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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF MEDICINAL RECIPES FROM INDIGENOUS WOMEN HEALERS OF BURKINA FASO

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ABSTRACT

Infectious and parasitic diseases were responsible for 9.2% of worldwide deaths in 2019. Research is focused on herbal preparations or medicinal plants to develop new and more potent antimicrobial compounds. In the present manuscript, the antimicrobial effect of four indigenous recipes from traditional women healers of Burkina Faso was assayed against various pathogens, including bacteria, yeast, and molds. Moreover, the phytochemical analysis and acute toxicity study were carried out. The paper disc diffusion method was used to screen the antimicrobial effect of the herbal recipes. The search for alkaloids, saponins, coumarins, tannins, flavonoids, triterpenoids, and steroids was performed using adequate standard qualitative methods. The acute toxicity of each recipe was investigated in female NMRI mice with a single oral dose of 5000 mg/kg. Based on the germs tested, the recipes presented an antibacterial effect with inhibition diameters ranging from 0 to 20.33 ± 0.57 mm. Recipe BMDA obtained from *Parkia biglobosa* exhibited strong antibacterial activity against various gram-positive and gram-negative bacteria. However, none of the recipes was potent on fungi germs. The recipes presented differences in phytoconstituents composition. Saponins and triterpenoids were present in all recipes, whereas coumarins were only seen in recipe BMDA. All the recipes showed no acute toxicity at the limit dose of 5000 mg/kg of body weight. Taken together, the results provide scientific validation regarding the traditional anti-infectious use of the recipes. Further investigations of antimicrobial action and identification of the main phytochemical compounds may be necessitated.

KEYWORDS: Indigenous recipes, infectious diseases, antimicrobial effect, phytochemical, acute toxicity.

INTRODUCTION

Worldwide, in 2019, 26.35% of deaths were attributable to communicable, maternal, neonatal, and nutritional diseases.[1] Contagious or infectious diseases are illnesses caused by various microorganisms that can spread directly or indirectly from one person to another through contact with air, contaminated surfaces, bodily fluids, blood products, or insect bites.[2] The Global Health Estimates (GHE) estimated deaths caused by infectious and parasitic diseases to be 9.2% of total deaths worldwide.[3] Most of these deaths occurred in developing countries, especially in sub-Saharan Africa.[1, 3, 4] In Burkina Faso, most infectious disease deaths are mainly caused by lower respiratory infections, malaria, and diarrhea.[3]

The use of antibiotics to prevent and control infectious diseases reduced the burden of most of these ailments. The antimicrobial compounds act diversely against microorganisms, especially bacteria and fungi. They depolarize the cell membrane and inhibit different metabolic pathways.[5] Antibacterial agents inhibit (i) cell wall synthesis, (ii) protein biosynthesis, (iii) folic acid metabolism, and (iv) prevent the replication of nucleic acid.[5,6] Most anti-fungi compound targets are the synthesis of ergosterol, an essential lipid of the yeast cell membrane, and chitin and β-glucan, two fundamental structural elements of the fungi cell wall.[7] Although these antimicrobial compounds have interesting beneficial therapeutic effects, their misuse or overuse has led to resistance and reduced efficacy.[6–9]

Antimicrobial resistance is a significant health problem worldwide. The Global Burden of bacterial antimicrobial resistance estimates that antimicrobial resistance kills more people than HIV and malaria.[10] Most of these deaths occur in sub-Saharan Africa, and the leading pathogens responsible for resistance are *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*,

and *Pseudomonas aeruginosa*.[10] The mechanisms by which microorganisms manifest resistance involve modification of a target molecule, changes in the outer membrane permeability, activation of efflux pumps, and inactivation of antibiotic molecules.[6] Strategies have been developed to combat or prevent antimicrobial resistance. Some involve reducing antibiotic prescriptions, using two or more antimicrobial compounds, and preventing infections through vaccination.[5, 8, 10]

Moreover, exploring herbal preparations or medicinal plants has been suggested to develop new, more effective antimicrobial compounds to face the resistance phenomenon.[11, 12] In most sub-Saharan African areas, people still rely on traditional medicine, mainly herbal medicine, for their primary health care.[13, 14] This effective use of traditional medicine is related to its affordability, availability, and accessibility.[15] Herbal medication, preparations containing parts or the plant's whole, or a selective isolated phytochemical are extensively used against infectious diseases.[15–17]

Prosopis africana (Fabaceae), *Ximenia americana* (Ximeniaceae), *Parkia biglobosa* (Mimosaceae), *Terminalia avicennioides* (Combretaceae), and *Acacia macrostachya* (Fabaceae) are parts of indigenous recipes used commonly against various ailments including infectious diseases in Burkina Faso traditional medicine. The present manuscript aimed to report preliminary antimicrobial activity, phytochemical analysis, and acute toxicity assessment of four (4) traditional recipes made of these plants.

MATERIAL AND METHODS

Collection of traditional recipes

The recipes were obtained from the indigenous women healers of Sanmatenga province (Burkina Faso). Before collection, the traditional women healers, all members of the Association of Sanmatenga Traditional Healers, were informed and gave their oral consent. They were surveyed using a semi-structured questionary about the remedies' indication, preparation, and administration. Sixty (60) women were interviewed, among which twenty-four were curing infectious diseases.[18] Additional information about the composition of the recipes was gathered from four (4) traditional women healers (Table 1).

Table1: **Selected traditional recipes used to treat infectious diseases in the Sanmatenga province.**

Codes*	Locality	Composition of recipe	Indication	Mode of preparation
BGSH	Barsalogho	Roots of *Prosopis africana* (Guill. & Perr.) Taub. (**Fabaceae**)	Diarrhea with important stools	Decoction
BMSP	Boussouma	Bark of *Ximenia americana* L. (**Ximeniaceae**)	Diarrhea, mycosis, fever	Decoction
BMDA	Boussouma	Bark of *Parkia biglobosa* (Jacq.) R.Br. ex G.Don (**Mimosaceae)**	Diarrhea, fever	Decoction
MSGA	Mane	Bark of *Terminalia avicennioides* Guill. & Perr. (**Combretaceae) +** *Ximenia americana* L. (**Ximeniaceae) +** *Acacia macrostachya* Rchb. ex DC. (**Fabaceae)**	Mycosis, diarrhea, fever	Decoction

* Codes, based mainly on the number of traditional healers and the name of the located area, assigned to recipes

Preparation of the recipes

The women prepared the recipes extemporarily following their protocols (Table 1). Then, recipes were sent to Ouagadougou, the capital city of Burkina Faso, at the Research Institute of Health Sciences (IRSS). There, the samples were immediately stored in the freezer for further use. 500 mL of liquid recipes were dried in vacuo using a rotatory evaporator.

Indicator microorganisms and growth conditions

The antimicrobial assays were performed using fourteen (14) indicator strains, including eleven (11) bacteria and three (3) fungi (Table 2). Bacteria strains were stored at -80 °C in Brain Heart Infusion (BHI) broth (Oxoid CM1135, Basingstoke, United Kingdom), except for *Micrococcus luteus* stocked in nutrient broth (Oxoid, CM0001, Basingstoke, United Kingdom) supplemented with 20% (v/v) glycerol. The yeast was maintained at -80 °C in yeast glucose peptone (YGP) broth [made of 1% (w/v) bactopeptone (211677, Becton, Dickinson, NJ, USA), 1% (w/v) glucose (Merck 38291142, Germany), 0.5% (w/v) yeast extract (Oxoid LP0021), pH 5.6 ± 2]. The molds were stocked in malt extract broth (Oxoid CM0057, Basingstoke, United Kingdom). Before use, bacteria were grown in Mueller-Hinton agar (Himedia, M173, India), yeast in Sabouraud Chloramphenicol agar (Biolab, SCC20500, Budapest, Hungary), and molds in potato dextrose agar (Biolife, MF0102, Milan, Italy).

Table 2: Microorganism strains used for antimicrobial activity testing and their growth conditions.

Microorganisms	Codes	Media/Temperature (°C)
Gram-positive bacteria		
Listeria monocytogenes NCTC 9863	*L. m* 98	MH/37
Enterococcus faecalis ATCC 19433	*E. faecalis*	MH/37
Bacillus cereus LMG13569	*B.c* LMG	MH/37
Streptococcus oralis	*S. oralis*	MH/37
Micrococcus luteus AT49732	*M. lut* 49	MH/30
Staphylococcus aureus toxin A+B	*S. aureus*	MH/37
Gram-negative bacteria		
Salmonella Typhimurium O:1036340P/t49	*S. typhi* O: 10	MH/37
Pseudomonas aeruginosa ATCC	*P. aeru*	MH/37
Yersinia enterocolitica BT3ST5,27	*Y. e* BT3	MH/37
Escherichia coli 81 nr.149 SKN 541	*E. coli* 12	MH/37
Shigella dysenteriae 370	*S. dys*	MH/30
Yeast and Molds		
Candida albicans	*C. alb*	Sab /25
Aspergillus fumigatus	*A. fum*	PDA/25
Aspergillus flavus	*A. fla*	PDA/25

MH: Mueller-Hinton agar; Sab: Sabouraud chloramphenicol agar; PDA: Potato Dextrose agar.

Preparation of the microorganism's inocula and propagation

The indicator organisms were cultured in BHI broth overnight at 37°C for bacteria or in YGP broth at 25°C for 48 H for *C. albicans*. The molds were cultivated in YGP during 2-5 days at 25°C. Then, 100 µL of suspension containing approximately 10^7 colony-forming units per ml (CFU/mL) of bacteria and 10^7spores/mL of molds were mixed in a Petri dish with 10 mL of Mueller-Hinton agar and 10 mL of potato dextrose agar, respectively. The culture of *C. albicans* (about 10^7 CFU/mL) was mixed with 10 mL of Sabouraud agar in a Petri dish.

The Petri dishes were then allowed to solidify.

Preparation of the extract and antimicrobial sensitivity test

The dried recipe (approximately 1.5 g) was dissolved in sterilized water (10 mL) to give a final concentration of 150 mg/mL. The solution was filtered using 0.45 µm pore size membrane filters.

The antimicrobial test was performed by disc diffusion.[19, 20] Blank discs of 5 mm in diameter were soaked in each recipe extract (15 µL per disc) for 15 minutes and left to dry in the oven at 45°C for 10 minutes. Then, discs (three discs per plate) were placed onto inoculated Petri dishes plates and incubated according to the growth conditions of each test organism (Table 2), 24 H for bacteria, 48 H for yeast, and 2-5 days for molds. Clear zones around the discs indicated an antimicrobial effect. The experiment was performed in triplicate. Based on the diameter of the inhibition zones, a substance is considered not sensitive for diameters less than 8 mm, sensitive for diameters between 9 and 14 mm, and very sensitive for diameters 15-19 mm. Above 20 mm diameter, the substance is defined as extremely sensitive.[21]

Phytochemical analysis of the recipes

2 g of each dried recipe was dissolved in 15 mL of distilled water. The extract was successively exhausted with hexane (2 × 15 mL), dichloromethane (2 × 15 mL), ethyl acetate (2 × 15 mL), and butanol (2 × 15 mL). The solvents were of technical grade from Merck (Darmstadt, Germany). The search for alkaloids, saponins, coumarins, tannins, flavonoids, triterpenoids, and steroids was performed using adequate standard qualitative methods.[22] Polar compounds including tannins, flavonoids, and alkaloids were detected in ethyl acetate and butanol extracts. Coumarins, steroids, saponins, and triterpenoids were researched in hexane and dichloromethane extracts.

Animals and acute toxicity

The acute toxicity was carried out on NMRI female strains mice (20-30 g). The animals were maintained under standard laboratory conditions (temperature of 22 ± 3°C, 12/12 h light/dark cycle, and relative humidity of 50–70%) with *ad libitum* access to water and food fortified with 29% protein. However, food was drawn back 4 hours before the experiment, but the animals still had free access to water.

Experimental protocols were strictly performed following the eighth edition of the "Guide for the Care and Use of Laboratory Animals".[23]

The OECD Guidelines 423 were used to assess the acute toxicity of the recipes.[24] Initially, two groups of 3 mice each were formed. The first group served as control and received distilled water orally, and the second was administered the recipe at the limited dose of 5000 mg/kg using a feeding tube. Two hours following the administration of the recipes and regularly during the first 24 hours, mice were observed to detect any eventual clinical signs of toxicity (change in general behavior, reflexes, movements, and mortality). Then, mice were

followed twice daily for 13 days. After the first two hours, mice had free access to food and water. The test was carried out in duplicate.

Data presentation and analysis

The antimicrobial assay was realized in triplicate. The diameters of clear zones (or inhibition zones) were measured in millimeters and presented as mean ± S.D.

The presence or absence of a specific phytochemical compound was assessed par positive (+) or negative (-) signs.

RESULTS

Antimicrobial effect of the four recipes

Table 3 presents the *in vitro* evaluation of the antimicrobial effect of the recipes obtained from traditional women healers.

Table 3: Antimicrobial effect of the four recipes against various microorganisms.

Recipes / Organism Tested	Diameter of growth inhibition zones*			
	MSGA	**BMDA**	**BGSH**	**BMSP**
Gram-positive Bacteria				
L. m 98	-	17.66 ± 0.57	-	-
E. faecalis	9 ± 0	19.66 ± 0.57	-	-
B. c LMG	11.66 ± 0.57	13.66 ± 0.57	-	11 ± 0
S. oralis	11.66 ± 0.57	10.66 ± 0.57	7.33 ± 0.57	11.33 ± 0.57
M. luteus 49	-	10 ± 0	-	-
S. aureus	9 ± 0	9.66 ± 0.57	-	-
Gram-negative Bacteria				
S. typhi O:10	20.33 ± 0.57	10 ± 0	11.66 ± 0.57	-
P. aeru	-	12 ± 0	19.66 ± 0.57	-
Y. e BT3	-	11.33 ± 0.57	11.33 ± 1.15	-
E. coli 12	-	16.66 ± 0.57	8.66 ± 0.57	-
S. dys	-	12.33 ± 0.57	-	-
Yeast and Molds				
C. albicans	-	-	-	-
A. fum	-	-	-	-
A. fla	-	-	-	-

(*****) Values are presented as means ± S.D. of three independent experiments; (-) no inhibition. For microorganism's names, see Table 2

Depending on the recipe and test organism, the inhibition zone diameter varied from 0 (no inhibition) to 20.33 ± 0.57 mm. BGSH was more effective on Gram-negative bacteria rather than on Gram-positive. Indeed, BGSH exhibited a significant inhibitory effect on *P. aeruginosa* with a diameter zone equal to 19.66 ± 0.57 mm. The recipe BMDA demonstrated an interesting antibacterial effect on Gram-positive and Gram-negative bacteria. As presented in Table 3, this recipe was potent against all strains of bacteria, with inhibition diameters varying from 9 mm (*Staphylococcus aureus*) to 19.66 mm (*Enterococcus faecalis*).

In contrast, the recipe BMPS was only active toward *Bacillus cereus* and *Streptococcus oralis* (11± 0 and 11.33 ± 0.57 mm, respectively). Like recipe BMPS, MSGA was more effective on Gram-positive than Gram-negative bacteria. However, with an inhibition halo of 20.33 ± 0.57 mm, recipe MSGA showed the highest inhibitory activity on the Gram-negative bacteria *Salmonella Tiphimurium*. Moreover, it can be noticed that none of the recipes tested exhibited any antifungal effect against the three fungi.

Phytochemical analysis

The traditional recipes present differences in their phytochemical composition. As presented in Table 4, the phytochemicals are all detected in the recipe BMSP except for coumarins. These compounds were only seen in the recipe BMDA. Moreover, saponins and triterpenoids are both phytoconstituents present in all recipes.

Table 4: Results of the preliminary phytochemical analysis of the four recipes.

	Recipes			
Phytoconstituent	**BGSH**	**BMSP**	**BMDA**	**MSGA**
Tannins	**ND**	**+**	**+**	**+**
Saponins	**+**	**+**	**+**	**+**
Steroids	**ND**	**+**	**ND**	**+**
Alkaloids	**ND**	**+**	**+**	**ND**
Flavonoids	**ND**	**+**	**ND**	**+**

Triterpenoids	+	+	+	+
Coumarins	**ND**	**ND**	+	**ND**

ND: not detected in our experimental conditions.

Acute toxicity study

The evaluation of the acute toxicity revealed that no mortality was observed in the mice at the limit dose of 5000 mg/kg body weight. Apart from slight drowsiness during the first 30 min after administering the recipes, no other significant changes in the general behavior were recorded in treated and control mice.

DISCUSSION

The several drawbacks of the extensive and inappropriate use of antibiotics led researchers to develop new antimicrobial agents. Research is being increasingly focused on plant-based medicines and other substances of natural origin.[20] Traditional medicine and mainly herbal medicine is a crucial system of care on which at least 70% of African people rely. It has been reported that more than fifty percent (50%) of the member states of WHO had a national policy on traditional and complementary medicine.[25] The results of a study implemented with the traditional healers of Sanmatenga province reported that thirty-four (34) traditional healers were granted a license to practice traditional medicine by 2019.[18] Among them, there were twenty-two (22) women involved in the treatment of infectious diseases. It is well known that in various rural areas, women healers are more likely to cure childhood illnesses including malaria, diarrhea, and some respiratory conditions. Based on an extended and current use against infectious diseases in the province of Sanmatenga, four recipes were selected to assess their antimicrobial, phytochemical, and toxicological properties. The recipes were prepared from local medicinal plants, including *Prosopis africana*, *Ximenia americana*, *Parkia biglobosa*, *Terminalia avicennioides*, and *Acacia macrostachya*. These recipes prepared extemporally and strictly by the traditional healers themselves for the treatment of patients might be considered traditional medicines of category one according to WHO guidelines set for the registration of traditional medicines.[26]

As presented in Table 1, extraction by decoction was the most used, thus confirming that decoction is the primary mode of preparation of herbal medicines.[27, 28]

The infectious diseases treated by the recipes include diarrhea and mycosis. Diarrheal conditions are significant causes of morbidity and mortality among children under five in low and less-developed countries.[29, 30] The microorganisms responsible for these diseases involve bacterial and fungal strains. Thus, bacterial strains involve *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholera*, *Bacillus cereus*, *Clostridium perfringens*, *Lactobacillus casei*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Fungal pathogens responsible for diarrheal diseases are *Trichoderma* spp., *Candida* spp., *Aspergillus* spp., and *Fusarium* spp.[31] The results indicated that all recipes inhibited most bacteria strains, including *Escherichia coli*, *Salmonella tiphi*, *Shigella dysenteriae*, *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Furthermore, the recipe BGSH strongly inhibited *Pseudomonas aeruginosa*, one of the six leading pathogens responsible for antibiotic resistance. Consequently, the traditional use of the recipes against diarrhoeal disease is confirmed. Nevertheless, none of the recipes exerted any antifungal effect.

Numerous techniques have been proposed to assess antimicrobial testing susceptibility. The most routinely used method remains agar disc-diffusion because of its simplicity, low cost, ability to test several microorganisms and antimicrobial agents, and ease of interpreting results.[20] For these reasons, agar disc diffusion was used for the preliminary antimicrobial evaluation of the four recipes. Overall, the results demonstrated a potent antibacterial effect of the recipes on the different pathogens tested.

The recipes are made from medicinal plants with previously reported antimicrobial activity. Indeed, different works have reported the antimicrobial activity of *Prosopis africana* (Fabaceae), *Ximenia americana* (Ximeniaceae), *Parkia biglobosa* (Mimosaceae), *Terminalia avicennioides* (Combretaceae), and *Acacia macrostachya* (Fabaceae).[32–36]

Altogether, the results provide scientific validation regarding the traditional anti-infectious use of the recipes.

The four recipes develop significant variations in their phytochemical composition. It has been reported that the antimicrobial activity of plant-derived recipes varies depending on the phytochemical composition.[37] The main phytoconstituents responsible for the antimicrobial effect of plant-derived materials involve polyphenols such as phenolics and flavonoids, alkaloids, coumarins, terpenes, and sulfur-containing compounds.[11, 37, 38] Most of these secondary metabolites are efflux pump inhibitors (most phenolic compounds), induce cell membrane disturbance (terpenes, alkaloids, coumarins), and inhibit metabolic pathways.[38] Based on the results, it can be assumpted that the antimicrobial effect did not depend on the content of secondary compounds. The interesting antimicrobial effect of the recipe BMDA could be attributable to the researched bioactive compounds such as tannins, saponins, alkaloids, triterpenoids, and coumarins. Although six secondary metabolites were present in the recipe BMSP made from the bark of *Ximenia americana*, a weak antibacterial effect was observed. In contrast, recipe BGSH, which contains only

saponins and triterpenoids, possesses a potent antibacterial activity mostly on gram-negative bacteria.

The acute toxicity evaluation gives no mortality and no significant changes in the general behavior of animals at the limit dose of 5000 mg/kg body weight. Therefore, the lethal dose of the recipes could be considered over 5000 mg/kg according to the OECD guidelines. Consequently, these recipes may be classified as substances of relatively low acute toxicity following the Globally Harmonized System of classification and labeling of chemicals.[39]

CONCLUSIONS

Since humankind, traditional medicine has treated several illnesses, including infectious diseases. Although effective among people, the scientific justification of the traditional use of medicinal plants or plant-derived recipes remains to be done. The present study results provide a scientific response to the safety and efficacy of four indigenous recipes from the traditional women healers of Sanmatenga. The recipes were mainly effective against bacterial strains. Based on the results, the recipe BMDA made of *Parkia biglobosa* was more potent than the three other recipes. The phytoconstituents present in most recipes may explain the antibacterial activity of the recipes, even though other mechanisms of antimicrobial action can intervene. Further studies are needed, including techniques of antimicrobial effect and identification of the main phytochemical compounds.

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