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RESEARCH ARTICLE

PHYTOCHEMISTRY AND BACTERIAL SENSITIVITY OF SOME PLANTS USED AGAINST JAUNDICE AND INFECTIOUS DERMATOSES INTO KINDIA PREFECTURE (GUINEA)

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Abstract

This work was undertaken to contribute to the management of dermatological infections, and aims mainly to determine antibacterial potentialof plants used by traditional practitioners at the Republic of Guinea. To this end, we used the classical method to qualitatively identify the chemical groups with recognized pharmacological properties of 6 medicinal plant species used to treat dermatoses: *Acanthospermumhispidum* DC, *Vernoniacolorata* (Wild) Drake, *Annona senegalensis* Pers., *Tamarindusindica* L., *Mimosa pudica* L., *Erythrinasenegalensis* DC. The crude extracts (aqueous, hydroethanolic and methanolic) were obtained by maceration method. Antibacterial effet was carried outby determining the antibiotic potency of crude extracts using the method of liquid microdilution. Phytochemical screening showed that all six plants are rich in secondary metabolites and share common alkaloids, flavonoids, terpenes, steroids, saponosides, mucilages and reducing compounds, all of which have antimicrobial properties. The hydroethanol extract of Erythrinasenegalensis DC gave the highest yield (14.34±1.03%), while the lowest value was 5.83±0.08% corresponding to *Tamarindusindica* L. (aqueous extract). Antibacterial potential showed that gram-negative strains were more resistant to the extracts tested, while the most sensitive were gram-positive. The most sensitive strain to the methanolic extract of *Mimosa pudica* L. was *Bacillus cereus* ATCC 14579 with a MIC=250µg/mL, and the most resistant strain to the several extracts was *Salmonella typhimorium* ATCC 2564 with MBC=1000 µg/mL.

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Introduction

Dermatoses represent a wide range of skin disorders affecting millions of people worldwide. They are defined as pathologies whose symptoms are visible and manifest themselves at the level of certain organs, namely the skin, mucous membranes and phanera. They are partly linked to poor hygiene, and their appearance is reinforced by the upsurge in opportunistic infections linked to HIV/AIDS (Mozouloua et al., 2011). These conditions range from mild irritations to serious, life-altering illnesses resulting from bacterial, viral, fungal or parasitic infections. Dermatoses are therefore a major public health problem worldwide, and in tropical countries where they account for 30% of consultations in rural areas (Diatta et al., 2013).

Scabies is a benign cutaneous parasitosis that is common in most countries. According to WHO epidemiological data, the annual number of new cases of scabies worldwide is around three hundred million. It affects men and women, on every continent. In industrialized countries, epidemics occur mainly in institutions (communities, nursing homes, retirement homes, etc.). In temperate regions, the disease occurs mainly during cold seasons, probably due to an increase in unhygienic conditions.

Infection is not systematically listed except in communities where broad information and prevention measures need to be put in place (Laffitte et al., 2017).

In African continent,HIV infection and fungal skin diseases were high score, as well as a growing number of people with cancer or chronic lung disease, and many others treated with medecines that actson to system of immune. Severe fungal diseases are often relatively silent and must be actively sought out to maximize chances of survival and minimize disability (GAFFI, 2022). The Republic of Guinea has been the scene of haemorrhagic viral infections in recent years (Mbaye et al., 2023). In Guinea, the number of hospitalizations is 106 cases for scabies and 420 cases for other dermatological diseases, while in pediatrics, there were 759 cases of infectious dermatoses (Ministry of Health, 2018). According to the health statistics yearbook, the hospitalization rate for dermatological diseases rose to 28801 cases in 2021 (MSHP, 2021).

Access to primary healthcare remains problematic in developing countries. Population growth and the inaccessibility of modern medicines in these countries are participated in promotingthe use of traditional products(Yimta, 2017; Ladoh-Yemeda et al., 2016). In Africa, this demand is not only the result of inaccessibility to modern equipment and the high costs of conventional medicine, but also of traditional medicine, which is very often considered as first-line medicine (Yimta, 2017).

In most developing countries, between 70 and 95% of the population rely on traditional medicine, which has always occupied a central place in the global healthcare system, to cure myriad ailments (Moses et al.; 2015). Plants therefore offer better prospects through the discovery of new pharmaceutical recipes in general and anti-infective agents in particular.

In Africa, several plants are used to treat many diseases such as: malaria, dermatitis, infections, stomach aches, female sterility (Nadembega et al., 2011).

In Guinea, botanical researchers conducted a nationwide on the plants indicated in the treatment of various pathologies, with the most frequently cited plants being those used to treat jaundice and skin infections (Barry et al., 2006). Little work has been done to scientifically valorize these species. With this in mind, the main objective of manuscript is to study the bioefficacy of extracts on pathogenic strains involved injaundice and skin diseases.

Material and Methods

Material:

Vegetable material

The plant material were fresh plant leaves (Table 1 below) harvested inKindia prefecture and brought to the laboratory, where they were dried in room at 22°Cwithout temperature variation for 14 days, after which they could be ground. The dry material is ground with a grinder.

Table 1: List of plants selected for analysis.

N°	Scientific name of species	Family	Common name	Diseases treated	Part used
1	*Acanthospermumhispidum* DC	ASTERACEAE	Bullèbaali (P)	Jaundice, dermatosis, wounds, malaria	leaves
2	*Vernonia colorata* (Wild) Drake	ASTERACEAE	Bantaraburuurè (P)	Jaundice, dermatosis	leaves
3	*Annonasenegalensis* Pers.	ANNONACEAE	Doukunmè (P)	Jaundice, dermatosis, wounds	leaves
4	Tamarindusindica L.	CEASALPINIACEAE	Dyabbhe(P);Tombi(M) ; Tombinnyi (S)	Jaundice, dermatosis, measles and chickenpox, purgative	leaves
5	Mimosa pudica L.	MIMOSACEAE	Fidanimaloyala (M)	Scabies, jaundice,	Whole plant
6	ErythrinasenegalensisDC	FABACEAE	Mbootyolla (P)	Jaundice, dermatosis	leaves ; bark and stem

M = Maninka ; P = Poular ; S = Soussou

Bacteriological material

A support of 6 bacteria of ATCC type, was used for the test. These bacteria represent the major groups of bacteria pathogenic to man, based on their physico-chemical characteristics, and cover the different modes of resistance. They are Bacillus cereus ATCC 14579; *Escherichia coli* ATCC 8739; *Klebsiellapneumoniae* ATCC 13883; *Pseudomonas aeruginosa* ATCC 15442; *Staphylococcus aureus* ATCC 6538 and *Salmonella typhimorium*ATCC 2564.

Methodology

Phytochemical study

The various chemical groups were identified using the tube method, showing staining and precipitation reactions (Table 2) characteristic of plant chemical compounds (Houngbèmè and al., 2014; Omboumaand al., 2021; Agbodjogbéand al., 2022; Deguenonand al., 2023).

Table 2: Identification reactions of different chemical group.

Active ingredient classes	Specific reaction
Alkaloids	-Dragendorffreagent: orange precipitate -Mayer reagent: yellowish precipitate
Tannins	-FeCl3 : dark-blue coloration
Flavonoids	-Shinoda reagent: orange-red coloration
Anthocyanins	-Red coloration (acid medium) -purplish-blue (alkaline medium)
Leucoanthocyanins	-Hydrochloric alcohol: cherry-red coloration
Quinone compounds	-Bornträger reagent : purplish-red coloration
Saponosides	Measurement of foam index
Steroids and terpenes	-Liebermann-Burchard reagent: violet -Keddereagent: purple red coloration
Cyanogenic compounds	-Guignard reagent : brown coloration

Preparation of crude extracts

Extracts were prepared by maceration for 72 hours under continuous stirring of (50g) of powder of each species in 500mL of solvent (water-ethanol mixture (4/6; volume per volume) for hydro-alcoohol extract; water for aqueous extract; and methanol for the methanol extract). The macerate obtained was filtered with hydrophilic cotton, then evaporated at 40°C under the rotavapor and the extract weighed to determine the mass percentage (Houngbeme et al., 2014).

In vitro antibacterial assay

We proceeded in two steps: preparation of the extract solutions (to be tested) and inoculation of the strains with the extracts. The different extracts of each of the six (06) plants were taken in distilled water at 100 mg/mL. The extracts were tested for their sterility by inoculating each solution on Mueller Hinton medium and incubating at 37°C for 24 hours. The absence of microbial growth within 48 hours confirmed that the extract stock solutions were sterile (Agbankpe et al., 2016; Deguenon et al; 2023). To determine the MIC we used the microplate dilution method (Kpadonou-Kpoviessi et al., 2012; Kpadonou-kpoviessi et al., 2013; Lavaee et al., 2019). Tests were carried out on successive dilutions with pure water, from a 2000 µg/mL stock solution. Final concentrations tested ranged from 1000 µg/mL to 31.25 µg/mL.One hundred microliter of the innoculum prepared at 0.5 Mc Farland scale on MH media was deposited in each well. Following MIC determination using tetrazolium as an indicator of germ growth, the wells were plated on MHA agar for determination the MBC corresponding to small concentration which killed all germs (Legba et al.,2017). The positive control used was griseofulvin® at 50 µg/mL, and pure distilled water represent the negative control. The BMC/MIC ratio was then calculated to express the antibiotic power(a.p.) of each extract tested. When this ratio is less than or equal to 4, the extract tested is said to possess antibiotic potency (Houngbeme et al., 2015). The essay wascarried out,3 times to ensure the fidelity of different parameters.

Results and Discussion

Phytochemical groups of plants

The table 3 noted the secondary metabolites identified in plants used

Table 3: Secondary metabolites of plants.

Chemical Groups	P1	P2	P3	P4	P5	P6	Total*
Catechic tannins	++	+	+	+	++	+	06
Gallic tannins	++	+	+	+	++	+	06
Flavonoids	+	+	+	+	++	+	06
Anthocyanins	++	+	-	+	-	+	04
Leucoanthocyanins	-	-	-	-	+	-	01
Alkaloids	+	-	-	-	+	+	03
Reducing compounds	-	+	-	-	+	-	02
Mucilage	+	-	+	-	+	+	04
Saponoside	+	-	+	+	-	+	04
Cyanogenic compounds	-	-	-	-	-	-	00
Triterpene	+	-	+	-	+	+	04
Steroid	+	+	+	-	+	-	04
Coumarin	-	+	+	+	-	-	03
Quinonic compounds	+	-	-	-	+	+	03
Free Anthracenics	-	-	-	-	-	-	00
C-Heteroside	-	-	-	-	-	-	00
O-Heteroside	-	-	-	-	-	-	00
Cardiotonic compounds	-	-	-	-	-	-	00

+: positive reaction ++: strongly positive reaction -: negative reaction

*Total number of species containing a given chemical group.

P1: *Acanthospermumhispidum* DC; P2: *Vernoniacolorata* (Wild) Drake; P3: *Annona senegalensis* Pers.;
P4: *Tamarindusindica* L.;P5: *Mimosa pudica* L.; P6: *Erythrina senegalensis*DC

This table shows that the six plants cited in traditional medicine to combat dermatitis are very rich in secondary metabolites. All the plants analyzed contain gallic tannins, catechic tannins and flavonoids. These three chemical groups can therefore be considered the most common, and are the source of the antimicrobial properties attributed by healers. Four out of six plants (4/6), i.e. 66.66% of all plants, contain anthocyanins, mucilages, saponosides, triterpenes and steroids, which have several antibacterial properties. Similarly, alkaloids, which are very strong antibiotic agents, have been identified in the leaves of *Acanthospermumhispidum*, *Mimosa pudica* and *Erythrinasenegalensis.*

Research work reports that the antimicrobial activity of plants is linked to certain chemical groups namely flavonoids, tannins, anthocyanins, alkaloids and coumarins, which are well represented here (Lègba et al., 2017).

Flavonoids are a widespread group of metabolites in plants and are of interest because of their several biological potentials, for example, antibacterial, antiviral, anti-allergic and antioxidant (Gandonou et al., 2017; Boko-Haya et al., 2017; Gnansounou et al., 2018). For other authors, antimicrobial activity is linked to the existence of flavonoids, tannins and saponins (Dzoyem et al., 2017).

The identification of metabolites of *Tamarindusindica*shows catechic and gallic tannins, flavonoids, anthocyanins and coumarins, in the fruit. But cyanogenic derivatives, alkaloids and cardiotonicheterosides are absent. These results corroborate those of De Caluwé et al, (2010) which revealed that *Tamarindusindica* pulp is rich in these metabolites. In addition, Bhadoriya et al, (2012) demonstrated the presence of flavonoids in *Tamarindusindica* fruits. Previous work has noted that coumarins and saponosidesare present in *Tamarindusindica* fruit pulp, as in the case of the results obtained in this work (Paula et al., 2009).

Our results are similar with the work of Arama (2005), who found catechic tannins, saponosides, cardiotonicheterosides, reducing compounds, terpenes and steroids, flavonoids, alkaloids and coumarins in the trunk and root bark of *Erythrinasenegalensis*.

Furthermore, our results concerning the chemical composition of *Acanthospermumhispidum* DC, are similar to those of Houngbeme et al. (2014) having shown the presence in the leaves, of gall tannins, alkaloids, flavonoids, terpenes, steroids and mucilages. Similarly, the presence of terpenes is confirmed by the work of Ganfon et al. (2012) when they determinedantiparasitic activity of diterpenes isolated from *A. hispidum* DC.

In 2005, Ouattara F. highlighted flavonoids, triterpenes, tannins and saponosides in *Annona senegalensis* leaves using the thin-layer chromatography method followed by revelation with characteristic chemical reagents. These 4 groups were also found in our work, i.e. the leaves of the plant harvested in Guinea. Our results are therefore justified, the only difference being that the Guinean sample also contains mucilages, steroids and coumarins. This difference can be explained by the influence of geographical and environmental parameters such as season, relief and climate, which vary from one ecological region to another.

ExtractionYield

Table 4 shown the mass percentages of residues obtained after evaporation and drying

Table 4: Mass percentage of plant extracts.

Plants	Crude extracts (% m/m)		
	aqueous	hydro-ethanolic	methanolic
Acanthospermumhispidum DC	7,54±0,12	8,42±0,06	7,68±0,08
Vernoniacolorata (Wild) Drake	8,37±0,21	7,83±0,32	6,48±0,16
Annona senegalensis Pers.	10,48±0,26	12,63±0,11	9,74±0,19
Tamarindusindica L.	**5,83±0,08**	11,46±0,03	7,65±0,05
Mimosa pudica L.	6,47±0,04	8,57±0,15	7,45±0,12
*Erythrinasenegalensis*DC	10,04±0,27	**14,34±1,03**	8,26±0,18

The solvents used in this work are all polar and have enabled us to extract the chemical principles contained in the different species, but in varying proportions. This variation is certainly linked to the non-identical appearance of compound groups from one species to another. Extraction with the binary water-ethanol system enabled a greater quantity of chemical compounds to be extracted, whatever the species. This is inherent in the fact that this mixture extracts both polar and apolar compounds as reported in several works (Houngbeme et al., 2014). The hydroalcohol extract of *Erythrinasenegalensis* DC shows the highest mass percentage (14.34±1.03%), while the lowest yield value is 5.83±0.08% corresponding to the aqueous extract of *Tamarindusindica* L. This finding regarding the extraction performance of the binary mixture corroborate with the work of Ouattara et al. (2005), when they showed that the hydroalcohol extract (70%) for root and trunk bark of *Erythrinasenegalensis*, had the highest yields of 40% and 28% respectively for both parts. We can deduce that this method achieves higher extraction yields than the most widely used traditional method (maceration or decoction). Moreover, the macerate with water of leaves of *Annonasenegalensis*yielded 16.26% according to the work of Ouayogode et al. in 2021, which is higher than the value (10.48±0.26) found for the same type of extract from the same plant harvested in Guinea.

Antimicrobial activity of extracts

Tables 5a and 5b below summarize the values of the various parameters indicating the bacteriostatic or bactericidal effects of the extracts.

Many extracts tested showed bacteriostatic activity on the strains. Minimum inhibitory concentrations ranged from 250 µg/mL for the methanolic extract of *Mimosa pudica* L. on *Bacillus cereus*, to 1000 µg/mL for several extracts and other reference strains used. Three of the six species (50%) showed bactericidal activity on the *Bacillus cereus* strain, with a minimum bactericidal concentration (MBC) equal to 1000 µg/mL for the hydroalcoholand methanolic extracts. These are essentially the hydro-alcohol extract of *Acanthospermumhispidum* DC, *Tamarindusindica* L., *Mimosa pudica* L. with the same antibiotic potency value p.a = 2, and the methanolic extract of *Mimosa pudica* L. with p.a = 4. However, these extracts are less active than the reference griseofulvin with a p.a = 1 value on gram+ bacteria and a.p = 2 for gram-negative strains. In view of these results, the most sensitive strain is Bacillus cereus. The most resistant strain is *Salmonella typhimorium* ATCC 2564, for which no extract showed inhibition at the highest concentration tested. The three other resistant strains after *S. typhimorium* were *Klebsiellapneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 15442 and *Escherichia coli* ATCC 8739, which were sensitive to a very small number of hydro-ethanol-type extracts and had no bactericidal power. The resistant strains on extract were gram-negative bacteria, while the most sensitive strain was gram-positive. In fact, gram-positive bacteria are characterized by the complexity of the chemical composition of their walls, including phospholipids (Yehouénou et al., 2010), which have no affinity with highly polar solvents such as water. However, alcohol dissolves lipids well, which would explain the activity of hydro-ethanol extracts on microbes in this case. This result corroborates the work of Ouayogode et al, (2021) who reported that hydroethanolic extracts from traditional recipes had demonstrated antiplasmodial activity on both susceptible and resistant strains.

The bactericidal effect exhibited by the hydroethanolic extract of *Acanthospermumhispidum* on Bacillus cereus is in line with the results obtained by Houngbeme et al., (2014) when they demonstrated that the plant's hydroethanolic extract is bacteriostatic and bactericidal on *S. aureus* and *C. albicans* strains.

The extract’s activity can be explained by a destabilization of microbial walls followed by the inactivation of enzymatic proteins formed by complexation with nucleophilic alpha amino acids (Buchholz et al., 2016).

This activity is also inherent in the various chemical groups identified in these plants, namely polyphenols (flavonoids, tannins), alkaloids and terpenoids.

The mechanism of action of these phytomolecules is likely to be the destruction of the bacteria's outer and inner membranes (Falconer and Brown, 2009). They may also act via other mechanisms, as secondary metabolites have been shown to involve various mechanisms of action that differ from those of conventional antibiotic molecules (Falconer and Brown, 2009; Tene et al., 2009; Yala et al., 2017).

Conclusion

We have shown in this work that the plants used in Guinea to treat jaundice and dermatoses are rich in secondary metabolites whose pharmacological properties justify their use. The most active extracts on the strains are derived from a water-ethanol mixture, extracting a large number of polar and apolar compounds whose synergistic effect is thought to underlie the imbalance of microbial cell walls. The three most active plants are potential candidates for bioguided fractionation to identify and isolate the active principles responsible for their antimicrobial properties.

Table 5a: Values in µg/mL for inhibitory and bactericidal concentrations and antibiotic power (a.p) of extracts.

Species	Extracts	Strains								
		Pseudomonas aeruginosa ATCC 15442			*Staphylococcus aureus* ATCC 6538			*Salmonella typhimorium* ATCC 2564.		
		MIC	MBC	a.p	MIC	MBC	a.p	MIC	MBC	a.p
Acanthospermumhispidum DC	Aqueous	>1000	-	-	1000	-	-	>1000	-	-
	Hydroalcohol	1000	-	-	1000	-	-	>1000	-	-
	Methanolic	>1000	-	-	1000	-	-	>1000	-	-
Vernoniacolorata (Wild) Drake	Aqueous	>1000	-	-	>1000	-	-	>1000	-	-
	Hydroalcohol	>1000	-	-	1000	-	-	>1000	-	-
	Methanolic	>1000	-	-	1000	-	-	>1000	-	-
Annona senegalensis Pers.	Aqueous	>1000	-	-	>1000	-	-	>1000	-	-
	Hydroalcohol	>1000	-	-	1000	-	-	>1000	-	-
	Methanolic	1000	-	-	1000	-	-	>1000	-	-
Tamarindusindica L.	Aqueous	>1000	-	-	1000	-	-	>1000	-	-
	Hydroalcohol	>1000	-	-	1000	-	-	>1000	-	-
	Methanolic	>1000	-	-	500	-	-	>1000	-	-
Mimosa pudica L.	Aqueous	>1000	-	-	1000	-	-	>1000	-	-
	Hydroalcohol	1000	-	-	1000	-	-	>1000	-	-
	Methanolic	1000	-	-	500	-	-	>1000	-	-
*Erythrinasenegalensis*DC	Aqueous	>1000	-	-	>1000	-	-	>1000	-	-
	Hydroalcohol	>1000	-	-	1000	-	-	>1000	-	-
	Methanolic	>1000	-	-	>1000	-	-	>1000	-	-

Table 5b: Values in µg/mL for inhibitory and bactericidal concentrations and antibiotic power (a.p) of extracts (continued)

Species	Extracts	Strains								
		Bacillus cereus ATCC 14579			*Escherichia coli* ATCC 8739			*Klebsiellapneumoniae* ATCC 13883		
		MIC	MBC	a.p	MIC	MBC	a.p	MIC	MBC	a.p
Acanthospermumhispidum DC	Aqueous	>1000	-	-	>1000	-	-	>1000	-	-
	Hydroalcohol	500	1000	2	1000	-	-	1000	-	-
	Methanolic	1000	-	-	1000	-	-	>1000	-	-
Vernoniacolorata (Wild) Drake	Aqueous	>1000	-	-	>1000	-	-	>1000	-	-
	Hydroalcohol	1000	-	-	>1000	-	-	>1000	-	-
	Methanolic	500	-	-	>1000	-	-	>1000	-	-
Annona senegalensis Pers.	Aqueous	>1000	-	-	>1000	-	-	>1000	-	-
	Hydroalcohol	1000	-	-	1000	-	-	>1000	-	-
	Methanolic	1000	-	-	>1000	-	-	>1000	-	-
Tamarindusindica L.	Aqueous	1000	-	-	>1000	-	-	>1000	-	-
	Hydroalcohol	500	1000	2	1000	-	-	>1000	-	-
	Methanolic	500	1000	2	1000	-	-	>1000	-	-
Mimosa pudica L.	Aqueous	1000	-	-	1000	-	-	>1000	-	-

	Hydroalcohol	500	1000	2	1000	-	-	1000	-	-
	Methanolic	250	1000	4	1000	-	-	>1000	-	-
*Erythrinasenegalensis*DC	Aqueous	>1000	-	-	>1000	-	-	>1000	-	-
	Hydroalcohol	1000	-	-	>1000	-	-	>1000	-	-
	Methanolic	1000	-	-	>1000	-	-	>1000	-	-

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Conflict of interest

The authors declare that there is no conflict of interest.

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