaporator. The hydro-ethanolic extracts obtained were stored at 4 °C away from light. A 100 mg/mL solution of the extracts was prepared by dissolving 5 g of dry extract in 50 mL of distilled water. This extract solution was filtered through a 0.45 µm millipore membrane. The sterilized extract was used for the antimicrobial assay. 100 µL of extracts were spread on the nutrient agar to

assess the sterility of the extracts.

Microorganisms tested

A total of 7 different microbial strains were isolated from human stool and urine at le "Miroire laboratory, "l'Amitié" private laboratory, "la Renaissance laboratory and at the "Bacteriology Laboratory of the Reference University Hospital of N'Djamena for this study: *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Shigella dysenteriae*, and *Candida albicans*. The positive control strains, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 35659, *Salmonella typhi* ATCC 35723, *Candida albicans* ATCC 90029, *Shigella dysenteriae* ATCC 13311, and *Enterococcus faecalis* ATCC 19433 obtained from CECOQDA Laboratory, from le "Miroire Laboratory, "l'Amitié", "la Renaissance laboratory and from the "Bacteriology Laboratory of the Reference University Hospital of N'Djamena were also tested.

Biochemical, physiological, and morphological tests were carried out to characterize the isolated bacteria and fungi using the VITEK 2 bio-Mérieux device [6].

Ampicillin (10 µg), oxacillin (5 µg), amoxicillin + clavulanic acid (20 + 10 µg), doxycycline (30 µg), ciprofloxacin (5 µg), rifampicin (5 µg), azithromycin (15 µg), Clotrimazole, Amphotericin B, Nystatin, and metronidazole (4 µg) were used to carry out antimicrobial susceptibility testing of the clinical isolates and positive control strains to determine the antibiogram profile [6].

The antimicrobial activity of the different plant extracts was evaluated by disc diffusion method (Kirby-Bauer). The microdilution method was used to determine the Minimum Inhibitory Concentrations (MIC) and the Minimum Bactericidal Concentrations (MBC).

Inoculum preparation

For inoculum preparation, each microbial culture was streaked on nutrient agar to obtain well isolated colonies. After incubation at 37 °C for 24 h, a few colonies were transferred with a platinum loop into a test tube containing distilled water. Optical densities were adjusted using a spectrophotometer at a wavelength of 625 nm. The optical density was at 0.09 CFU/mL equivalent to 10^8 CFU/mL. The inoculum thus prepared was diluted to 1/100 in physiological water. The final optical density obtained from the inoculum was equivalent to McFarland standard 10^6 CFU/mL [6].

Disc diffusion method

Mueller Hinton agar for bacteria and Sabouraud dextrose agar for fungi were inoculated by flooding the prepared inoculum of test organisms. Sterile 6 mm diameter Whatman paper was impregnated with 50 µL of the extracts at concentrations of 100, 50, 25, and 12.5 mg/mL and dried in an oven at 30 °C for 30 min.

The discs were then aseptically placed on the already inoculated agar and incubated at 37 °C for 24 h for bacteria and 48 h for fungi. The diameters of zones of inhibition were measured using a ruler and compared to that of positive control (gentamicin). The tests were performed in triplicates and an average was made.

Determination of Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC) and Manimum Fungicidal of the Plant Extracts

Sterile 96-well microplates were placed in a laminar flow hood where the first column wells were left empty, and 100 µL of Müller Hinton broth was distributed to the remaining wells. Then, 200 µL of 100 mg/mL concentration extracts were introduced into the empty well of the first column, and 2-fold dillutions were performed successively using 100 µL taken from the 200 µL of extracts and 100 µL of the microbial suspension was then added to each well. Negative controls were made by adding 100 µL of Müller Hinton broth only to one well and 100 µL of extracts supplemented with 100 µL of Müller Hinton broth to the other. MIC was determined by reading the plates. The MIC was determined as the lowest concentration of the extract in the well that did not show visible culture to the naked eye. The determination of the MBC follows the counting of the colonies on the Petri dishes. The MBC was determined as the concentration of the well extract that inhibits 99.99% of the starting inoculum [6].

Time-kill assay

The three strains on which the plant extracts were active, namely *Escherichia coli*, *Shigella dysenteriae*, and *Salmonella typhi*, were tested by a single concentration of 2 MIC of the extracts of *Bauhinia rufescens*, the most active plant. 100 L of each microbial suspension of 10^8 CFU/mL were mixed with 100 L of extracts (100 mg/mL) at time t =0.10 L samples were taken, spread on Muller Hinton Agar at times t =0 min, t =15 min, t =30 min, t = 45 min t =1 h, and t =24 h. Plates were incubated at 37 °C and colonies were counted in 24 h. Negative control with microbial suspensions without extract were also made up.

Qualitative phytochemical screening

Phytochemistry screenings have been carried out on the extract using standard procedures to identify constituents. Qualitative analysis of the crude extract was carried out as described by Harbone (1999) to identify the presence of secondary metabolite classes (saponins, polyphenols, flavonoids, terpenes/steroids and alkaloids) [6].

Estimation of saponins

Saponins content of the plant extracts were estimated by dissolving 5 mg of extract in 10 mL of hot distilled water (50 °C). Following vigorous shaking, it was allowed to rest for five min. Saponins were detected in the form of foam [7].

Estimation of polyphenols

Five mg of each extract were dissolved in 1 mL of distilled water and then a few drops of ferric chloride 2 % was added. The green color indicated the presence of polyphenols [7].

Estimation of flavonoids

The Shibata's test was used to test flavonoids in the plant extracts. 5 mg of each extract were dissolved in 3 mL of methanol and then treated with a drop of concentrated hydrochloric acid and 0.5 g of magnesium chips. 3 min later, a red coloration indicated the presence of flavonoids [7].

Estimation of steroids and triterpenes

The Liebermann-Burchard test was used to screen steroids and terpenes. 5 mg of each extract were dissolved in 5 mL of distilled water, 5 mL of acetic anhydride and a few drops of concentrated sulfuric acid. 30 min later, steroids gave a red color with this reaction, whereas the appearance of a green color indicated the presence of triterpenes [7].

Estimation of alkaloids

The Dragendorff's test was used to characterize alkaloids in the plant extracts. 5 mg of the extract was dissolved in a test tube in 2 mL of 2% hydrochloric acid. After the addition of a few drops of Dragendorff reagent, the presence of alkaloids was manifested by the development of a cloud or precipitate in the tube [7].

Data analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using using Epi-info version 7.2 software.

Results

Ethnobotanical survey

The results collected from the traditional healers (n =100) in the markets of N'Djamena city indicating information of the plant species, the diseases they treat and the number of times they were mentioned by the people interviewed are presented in Table 1.

The results of ethnobotanical survey, showed that 12 plant species were identified. *Bauhinia rufescens* was the most cited plant (out of (n =100) respondents, 94 cited this plant, (94%)). This was followed by *Ocimum basilicum* (n =90 people (90%)), and *Salvadora persica* (n =86 people (86%)). *Psidium guajava* was the least cited plant (n =15 people (15%)).

After analysis of the data, three plants were chosen based on their frequency of occurrence in the mention by the respondents

Table 1 List of plants and their uses in the traditional pharmacopoeia in N'Djamena

Family	Species	Parts used	Application	Treated diseases	N Ind.
Fabaceae	*Bauhinia rufescens*	Leaves, pods	Oral use (decoction)	Gastro-enteritis, Urinary tract infection, Venereal infection, malaria, dysentery, inflammation	94
Limiaceae	*Ocimum basilicum*	Leaves, stem	Oral use (decoction)	Gastro-enteritis, Urinary tract infection, dysentery, inflammation, skin infection, malaria	90
Salvadoraceae	*Salvadora persica*	Leaves, roots	Oral use (decoction), Local use	Mouthwash, Toothache, Gastro-enteritis, Urinary tract infection, Burn, dysentery, inflammation	86
Renunculaceae	*Nigella sativa*	Seeds, leaves	Oral use (decoction), Local use	Asthma, ear infections, inflammation	70
Amaryllidaceae	*Allium salivum*	Whole plant	Oral use (decoction)	Respiratory tract infection, Dysentery, Tuberculosis, Chronic bronchitis, High blood pressure	70
Capparaceae	*Boscia senegalensis*	Leaves, fruits	Oral use (decoction)	Diabetes, Gastro-enteritis, Urinary tract infection	70
Fabaceae	*Vachellia nilotica*	Leaves, Pods, Bark	Oral use (decoction)	Gastro-enteritis, ear infection, skin infection, sore throat, malaria	58
Caricaceae	*Carica papaya*	Leaves, seeds	Oral use (decoction)	skin ulcers, gastrointestinal tract disorders, Malaria, intestinal parasite infections	47
Asteraceae	*Artmesia afra*	Whole plant	Oral use (decoction, Maceration)	Dysentery, heartburn, Chest pain, malaria	32
Fabaceae	*Trigonella foenum-graecum*	Seeds, Leaves	Oral use (decoction, Maceration)	Gastro-enteritis, Malaria, Venereal infection	28
Poaceae	*Cybopogon citrarus*	Whole plant	Oral use (decoction, Maceration)	Respiratory tract infection, Tuberculosis, Chronic, bronchitis	22
Myrtaceae	*Psidium guajava*	Leaves, Bark	Decoction	Gastro-enteritis, Urinary tract infection, Venereal infection, malaria, dysentery	15

N Ind = number of people who mentioned the plant

and diseases treated (digestive infections, gastroenteritis, urinary infection). The plants chosen are: *Bauhinia rufescens*, *Ocimum basilicum*, and *Salvadora persica*.

Determination of the extraction yield

The hydro-ethanolic extracts recovered after evaporation were weighed to determine the resulting dry weight. The yield was determined for 100 g of plant material. Let 'M' be the mass of powder used for extraction, m the mass of extract obtained after extraction. The extraction yield (%) was then estimated using the following formula:

$$\text{Yield } (\%) = (m/M) \times 100$$

m: mass of extract obtained, M: extraction test sample

The leaves of *Bauhinia rufescens* gave the highest yield (20%) followed by the leaves of *Ocimum basilicum* (17%). *Salvadora persica* root barks gave the lowest yield with 8.8%.

Antimicrobial effects of plant extracts (Disc diffusion method)

Three plant extracts were tested on six bacterial isolates (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Shigella dysenteriae*) and one yeast (*Candida albicans*). Each species was represented by two strains (a clinical isolate strain and a standard strain). After 24 h of incubation for bacteria and 48 h for yeast, the zones of inhibition diameter were measured in mm (Tables 2-4).

Ocimum basilicum leaves and *Salvarora persica* root barks showed activity against *Escherichia coli* and *Salmonella typhi* with zones of inhibition diameters ranging from 18.650.32 to 10.550.1 mm at 100 mg/mL concentration respectively. However, *Bauhinia rufescens* leaves were the most active on *Escherichia coli* (Fig. 1A) and *Salmonella typhi* (Fig. 1B) with diameters of 20.500.2 and 150.1 mm respectively. *Bauhinia rufescens* leaves were also the only ones active on *Shigella dysenteriae* with a diameter of 130.16 mm. On the other hand, all extracts showed no efficacy against the other four microorganisms tested (*Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Shigella dysenteriae*).

Approximately the same results were obtained with standard microorganisms as indicated in Tables 5, 6, and 7.

Antimicrobial effects of plant extracts (micro-dilution)

The results of antimicrobial tests performed using the micro-dilution plate method are reported in Tables 8-13. These results express the effectiveness of the extracts on the tested micro-organisms. The effect of the plant extract on a microbial strain is assessed by the MBC/MIC ratio. If the MBC/MIC ratio is equal to 1, the extract is considered bactericidal. On the other hand, if the MBC/MIC ratio is greater than 1, the extract is defined as bacteriostatic.

The extracts of *Bauhinia rufescens*, *Ocimum basilicum*, and

Table 2 Disc diffusion method for isolates strains with *Bauhinia rufescens* extracts

Microorganisms	Inhibition Zone (mm*)				Gentamycin
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	30 µg
S. aureus	06±00	06±00	06±00	06±00	19
E. coli	20.50±0.2	15±0.1	06±00	06±00	36
K. pneumoniae	06±00	06±00	06±00	06±00	30
S. typhi	10±0.14	06±00	06±00	06±00	23
C. albicans	06±00	06±00	06±00	06±00	23
E. faecalis	06±00	06±00	06±00	06±00	25
S. dysenteriae	13±0.16	06±00	06±00	06±00	22

*Disc diameter = 6.0 mm; 6 mm = no inhibition; 7 to 10 mm = weak activity; 11 to 14 mm = moderate activity and >15 mm = strong activity
Extract concentrations in 50 L of volume impregnated on the discs

Table 3 Disc diffusion method for isolates strains with *Ocimum basilicum* extracts

Microorganisms	Inhibition Zone (mm*)				Gentamycin
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	30 µg
S. aureus	06±00	06±00	06±00	06±00	19
E. coli	18.65±0.32	12.38±0.30	06±00	06±00	36
K. pneumoniae	06±00	06±00	06±00	06±00	30
S. typhi	06±00	06±00	06±00	06±00	23
C. albicans	06±00	06±00	06±00	06±00	23
E. faecalis	06±00	06±00	06±00	06±00	25
S. dysenteriae	06±00	06±00	06±00	06±00	22

*Disc diameter = 6.0 mm; 6 mm = no inhibition; 7 to 10 mm = weak activity; 11 to 14 mm = moderate activity and >15 mm = strong activity
Extract concentrations in 50 L of volume impregnated on the discs

Table 4 Disc diffusion method for isolates strains with *Salvadora persica* extracts

Microorganisms	Inhibition Zome (mm*)				Gentamycin
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	30 µg
S. aureus	06±00	06±00	06±00	06±00	19
E. coli	18.32±0.2	10.55±0.1	06±00	06±00	36
K. pneumoniae	06±00	06±00	06±00	06±00	30
S. typhi	06±00	06±00	06±00	06±00	23
C. albicans	06±00	06±00	06±00	06±00	23
E. faecalis	06±00	06±00	06±00	06±00	25
S. dysenteriae	06±00	06±00	06±00	06±00	22

*Disc diameter = 6.0 mm; 6 mm = no inhibition; 7 to 10 mm = weak activity; 11 to 14 mm = moderate activity and >15 mm = strong activity
Extract concentrations in 50 L of volume impregnated on the discs

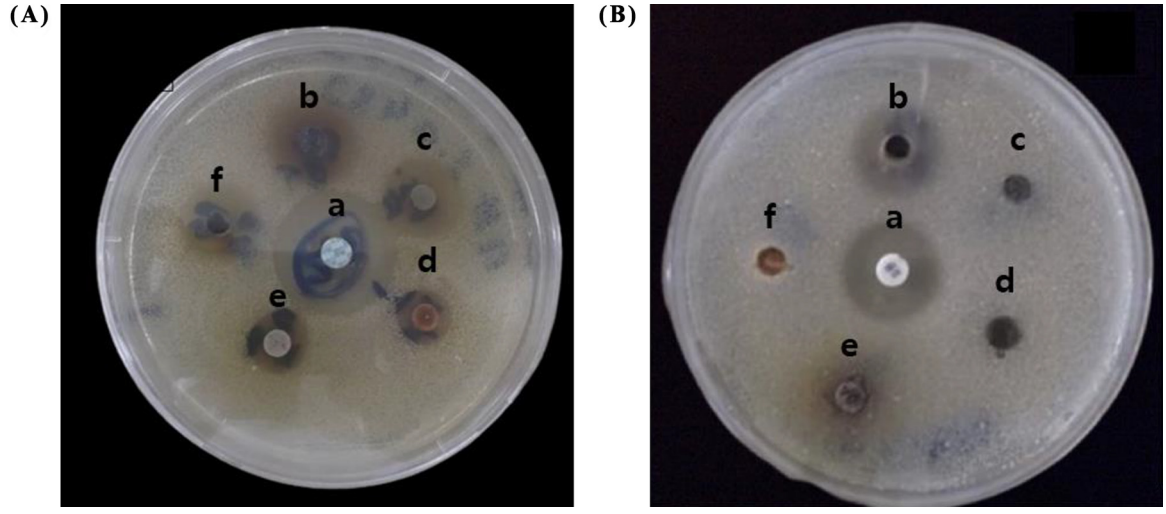

Fig. 1 Discs with *Bauhinia rufescens* extracts showing activities against *Escherichia coli* (A) and *Salmonella typhi* (B). a, Gentamycin 30 µg; b, extract 100 mg/mL; c, extract 50 mg/mL; d, extract 25 mg/mL; e, extract 12.5 mg/mL; f, negative control

Salvadora persica inhibited the *in vitro* growth of all the germs tested. The MICs observed vary from 12.5 to 50 mg/mL. However, they are very effective on both isolated and ATCC reference strains of *Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae* with a MIC of 6.25 mg/mL.

Time-kill assay

In order to follow the antimicrobial activity of *Bauhinia rufescens* extracts as a function of time, a Kinetic study was performed on *Escherichia coli*, *Shigella dysenteriae*, and *Salmonella typhi*. The results are presented in Table 14.

Phytochemical screening of plant extracts

The phytochemical study reveals that the hydro-ethanolic extracts of the leaves of *Bauhinia rufescens* and *Ocimum basilicum* as well as the roots of *Salvadora persica* contain all the major groups of chemical compounds of interest which are Alkaloids, Flavonoids, polyphenols, Saponins and Triterpenes/Steroids as shown in Table 15.

Discussion

The main objective of this study was to evaluate the antimicrobial potential of *Bauhinia rufescens*, *Ocimum basilicum*, and *Salvadora persica* on clinical strains of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Enterococcus faecalis*, *Klepsiella pneumoniae*, *S. dysenteriae*, and *Candida albicans* and their standard strains. The choice of these plants as study material is guided by their common use in traditional medicine in Africa to treat various diseases [8].

Hydro-ethanol extractions (alcohol 70%, water 30%) of different parts of these plants were performed and their antimicrobial properties were evaluated by the agar diffusion method (Kirby-Bauer technique) and their MICs and MBCs were determined by the well microdilution method. In order to ascertain whether the antimicrobial effect was bactericidal or bacteriostatic, the MBC/MIC ratio was performed. An MBC/MIC ratio greater than 1 was considered bacteriostatic and a MBC/MIC ratio equal to 1 was

Table 5 Disc diffusion method for standard strains with *Bauhinia rufescens* extracts

Microorganisms	Inhibition Zone (mm*)				Gentamycin
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	30 µg
S. aureus ATCC 29213	06±00	06±00	06±00	06±00	20
E. coli ATCC 25922	20.33±0.56	15±0.45	06±00	06±00	35
K. pneumoniae ATCC 13883	06±00	06±00	06±00	06±00	30
S. typhi ATCC 35723	11.50±0.31	06±00	06±00	06±00	20
C. albicans ATCC 90029	06±00	06±00	06±00	06±00	25
E. faecalis ATCC 29212	06±00	06±00	06±00	06±00	30
S. dysenteriae ATCC 13311	06±00	06±00	06±00	06±00	21

*Disc diameter = 6.0 mm; 6 mm = no inhibition; 7 to 10 mm = weak activity; 11 to 14 mm = moderate activity and >15 mm = strong activity
Extract concentrations in 50 L of volume impregnated on the discs

Table 6 Disc diffusion method for standard strains with *Ocimum basilicum* extracts

Microorganisms	Inhibition Zone (mm*)				Gentamycin
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	30 µg
S. aureus ATCC 29213	06±00	06±00	06±00	06±00	20
E. coli ATCC 25922	17.34±0.55	10.46±0.70	06±00	06±00	35
K. pneumoniae ATCC 13883	06±00	06±00	06±00	06±00	30
S. typhi ATCC 35723	06±00	06±00	06±00	06±00	20
C. albicans ATCC 90029	06±00	06±00	06±00	06±00	25
E. faecalis ATCC 29212	06±00	06±00	06±00	06±00	30
S. dysenteriae ATCC 13311	06±00	06±00	06±00	06±00	21

*Disc diameter = 6.0 mm; 6 mm = no inhibition; 7 to 10 mm = weak activity; 11 to 14 mm = moderate activity and >15 mm = strong activity
Extract concentrations in 50 L of volume impregnated on the discs

Table 7 Disc diffusion method for standard strains with *Salvadora persica* extracts

Microorganisms	Inhibition Zone (mm*)				Gentamycin
	100 mg/mL	50 mg/mL	25 mg/mL	12.5mg/mL	30 µg
S. aureus ATCC 29213	06±00	06±00	06±00	06±00	20
E. coli ATCC 25922	19.54±0.30	11.67±0.4	06±00	06±00	35
K. pneumoniae ATCC 13883	06±00	06±00	06±00	06±00	30
S. typhi ATCC 35723	06±00	06±00	06±00	06±00	20
C. albicans ATCC 90029	06±00	06±00	06±00	06±00	25
E. faecalis ATCC 29212	06±00	06±00	06±00	06±00	30
S. dysenteriae ATCC 13311	06±00	06±00	06±00	06±00	21

*Disc diameter = 6.0 mm; 6 mm = no inhibition; 7 to 10 mm = weak activity; 11 to 14 mm = moderate activity and >15 mm = strong activity
Extract concentrations in 50 L of volume impregnated on the discs

considered bactericidal. The phytochemical screening of the extracts of the three plants was also conducted.

The choice of the extraction solvent was based on the fact that 70% ethanol gives a better yield [9]. One of the advantages of this solvent mixture is that it consists of water and ethanol, which are all polar and allow the extraction of compounds that are also polar, such as phenols, which are mostly antibiotics [10]. The other advantage is that the extracts obtained are soluble in water, so their solution is easy to prepare. The purity of the microorganisms is verified at each step by systematically performing the Gram control.

The yields obtained in the hydroethanolic extraction of the leaves *of Bauhinia rufescens*, *Ocimum basilicum*, and *Salvadora persica* roots were respectively 20, 17, and 8.8%. These results allow us to deduce that the yields of crude extracts are variable according to the species and the plant parts used. These results are somehow similar to the results obtained by Mahamat et al. [11] with acetonic extracts of *Bauhinia rufescens* (22.67%), by Coelho et al. [12]. with methanolic extracts of *Ocimum basilicum* (17.809%) and by Aissaoui and Maamri [13] with extracts of *Salvadora persica* (7.8%).

Bauhinia rufescens extracts showed activities against *Escherichia*

Table 8 Antimicrobial effects of the hydroethanolic of *Bauhinia rufescens* extracts on Isolate strains (Microdilution Method)

Microorganisms	*Bauhinia rufescens* extracts		
	MIC (mg/mL)	MBC (mg/mL)	Effect
S. aureus	12.5	25	Bacteriostatic
E. coli	6.25	6.25	Bactericidal
K. pneumoniae	100	100	Bactericidal
S. typhi	6.25	6.25	Bactericidal
C. albicans	12.5	12.5	Fungicidal
E. faecalis	25	50	Bacteriostatic
S. dysenteriae	6.25	6.25	Bactericidal

Table 9 Antimicrobial effects of the hydroethanolic extracts of *Ocimum basilicum* on Isolate strains (Microdilution Method)

Microorganisms	*Ocimum basilicum* extracts		
	MIC (mg/mL)	MBC (mg/mL)	Effect
S. aureus	50	>50	Bacteriostatic
E. coli	12.5	12.5	Bactericidal
K. pneumoniae	100	100	Bactericidal
S. typhi	6.25	6.25	Bactericidal
C. albicans	12.5	50	Fungistatic
E. faecalis	100	100	Bactericidal
S. dysenteriae	6.25	6.25	Bactericidal

Table 10 Antimicrobial effects of the hydroethanolic extracts of *Ocimum basilicum* on Isolate strains (Microdilution Method)

Germs	*Salvadora persica* extracts		
	MIC (mg/mL)	MBC (mg/mL)	Effect
S. aureus	12.5	25	Bacteriostatic
E. coli	12.5	12.5	Bactericidal
K. pneumoniae	50	50	Bactericidal
S. typhi	12.5	12.5	Bactericidal
C. albicans	12.5	12.5	Fungicidal
E. faecalis	25	50	Bacteriostatic
S. dysenteriae	6.25	6.25	Bactericidal

coli, *Escherichia coli* ATCC 25922, *Salmonella typhi*, *Salmonella typhi* ATCC 35723, and *Shigella dysenteriae* with inhibition zones ranging between 20.500.2 and 100.14. It was the plant which showed the high activities against the strains tested, specially against *Escherichia coli* and *Salmonella typhi* (Fig. 1). These results are similar to the results obtained by Kwa et al. [14]. who studied the antibacterial activities of aqueous and ethanolic extracts of the stem bark and leaves of *Bauhinia rufescens* on a range of bacteria including *Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae*.

Ocimum basilicum leave extracts and *Salvarora persica* root bark extracts showed activities against *Escherichia coli*, *Escherichia coli* ATCC 25922, and *Salmonella typhi* with inhibition diameters ranging from 18.650.32 to 10.550.1 mm at 100 mg/mL concentration.

Table 11 Antimicrobial effects of hydroethanolic extracts of *Bauhinia rufescens* on standard strains (Microdilution Method)

Microorganisms	*Bauhinia rufescens* extracts		
	MIC (mg/mL)	MBC (mg/mL)	Effect
S. aureus ATCC 29213	12.5	25	Bacteriostatic
E. coli ATCC 25922	6.25	6.25	Bactericidal
K. pneumoniae ATCC 13883	100	100	Bactericidal
S. typhi ATCC 35723	6.25	6.25	Bactericidal
C. albicans ATCC 90029	12.5	12.5	Fungicidal
E. faecalis ATCC 29212	25	50	Bacteriostatic
S. dysenteriae ATCC 13311	6.25	6.25	Bactericidal

Table 12 Antimicrobial effects of hydroethanolic extracts of *Ocimum basilicum* on standard strains (Microdilution Method)

Germs	*Ocimum basilicum* extracts		
	MIC (mg/mL)	MBC (mg/mL)	Effect
S. aureus ATCC 29213	25	50	Bacteriostatic
E. coli ATCC 25922	12.5	12.5	Bactericidal
K. pneumoniae ATCC 13883	100	100	nd
S. typhi ATCC 35723	6.25	6.25	Bactericidal
C. albicans ATCC 90029	25	50	Fungistatic
E. faecalis ATCC 29212	100	100	Bactericidal
S. dysenteriae ATCC 13311	6.25	6.25	Bactericidal

Table 13 Antimicrobial effects of hydroethanolic extracts of *Salvadora persica* on standard strains (Microdilution Method)

Germs	*Salvadora persica* extracts		
	MIC (mg/mL)	MBC (mg/mL)	Effect
S. aureus ATCC 29213	25	25	Bacteriostatic
E. coli ATCC 25922	6.25	6.25	Bactericidal
K. pneumoniae ATCC 13883	100	100	Bactericidal
S. typhi ATCC 35723	6.25	6.25	Bactericidal
C. albicans ATCC 90029	12.5	50	Fungistatic
E. faecalis ATCC 29212	12.5	12.5	Bactericidal
S. dysenteriae ATCC 13311	6.25	6.25	Bactericidal

These results are consistent with those obtained by Hossain et al. [15] with extracts of *Ocimum basilicum* on a wide range of bacterial strains which include *Escherichia coli* and *Salmonella typhi* with zones of inhibition varying from 16.30.6 to 11.20.5 mm and with the results obtained by Swamy and Lasiti [16]. with ethanolic extracts of *Salvarora persica* against selected pathogens, including *Escherichia coli* and *Salmonella typhi* with zones of inhibition of 32.1660.167 and 27.5000.289 mm respectively. However, extracts of the three plants did not show any antimicrobial activities against *S. aureus*, *K. pneumoniae*, *C. albicans*, *E. faecalis*, *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *S. typhi* ATCC 35723, *C. albicans* ATCC 90029, *E.*

Table 14 Viability of bacteria as a function of time of exposure to hydroethanolic extracts of *Bauhinia rufescens* (Time-kill assay)

Bacteria	Time					
	0 min	15 min	30 min	45 min	1 h	24 h
E. coli	10^6	1700±128	881±108	57±1	-	-
S. typhi	10^6	1086±98	710±50	-	-	-
S. dysenteriae	10^6	800±56	510±38	200±10	-	-

-: No visible bacterial growth

Table 15 Results of qualitative chemical analysis of extracts

Plant/parameter	Alkaloids	Flavonoids	Polyphenols	Saponin	Terpenes/Steroids
B. rufescens	+++	+++	+++	++	++
O.basilicum	+++	++	+++	+++	+++
S. persica	+	+	+	+++	++

Note: + means slight presence of the compound in the extract, ++ means presence of the compound in the extract in an intermediate amount, +++ means abundant presence of the compound in the extract

faecalis ATCC 29212, and *S. dysenteriae* ATCC 13311.

The sensitivity of the microbial strains tested varied from one species to another towards the extracts of the three plants (different MICs). The most effective extracts were those with the lowest MICs.

The MIC and MBC values observed in this study vary from 100 to 6.25 mg/mL. The three plant extracts exerted a bactericidal activity on some isolates tested, and bacteriostatic on others.

Ocimum basilicum showed effectiveness with the well microdilution on *S. aureus*, *C. albicans*, *S. dysenteriae*, *E. coli*, *S. typhi*, *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *S. typhi* ATCC 35723, *C. albicans* ATCC 90029, *S. dysenteriae* ATCC 13311, *K. pneumoniae*, *K. pneumoniae* ATCC 13883, *E. faecalis*, and *E. faecalis* ATCC 29212. The MIC ranged from 100 to 6.25 mg/mL. Beatobic et al. [17] assessed the antimicrobial activities of different essential oils from *Ocimum basilicum* leaves against several microorganisms among which *S. aureus*, *S. typhimurium*, *E. coli*, and *E. faecalis*. The results showed inhibiting action on the bacteria tested with MICs ranged from 11.74 to 0.18 µg/mL for these bacteria.

The hydroethanolic extracts of *Bauhinia rufescens* were active on *S. aureus*, *C. albicans*, *S. dysenteriae*, *E. coli*, *S. typhi*, *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *S. typhi* ATCC 35723, *E. faecalis*, *C. albicans* ATCC 90029, *E. faecalis* ATCC 29212, *S. dysenteriae* ATCC 13311, *K. pneumoniae*, and *K. pneumoniae* ATCC 13883 with MIC ranged from 100 to 6.25 mg/mL.

Similar to the extracts of the other two plants, *Salvadora persica* extracts were also active against all the tested bacteria and fungi with well microdilution method with MIC ranging from 100 to 6.25 mg/mL. These results corroborate the results obtained by Aissaoui and Maamari [14] who studied the antimicrobial activities of *Salvadora persica* extracts. Thes authors obtained significant antimicrobial activity on *Candida albicans* and *Escherichia coli* among others. Rahmoun et al. [18] showed that extracts of the same plant inhibit the *in vitro* growth of several germs including *Staphylococcus aureus* ATCC 25923, *Candida albicans*, and *Escherichia coli* ATCC25922 with MICs ranging from 175 to 0.35 mg/mL.

Regarding the time-kill assay, the hydroethanolic extracts of *Bauhinia rufescens* were effective on all three bacteria tested at the concentration of 100 mg/mL using the well microdilution method. Thus, after 45 min of contact, the extract completely inhibited the in vitro growth of *Salmonella typhi*. The bactericidal effect was observed one h after the extracts were put in contact with the *E. coli* and *S. disenteriae* strains.

Comparing the disc diffusion method to the microdilution method used in this study, it can be seen that the microdilution method is the most efficient method. Indeed, with the disc diffusion method, the discs of paper blotter were manually impregnated and dried before their use; this could cause losses of the active ingredient due to the size of the discs; whereas for the well microdilution technic, a precise volume of 100 µL was deposited in the wells with less possible loss. In addition, with the disc method, migration of the active compounds from the paper to the agar might not be achieved properly.

Characterization tests using specific reagents on the hydro-ethanol extracts of three plants identified polyphenols, alkaloids, saponins, flavonoids and terpenes/steroids in all three plants. The presence of these major potentially pharmacologically active chemical groups in these plants has also been reported by Garbi et al. [19] and Boshra et al. [20] who worked on these same plant species.

The biological activities of the plants would be related to their secondary metabolite composition. Indeed, flavonoids, tannins, alkaloids and terpenes are endowed with antimicrobial and antioxidant properties [21].

In this study, the antimicrobial activities and phytochemical screening of *Bauhinia rufescens*, *Ocimum basilicum*, and *Salvadora persica* were investigated. The overall results of the present study

showed that using the well micro-dilution method, the hydro-alcoholic leaf extracts of *Bauhinia rufescens* and *Ocimum basilicum* and the root extracts of *Salvadora persica* possess notable antimicrobial activities, indicating that *Bauhinia rufescens*, *Ocimum basilicum*, and *Salvadora persica* should be considered as a useful source of antimicrobial agent. Furthermore, the results showed that *Bauhinia rufescens*, *Ocimum basilicum*, and *Salvadora persica* could be useful as a source of natural antimicrobial agents.

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