

Phytochemical Investigation and Antimicrobial Activity of Six Plants Used in Children's Ailments Treatment in Niger

Mahamane Idi Issa Abdoulahi^{1,*}, Bakasso Sahabi¹, Alio Sanda Abdelkader²,
Ilagouma Amadou Tidjani¹, Yaou Chaïbou⁴, Moutar Chaibou¹, Adamou Ibrahim Maman Laouali²,
Kiendrebeogo Martin³, Sadou Hassimi⁴

¹Department of Chemistry, Faculty of Science and Technology, Abdou Moumouni University, Niamey, Niger

²Department of Biology, Faculty of Science and Technology, Abdou Moumouni University, Niamey, Niger

³Life and Earth Sciences Training And Research Unit, Faculty of Science and Technology, University of Ouagadougou, Ouagadougou, Burkina Faso

⁴Department of Chemistry, Faculty of Science and Technology, Abdou Moumouni University, Niamey, Niger

Email address:

m_abdoulahi@yahoo.com (M. I. I. Abdoulahi), b_sahabi2000@yahoo.fr (B. Sahabi), aliosanda@yahoo.fr (A. S. Abdelkader),
ilagoumat@mail.com (I. A. Tidjani), martinkindrebeogo@yahoo.co.uk (K. Martin), hassimi@yahoo.com (S. Hassimi)

*Corresponding author

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Abstract: Resistance phenomena of infectious agents to synthesis drugs and the high cost of these drugs led to medicinal plants as an alternative against infectious disease. The objective of this study is to characterize different classes of secondary metabolites and to test the antibacterial activity of six plants: *Bauhinia rufescens* Lam, *Blepharis linariifolia* PERS, *Chrozophora brocchiana* Vis, *Gardenia ternifolia* Schum and Thonn, *Indigofera astragalina* DC, *Phyllanthus pentandrus* Schumacher & Thonn, used in traditional medicine for children treatment. The extracts are obtained by aqueous decoction with the 1/10 model (50 g of each sample in 500 ml of solvent). Phytochemical screening of these six plants was carried out by standards tests, which are reactions of colorations, precipitation and complexation. The agar medium diffusion was used for the sensitivity test of the aqueous extracts of these plants against two strains of bacteria (two isolates of *Salmonella typhimurium* lettuce and clinical). Phytochemical screening showed the presence of polyphenols, tannins, flavonoids, coumarins, alkaloids, sterols/triterpenes in all tested extracts. All extracts contain saponins except *Phyllanthus pentandrus*. For free quinones, only *Phyllanthus pentandrus* and *Indigofera astragalina* showed a negative test. Among these six plants, five (5) showed an activity on *Salmonella typhimurium* at 200 mg/mL. *C. brocchiana* and *P. pentandrus* showed the best activity on *S. typhimurium* (lettuce) with 19.5 ± 0.70 mm and 17.5 ± 0.70 mm, respectively. At the concentration of 50 mg/mL, only these two plants are also showed antibacterial activity on *S. typhimurium* (lettuce). The results obtained indicate that these plants contain some secondary metabolites, some have antimicrobial activity and constitute a scientific database for the research in the field of phytomedicines. These results will justify the use of these plants in the treatment of some infections.

Keywords: Phytochemistry, *Salmonella typhimurium*, Infectious Agents, Niger

1. Introduction

Infectious diseases are a public health problem because of their frequency and severity. *Salmonella* is one of infectious agents, present in food and our environment. The distribution

analysis of annual number of *Salmonella* gastroenteritis cases estimates that 93.757.000 cases of which causes 155,000 deaths [1]. In Niger, isolated *Salmonella* prevalence from foods is very high. The prevalence of *Salmonella* found in lettuce in Niger is 36.94% and 38.06% from poultry [2, 3].

The discovery of antibiotics was a real relief to humanity because this class of drugs has helped in reducing the damage caused by infectious diseases, especially in developed countries. But the misuse of these drugs has led to the development of resistance in infectious agents. Moreover, a multi-resistance to the antibiotic of isolated *Salmonella* strains in human and in food have been proved [4]. Conventional medicines generally have undesirable effects and are expensive.

Considering the constantly high cost of available drugs, associated with the emergence of multi-resistant pathogens, the African Pharmacopoeia has been recognized [5, 6]. In Africa, up to 80% of the population uses traditional medicine to manage their health [7]. Plants are generally presented as having efficacy; they are low cost and available.

The aim of the present study is to characterize the different classes of secondary metabolites extract from the six plants which are commonly used in Niger in the management of child care and to evaluate the antibacterial activity on two strains of *Salmonella*.

2. Material and Methods

2.1. Plant Material

The study samples are made up of: *Bauhinia rufescens* Lam (leaves), *Indigofera astragalina* DC (whole plant), *Phyllanthus pentandrus* Schumacher & Thonn (whole plant), *Chrozophora brocchiana* Vis (whole plant), *Blepharis linariifolia* PERS (whole plant without seeds) and *Gardenia ternifolia* Schumacher & Thonn (fruits) (Figure 1). After identification of plants species, samples were washed and then left to dry in a ventilated room at room temperature. They were then crushed using a mechanical grinder and the resulting grind (powder) was stored in a dry place, away from moisture and light.

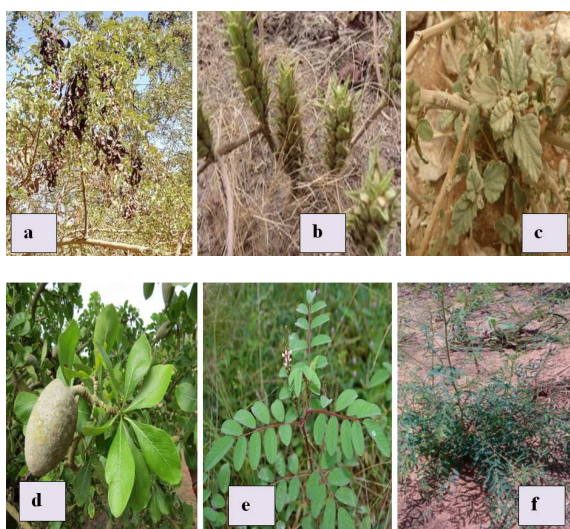


Figure 1. (a) *B. rufescens*; (b) *B. linariifolia*; (c) *C. brocchiana*; (d) *G. ternifolia*; (e) *I. astragalina*; (f) *P. pentandrus*.

2.2. Biological Material

Two isolates of *Salmonella typhimurium* was used to test the activity of these plants. One isolated from lettuce (lettuce) and another from patients at the national hospital of Niamey (clinical strain). The bacteria were cultured on Muller-Hinton agar at 37°C.

2.3. Extraction

The 50 g of powder obtained from each plant was placed in a one-litre flask containing 500 mL of distilled water. The mixture is heated at 60°C for 1 hour. After cooling, the mixture is filtered in a beaker using a funnel and filter paper. The sand bath is used to evaporate the water in order to obtain a dry extract.

2.4. Phytochemical Tests

Phytochemical screening is carried out by tube reactions according to techniques described by Ciulei in 1982; Wagner and Bladt in 1996 with some modifications [8, 9].

1. Tannins have been identified by Stiasny's reagent.
2. Polyphenols have been identified by the FeCl_3 test.
3. Flavonoids by the cyanidin reaction.
4. Saponosides by the foam test.
5. Coumarins by the NH_4OH and observation under the UV lamp at 366 nm.
6. Quinones by the Bornträger test.
7. Triterpenes and the steroids by the Liebermann-Burchard test.
8. Alkaloids by the Mayer, Dragendorff tests and Wagner.

2.5. Determination of Antibacterial Activity

2.5.1. Preparation of Culture Medium

The medium used in this study is Agar Muller Hinton (MH) obtained by dissolving 38 g of MH medium in 1L of distilled water (pH 7.5 ± 0.2). This medium was autoclaved at 121°C for 15 min.

2.5.2. Disc Method for Sensitivity Testing

After sterilization, Muller Hinton Agar (MH) was poured into Petri dishes (diameter 90 mm). For each bacterial strain, a bacterial suspension of 0.5 Mac Ferland turbidity was prepared using physiological water. The bacterial inoculum was swabbed and inoculated into plate agar by spreading. Discs of blotting sheets, diameter 6 mm, previously impregnated with 25 μL of each extract of plant to be tested (200 mg/mL and 50 mg/mL) and antibiotics (positive control) were placed on the sterile plates agar. The discs were prepared 24 hours before the test and incubated at 37°C. The positive control used was Ciprofloxacin (2 mg/mL). The DMSO 25% was used as negative control. The inoculated plate agar was incubated at 37°C for 24 hours. The antibacterial activity was determined by measuring the diameter of the inhibition zone induced by the extracts or antibiotics. Extracts or antibiotics that have induced an inhibition zone over than three diameters (> 3 mm) around the disc are considered to have antibacterial activity. Each test was repeated 3 times in a row [10-12].

2.6. Data Analysis

All data were analyzed using Excel to calculate means, standard deviations and obtain the graph and correlations. The analysis of variance (ANOVA) from the XLSTAT software was used to measure the statistical difference.

3. Results and Discussion

3.1. Extraction Yield of Six Plant Samples

Distilled water was used as solvent. The best yield is observed with *Bauhinia rufescens* and *Phyllanthus pentandrus*. The other four plants gave more or less low yields (Figure 2).

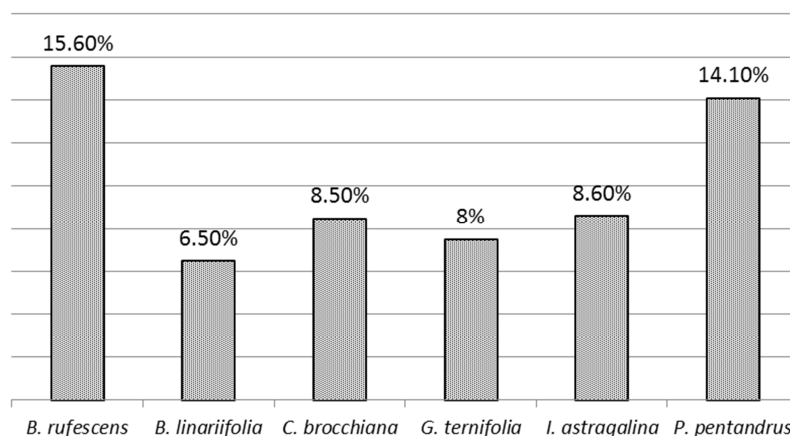


Figure 2. Extraction yield of aqueous extracts.

3.2. Phytochemical Screening

The results of the phytochemical screening of six plant samples are reported in Table 1.

Table 1. Results of phytochemical screening of six plant samples.

Species	Family	Organ (s)	Alc		Tan	Flv	Sap	Quin	Ter/str	Poly	Cou
			D	M							
<i>B. rufescens</i>	Fabaceae	Le	+	-	+	+	+	+	+	+	+
<i>B. linariifolia</i>	Acanthaceae	Wps	-	-	+	+	+	+	+	+	+
<i>C. brocchiana</i>	Euphorbiaceae	Wp	+	-	+	+	+	+	+	+	+
<i>G. ternifolia</i>	Rubiaceae	Fr	+	-	+	+	+	+	+	+	+
<i>I. astragalina</i>	Fabaceae	Wp	+	+	+	+	+	-	+	+	+
<i>P. pentandrus</i>	Euphorbiaceae	Wp	-	-	+	+	-	-	+	+	+

Alc=alkaloids; Tan=tannins; Flv=flavonoids; Sap=saponins; Quin=quinone; Ter/str=terpene/sterol; Poly=polyphenols; Cou=coumarin; absence=-; presence=+; Wp=Whole plant; Fr=fruit; Le=leaves; Wps=Whole plant without seeds D=Dragendorff; M=Mayer.

Phytochemical screening revealed the presence of tannins and flavonoids in all extracts of plants. The phytochemical screening of *B. rufescens* performed by Aliyu *et al.* (2009) on the methanolic extract of leaves showed the presence of flavonoids, tannins, alkaloids, triterpenes and saponins [13]; these results are similar to those obtained in the present study but with the aqueous extract. This could be explained by the fact that methanol and water are polar solvents. The study carried out by Yolidjé *et al.* (2019) on *Chrozophora brocchiana* aqueous and methanolic extracts showed the presence of flavonoids, tannins, saponins, sterols and polyterpenes in the aqueous extract [14]. However, the presence of coumarins and quinones is observed. Some work has also shown an absence of alkaloids in the aqueous and ethanol extracts of this plant [15, 16].

The phytochemical screening of the aqueous extract of *Gardenia ternifolia*, showed the presence of the groups sought. Kallo *et al.* (2018), found flavonoids, tannins and

alkaloids in the aqueous extract [17].

For the plant *I. astragalina*, the results of the present study are the same as those found by Yolidjé *et al.* (2019), which showed the presence of flavonoids, tannins, alkaloids and sterols/polyterpenes in the aqueous extract [14].

Their high use and efficiency reported by Bilan *et al.* (2018) could be explained by the fact that these plants simultaneously contain these two groups of secondary metabolites (tannins and flavonoids) [18]. Tannins have antidiarrheal properties and are enzyme inhibitors and flavonoids have anti-inflammatory properties [19]. The synergistic action between antidiarrheal, anti-inflammatory properties and the inhibition of the development of certain microorganisms is a primordial importance in the fight against certain diseases [19].

The phytochemical study also showed the presence of other groups of secondary metabolites (sterols/triterpenes, quinones and saponins) which may also be very important for

the proper functioning of the human body. The different groups of secondary metabolites detected, could justify the use and possible interesting biological activities which could intervene in the treatment of some affections of the children.

3.3. Antibacterial Activity

The results of the sensitivity test are recorded in the Table 2.

Table 2. Diameter of inhibition of the development of bacteria (mm).

Strains	Plants / witnesses	Inhibition diameter	
		200 mg/ml	50 mg/ml
<i>S. Typhimurium</i> (lettuce)	Ciprofloxacin (2 mg/mL)	23,5±0,70a	23,5±0,70a
	<i>C. brocchiana</i>	19,5±0,70b	10±1,41b
	<i>P. pentandrus</i>	17,5±0,70b	9±2,82b
	<i>B. linariifolia</i>	13,5±2,12c	0c
	<i>B. rufescens</i>	12±0,70c	0c
	<i>I. astragalina</i>	9,5±0,70cd	0c
	<i>G. ternifolia</i>	0e	0c
	Negative control	0e	0c
	Ciprofloxacin (2 mg/mL)	23,5±0,70a	23,5±0,70a
<i>S. Typhimurium</i> (clinical)	<i>B. linariifolia</i>	7,5±0,70c	0b
	<i>B. rufescens</i>	0d	0b
	<i>C. brocchiana</i>	8,5±0,70c	0b
	<i>G. ternifolia</i>	0d	0b
	<i>I. astragalina</i>	0d	0b
	<i>P. pentandrus</i>	11,5±0,70b	0b
	Negative control	0d	0b

Values are means±standard deviation of three determinations (zone of inhibition in mm). Columns with different letters (a, b, c, d, and e) indicate statistical differences ($P < 0.05$).

These results of antimicrobial test appeared to be statistically different ($p < 0.05$) from plant to plant and variation of concentration.

No inhibition zones were found around the disc impregnated with 25% DMSO (Figure 3c). For *S. typhimurium* isolated from lettuce, five (5) plants showed activity at 200 mg/mL and the diameter ranged from 9.5±0.70 mm to 19.5±0.70 mm. The 23.5±0.70 mm were obtained with Ciprofloxacin (Figure 3c). The *C. brocchiana* and *P. pentandrus* were the plants which showed the highest antimicrobial activity on *S. typhimurium* (lettuce) with 19.5±0.70 and 17.5±0.70 mm diameter respectively (Figure 3a and 3b). On *S. typhimurium* (clinical), three (3) plants, *P. pentandrus*, *C. brocchiana* and *B. linariifolia*, showed activity with diameters of 11.5±0.70 mm, 8.5±0.70 mm and 7.5±0.70 mm, respectively. *G. ternifolia* showed no activity. These results indicate that the inhibition zones varied from strain to strain and extract to extract; thus, the clinical strain was more resistant than the strain isolated from lettuce. The statistical analyses show that the diameters of inhibition zones exhibited by *S. Typhimurium* (lettuce) was found to be significantly different ($p < 0.05$) between all extracts of plants with Ciprofloxacin (CIP). However, insignificant different ($p < 0.05$) was noted between the extracts of *C. brocchiana* and *P. pentandrus*. The diameters of inhibition zones from the extracts of *B. linariifolia*, *B. rufescens* and *I. astragalina* are not statistically different.

On *S. Typhimurium* (clinical) plants extracts are statistically different with Ciprofloxacin (CIP). Thus, the extracts of *C. brocchiana* and *B. linariifolia* are not statistically different though significantly different with the extracts of *P. pentandrus*.

The study carried out by Mahamane et al. (2020) on the

same type of extract of these plants showed that *C. brocchiana* and *P. pentandrus* are the plants which contain more flavonoids with values of 51,60±0,28 and 47,68±0,63 mg QE/g (milligram quercetin equivalent per gramme) respectively [20]. The low content of flavonoids was obtained with the extract of *G. ternifolia*. The results of the determination of total polyphenols shows that *C. brocchiana* and *P. pentandrus* are the plants which contain more total polyphenols with values of 260.05±0.07 and 256.08±1.07 mg GAE/g (milligram gallic acid equivalent per gram) respectively. The low content of total polyphenols was obtained with the extract of *G. ternifolia* [20].

The results of this study are in agreement with those of the study carried out by Odeyemi et al. (2014) in which showed that leaf, flowers and roots extracts of *Tithonia diversifolia* have antibacterial activities due to the presence of polyphenols [21]. Bruneton (1999) have shown that bacterial growth decreases with the lowering of the concentration of flavonoid fractions the anti-bacterial activity of flowering heads of *Origanum glandulosum* [22]

Phyllanthus pentandrus extract showed antibacterial activity against strains of *S. Typhimurium* (Lettuce) and *S. Typhimurium* (Clinical). Our results corroborate with those found by Alio [12].

Some authors show that the anti-salmonella activity is linked to the polyphenolic compounds. According to the study conducted by Bolou et al. (2011), the antibacterial activity of the AcOEt fraction of *Terminalia glaucescens* Planch on *Salmonella typhi* and *Salmonella typhimurium* is based on the presence of triterpenoids, polyphenols, quinones and alkaloids [23]. But for Javanmardi et al. (2002), the antibacterial activity of plant extracts would be attributed to secondary metabolites such as polyphenols and triterpenoids

[24]. In the study carried out by Akomoun *et al.* (2019), they shown that phenolics and flavonoid compounds are responsible for the *anti-salmonella* activity of certain plants; they deduce that the flavonoids could be responsible for the *anti-Salmonella* activity of the roots of *Zanthoxylum zanthoxyloides* and the leaves of *Momordica charantia* [25].

It has been shown that the mechanism of toxicity of flavonoids to microorganisms is either by deprivation of metal ions such as iron, or by non-specific interactions such as the establishment of hydrogen bridges with the proteins of

the cell walls of microorganisms (adhesins) or enzymes [26].

Phytochemical screening showed the presence of polyphenols in all six aqueous extracts, *G. ternifolia* contains polyphenols with no antimicrobial activity. Antimicrobial activity does not only depend on the presence of phenolic compounds, but also on the presence of other secondary metabolites [27]. Flavonoids have antifungal and antibacterial properties. In addition to these groups, there is also the group of quinones and derivatives that possess antimicrobial properties.

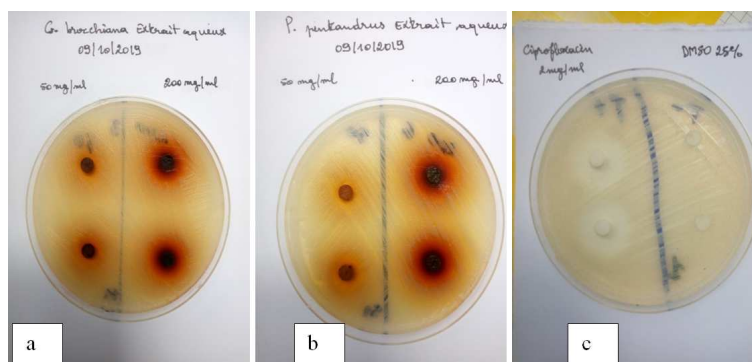


Figure 3. Some results on the diameter of inhibition of the development of bacteria from plant extracts (a and b) and controls (c).

4. Conclusion

This study has shown the presence of an important class of secondary metabolites that could justify the traditional use of these plants. Phytochemical screening revealed the presence of secondary metabolites: polyphenols, flavonoids, tannins, coumarins, alkaloids, saponins, quinones, sterols and terpenes. *C. brocchiana* and *P. pentandrus* have been shown the best antibacterial activity against the two strains used. The antimicrobial activity tested would justify the therapeutic use of these plants in traditional medicine in cases of bacterial infections caused by *S. typhimurium*. It is important in the next studies to determine minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and test the extract from these plants on other bacterial strains. Other work must also be carried out in order to prove the safety of these plants and carry out a bioguided fractionation in order to isolate the molecules responsables for these activities.

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