Caesalpinia Bonduc (L) Roxb Seed, An Alternative to Antimicrobial Control

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***Abstract*—**Background: Nowadays, there are different varieties of antibiotics due to the phenomenon of the emergence of antibiotic resistance to different pathogens.

Objectives: To evaluate the anti-erythematous properties of *Caesalpinia bonduc* seeds used in traditional medicine in Benin.

Methods: The determination of the chemical composition of *Caesalpinia bonduc* seeds and its antimicrobial activities could lead to formulations of phytomedicines but also to the preservation of foodstuffs. In the realization of the works, the method used is the dilution method in liquid medium and determination of MIC.

Results: The leaves of *Caesalpinia bonduc* (L) Roxb. are not used very much (40% of responses) but when people come to use it most often, it is to reduce abdominal pain related to menstruation in women. The roots of *Caesalpinia bonduc* (L) Roxb. are used to treat prostate problems, as the survey in the four cities shows, with 44% of the answers given. All extracts (both hull and almond) are fungicidal on *Candida albicans*.

Conclusions: At the end of the present study aiming to verify the antimicrobial properties of the seeds of *Caesalpinia bonduc* (L.) Roxb., we can retain that :- all parts of *Caesalpinia bonduc* are used in traditherapy (seeds, leaves and roots); the most antimicrobial extracts are the total aqueous and ethyl acetate extracts from the shell; - all extracts are bacteriostatic on *Enterococcus faecalis* except the petroleum ether extract which is bactericidal.

***Keywords*—**Candidiasis, Nappy Rash, Pharmacognosy, Plant Extract, Traditional Medicine.

I. Introduction

Today, there is a great diversity of antibiotics; however, this diversity masks another reality: most antibiotics only correspond to a small number of different families and the new molecules, the most advanced in clinical trials, are often only variants of already known compounds [1]. It is clear that this line of defense can no longer control the emergence of resistant pathogenic microorganisms. Faced with the appearance of these new therapeutic problems, the development of particularly innovative families of antibiotics is now a priority. It is therefore necessary to go back to nature so that it can give us its secrets and the solution to our concerns through traditional ancestral knowledge [2].

In the ancient fight against diseases, the African continent abounds in traditions, formulations and remedies still poorly known to the scientific world.

In Benin, several virtuous plants are used as antibacterial, antifungal, antiseptic and have been the subject of several studies. Others on the other hand have lost their history, the oral tradition on their properties and their different uses tend to disappear.

Among these plants is *Caesalpinia bonduc (L)* Roxb, a plant whose different parts (seeds, roots, leaves) are used for the treatment of several diseases. But it is threatened with extinction.

It is taking into account these realities that this work was interested in the antimicrobial properties, supposedly, of the seeds of *Caesalpinia bonduc (L*) Roxb. Through a brief ethnobotanical survey, a phytochemical study and microbiological tests on microbial strains responsible for diaper rash (*Staphylococcus aureus, Enterococcus faecalis and Candida albicans*).

II. Material and Methods

A. Ethnobotanical survey

Please A survey was carried out among tradipracticiants practicing as vendors of medicinal plants in the cities of Bohicon, Porto-Novo, Cotonou, Ouidah and periphery (Fig. 1). In each city, the following markets were targeted. These are:

- AHOUANGBO, AHIDAHO and OUANDO markets in Porto-Novo ;
- VEDOKO, MENONTIN and WOLOGUEDE markets in Cotonou
- ZOBE, KPASSE and PAHOU markets in Ouidah ;
- the central market of Bohicon.

The purpose of this survey is to learn about the different uses of *Caesalpinia bonduc* (L) Roxb in the field of pharmacopoeia in Benin.

The choice of these localities is guided by the importance given to the plant in the habits and customs of the "Gbé" continuum of the "KWA" linguistic group (fongbe, gungbe, gengbe, wacigbe, etc.) located in the southern part of Benin.

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This group, which shares many habits and customs, is renowned for its knowledge and mastery of herbal medicine and the divinatory arts [3].

Thus, the cities mentioned above are the regions of origin of the ethnic groups of the KWA linguistic group.

B. Vegetal material: Caesalpinia bonduc (L) Roxb

The seeds sampling was done in the city of Bohicon in central Benin.

It consisted of purchasing *Caesalpinia bonduc* seeds in the central market of the city of Bohicon. We bought 3.5 kg of seeds. The choice of this site was guided by the existence in the locality of nascent nurseries of *Caesalpinia bonduc*.

C. Microorganisms

Candida albicans, yeast, the main cause of erythema infectiosum;

Staphylococcus aureus and *Enterococcus faecalis*, also responsible for erythema infectiosum but also for the alteration of the microbiological quality of foodstuffs as evidence of a lack of hygiene and contamination of faecal origin.

D. Chemical analysis

The chemical analysis consisted in the realization of a phytochemical screening on the kernel and on the shell of seed of *Caesalpinia bonduc*. According to the method of Hougton and Raman [4].

Preparation of the Different Extracts

The different extracts carried out are those aqueous, ethanolic and with solvents of increasing polarity on the shell powder and the seed kernel of *Caesalpinia bonduc*.

Decoction

To 50 grams of powder, 1 Liter of water are added and the whole was brought to boil for 60 minutes, then filtered on Wathman R1 filter paper. The filtrate was concentrated in Rotavapor at 50 °C.

The concentrated filtrate was oven dried at 40°C. The lyophilisate was stored in sterile, clean, dry bottles in the refrigerator at 4°C.

Preparation of Alcoholic Extracts

100g of powder was taken and added to Preparation of alcoholic extracts

100g of powder is taken and 500 mL of ethanol at 70° is added. The whole is stirred and left to macerate for one week (seven days); then the mixture is filtered, and the filtrate is passed to the Rotavapor at 50°C and dried in the oven at 40°C. The deposit after filtration was also oven dried. The two were mixed and the resulting hydroalcoholic extract was stored in a sterile, clean, dry bottle at 4°C in a refrigerator.

Preparation of extracts with solvents of increasing polarity

50g of powder is taken and left to macerate for 5 days in ethanol at 70°C. After filtration it passed to the Rotavapor to eliminate the alcohol. On the extract plus water it was successively carried out extracts with 50mL of solvents of increasing polarity (petroleum ether, ethyl acetate, butanol) (fig.2).

E. Antimicrobial activity

The determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) was carried out.

In the realization of the works, the method used is the dilution method in liquid medium.

For the determination of the MIC a total of 864 hemolysis tubes were used, i.e. 432 tubes per test (2).

For safety reasons, all tubes were inoculated for the determination of BMC and FMC. The same number of Petri dishes was used, 432 per test and per strain, in addition to an environmental control, giving a total of 866 dishes.

The different suspensions obtained are calibrated and the following dilutions are used as inoculum for the tests with the extracts: dilution 10^{-6} for bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and 10^{-7} for fungi (*Candida albicans*) which corresponds to a number of germs between 104 and 106 germs per millilitre [5].

Determination of MIC by Macromethod

Add 50 µL of inoculum to this series of sterile tubes containing 950 µL of each dilution of the extract. Incubate 24 hours at 37°C for bacteria and 72 hours at 27°C for fungi.

The Minimum Bactericidal Concentration or MBC is the lowest concentration of the extract allowing to destroy 99.99% of the bacteria present at the beginning (i.e. one surviving bacterium out of 10000). If CMB < 5 CMI the extract is very effective.

On the contrary, if BMC > 10 MIC, it is considered not very effective.

To determine the BMC or MFC it is necessary to count the survivors on agar medium (nutrient broth) for all clear tubes (without dilution):

- Inoculate 50µL of each clear tube on this agar: dry 30 min;
- Incubate the agars according to the culture conditions of strains.

The BMC is read by comparing the number of survivors counted in each clear agar tube to that of the control agar. The BMC is the lowest concentration of extract for which there is no more than 0.01% of surviving germs.

III. RESULTS

A. Ethnobotanical Survey

Caesalpinia bonduc commonly called Adjikwin in Fon, the most widely spoken language in Benin [6] was and is widely used. In the rural areas of southern Benin, the seeds are pierced and attached to the neck and hip of children and sometimes adults to combat erythema. The other uses of the plant constitute the knowledge of the African and Beninese phytotherapists. The use of this medicinal plant for the cure of

ailments is still traditional and little known in the scientific field. Previous studies have focused on the chemical composition and certain properties of the leaves, barks and roots. But until now, no phytopharmacological study has been done on the seed. This justifies the choice of our topic, to verify the antimicrobial properties attributed to the seeds. The general objective is to verify the antimicrobial properties of the seeds of *Caesalpinia bonduc* (L) Roxb., commonly used in rural areas, for the treatment of diaper rash.

The determination of the chemical composition of *Caesalpinia bonduc* seeds and its antimicrobial activities could lead to formulations of phytomedicines but also to the preservation of foodstuffs.

In all markets, the traditional healers we met were women, ranging in age from 30 to 60 years.

The responses of the women vendors were confirmed by a professional traditherapist who works in a traditherapy center in the city of Cotonou; a total of 40 respondents. Figure 1 presents the results of the survey phase.

The leaves of *Caesalpinia bonduc* (L) Roxb. are not used very much (40% of responses) but when people come to use it most often, it is to reduce abdominal pain related to menstruation in women.

The roots are used to treat prostate problems, as the survey in the four cities shows, with 44% of the answers given.

It should be noted that the only part of the plant used for the treatment of erythema is the seed and according to the same method of use, either pierced and attached to the folds presenting these erythema.

But the roots and the seeds are the most used in traditherapy respectively for prostate problems and for erythema

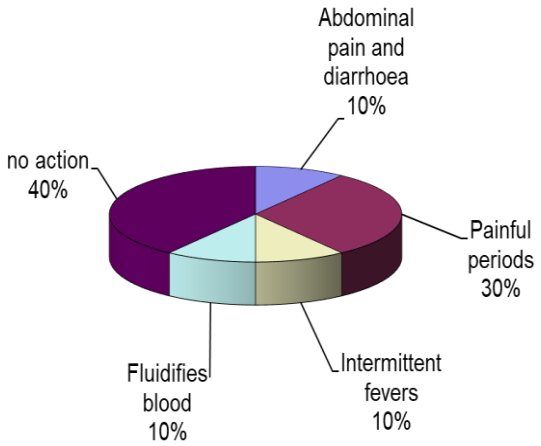

Fig. 1 Ethnobotanical survey

B. Phytochemical screening

The seeds of *Caesalpinia bonduc* (L) Roxb have been collected in the city of Bohicon (GPS 7,1805006, 2,0351144) after identification of the plants at the national herbarium under the number YH 958 / HNB.

The results of the phytochemical screening carried out separately on the kernel and on the shell of the seed of *Caesalpinia bonduc* are presented in the following table I.

TABLE I: RESULT OF PHYTOCHEMICAL SCREENING OF C. BONDUC SHELL AND SEED KERNEL

Compounds	Shell	Almonds
Alkaloids	+	+
Polyphenolic compounds		
➢ Tannins	+++	+
✓Catechic Tannins	+++	+++
✓Gallics Tanins	+++	-
➢ Flavonoïds	+	+++
Quinones derivatives	-	-
Anthocyanins	-	-
Leucoanthocyanins	++	-
Cyanogenics derivatives	-	-
Saponosides	Foam height = 1cm	Foam height = 0,5cm
Triterpenoids and steroids	+++	++
➢ Triterpenoïds	-	-
➢ Steroid	+	-
➢ Cardenolides		
Mucilages	+++	+++
Coumarines	++	++
Anthracene compounds		
➢ Combined anthracenes	+	-
✓ O-heterosides	-	-
✓ Reduced-genin heterosis	+++	+++
✓ C-heterosides	- ++	- ++
➢ Free anthracenics		
➢ Essentials oils		

- : Absence; + : Trace; ++ : Weak or medium presence; +++ : Strong presence

The analysis of the table shows that the shell is rich in tannins (gall tannins), leucoanthocyanins, triterpenoids and anthracene compounds than the kernel. On the other hand, the almond is only richer in flavonoids than the shell.

The observation of Table 2 shows that the highest yield is obtained with the hydroethanolic extract of almond (20.05%) followed by the aqueous extract of hull (17.16%).

The lowest yield was obtained with the petroleum ether extract of the hull (0.66%) in the liquid-liquid extraction followed by the butanolic extract (0.8%).

A first series of tests was performed on *Candida albicans*, *Staphylococcus aureus* and *Enterococcus faecalis* with the hydro-ethanolic and total aqueous extracts of shell and seed kernel of *Caesalpinia bonduc* (L) Roxb.

The results showed a more evident antimicrobial activity in the hull extract (Table 2).

TABLE II
RESULTS OF THE ACTIVITY OF HYDRO-ETHANOLIC EXTRACTS ON STAPHYLOCOCCUS AUREUS, ENTEROCOCCUS FAECALIS AND CANDIDA ALBICANS

	Extracts	Staphylococcus aureus		Enterococcus faecalis		Candida albicans	
		MIC (mg/mL)	BMC (mg/mL)	MIC (mg/mL)	BMC (mg/mL)	MIC (mg/mL)	BMC (mg/mL)
Almond	Hydro-ethanolic	ND	ND	12.5	ND	ND	ND
Hull	Hydro-ethanolic	1.965	3.925	0.098	15.7	0.098	3.925

ND: Not determined

The BMC/MIC ratio is used to determine the bactericidal or bacteriostatic activity of the extracts. When the ratio is less than or equal to 4, the extract is said to be bactericidal, when it is greater than 4, it is qualified as bacteriostatic (Bouraima, 2005).

Analysis of the results obtained (Tables III and IV) with solvents of increasing polarity show that ethyl acetate extract exerts a bactericidal action at 0.017125mg/mL on *Staphylococcus aureus*. On the same germ, the final aqueous shell extract shows a bacteriostatic activity at 0.164mg/mL as well as the butanolic extract at 0.0685mg/mL.

TABLE III
MICROBIOLOGICAL TEST RESULTS OF EXTRACTS ON S. AUREUS, E. FAECALIS AND C. ALBICANS

		Staphylococcus aureus		*Enterococcus faecalis*		*Candida albicans*	
	Extracts	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Almond	Hydro-ethanolic	-	-	12.5	-	-	-
	Total aqueous	0.0685	8.77	0.274	17.54	8.77	8.77
Hull	Total aqueous	1.096	7.02	0.219	7.025	0.548	0.548
	Hydro-ethanolic	1.965	3.925	0.09812	15.7	0.098124	3.925
		0.1096	7.0175	7.0175	7.0175	1.754	3.508
	Ethyl acetate	0.0171	0.0171	0.0685	8.77	8.77	8.77
	Butanolic	0.0685	17.54	1.096	17.54	0.548	0.548
	Final aqueous	0.164	21.05	1.315	10.525	0.657	0.657

Staphylococcus aureus (MIC=0.017mg/mL and BMC=0.017mg/mL) with ethyl acetate extract of hull;
Enterococcus faecalis (MIC= 0.219mg/mL and BMC= 7.025mg/mL) with total aqueous shell extract; and Candida albicans (MIC=0.548mg/mL and BMC= 0.548mg/mL) with butanol extract.

TABLE IV
BMC/MIC RATIO

	Almond extract		Hull extract					
Micro organisms	Hydro-ethanolic	Aq total	Aq total	Hydro-ethanolic	Petroleum Ether	Ethyl Acetate	Butan	Aq final
S. aureus	-	128.03 **BS**	6.40 **BS**	2 **BC**	6.40 **BS**	1 **BC**	256 **BS**	124.55 **BS**
E. faecalis	-	64.015 **BS**	32.07 **BS**	160.0 **BS**	1 **BC**	128 **BS**	16 **BS**	8 **BS**
C. albicans	-	1 **FC**	1 **FC**	40 **FS**	2 **FC**	1 **FC**	1 **FC**	1 **FC**

BS: Bacteriostatic; Aq total: Aqueous total; BC: Bactericide; FC: Fungicide; Hydro-ethan: Hydro-ethanolic; Butan: Butanolic; FS: Fungistatic; Aq final: Aqueous final.

For *Enterococcus faecalis*, the petroleum ether extract has a bactericidal activity at a concentration of 7.0175mg/mL. While ethyl acetate extract appears to have bacteriostatic activity at a concentration of 0.0658 mg/mL.

On *Candida albicans*, the butanolic extract at a concentration of 0.548mg/mL is fungicidal while the ethanolic extract at a concentration of 98.125µg/mL exerts fungistatic activity.

IV. DISCUSSION

The ethnobotanical survey allowed to realize that the seeds

of *C. bonduc* are not only used for the treatment of diaper rash but also for the treatment of other ailments such as insect bites and olfactory infections.

It allowed us to know the therapeutic use of the other parts of the plant, in particular the root which is used to treat the problems of prostate in the men and the sheets to avoid the gastric or colic pains.

Analysis of the survey responses shows that the remedies are similar but that the dosage differs from one practitioner to another.

Outside Benin, the seeds of *C. bonduc* (L) Roxb. are used in tropical countries (India, Pakistan, ...) for the treatment of intermittent fevers and malaria [7], anti-inflammatory and analgesic [8] but especially as a plaything [9].

The phytochemical study showed in the almond an important presence of alkaloids, catechic tannins, triterpenoids, flavonoids, mucilages, coumarins and anthracene derivatives c-heterosides; while in the shell, we note the presence in very high proportion of gall tannins, flavones, triterpenoids, mucilages and anthracene derivatives c-heterosides, but in lesser quantity of leucoanthocyanins, coumarin and anthracene derivatives o-heterosides [10].

These results partly corroborate those obtained by [11] who obtained a chemical composition of the whole seed not by part. Similar results were observed for the presence of flavonoids (bonducellin), terpenoids (caesalpin F, α-caesalpin, β-caesalpin, γ-caesalpin, ε-caesalpin), lipids and alkaloids [12]. Our analyses show a total absence of reducing sugars in contrast to Panda [14] who found some.

The presence of tannins, which have the action of waterproofing the external layers of the skin protecting the underlying layers (property of tanning the skin) and of being vasoconstrictors of the small vessels of the skin, associated with the presence of flavonoids and coumarins with vasculoprotective and venotropic effect can justify the cases of cure noted in the subjects reached of erythema related to a vasoconstriction of the small superficial vessels and a fold of skin presenting important rednesses. Moreover, the mucilages raised in seeds can by their lenitive and antipruriginous property, intervene in the treatment of the dermatoses (what is the case for the erythemas of the buttocks), the appeasement of the irritations caused by the prickles and ocular discomfort.

These properties and observations are used in the therapeutic treatment of the in vivo study with a 5% aqueous solution of total cockle extract.

Finally, the local anesthetic, antibacterial, antifungal, antiseptic, anti-infectious, anti-inflammatory, anti-oedematous, analgesic, tonic properties of alkaloids, saponosides, triterpenoids and Essential Oils are used in the treatment of diaper rash.

The total aqueous extract of the hull showed a more important antimicrobial action, with a yield of 17.16%, on *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans*. According to BALANSARD [13,15], during a primary screening 3 to 4 strains including 1 fungus are sufficient for antimicrobial activity tests.

These encouraging results of the shell led us to perform a series of extraction with solvents of increasing polarities tested on the three strains. These tests showed that on *Staphylococcus aureus*, the acetate extract is the most bactericidal and the butanolic one presents a high bacteriostatic power. On *Enterococcus faecalis*, except for the petroleum ether extract which is bactericidal, the other extracts present a strong bacteriostatic activity (the most bacteriostatic being that of ethyl acetate). All the extracts present a fungicidal activity on *Candida albicans*.

From the above analysis, it appears that the ethyl acetate extract from the seed shell of *Caesalpinia bonduc* is the fraction with the highest antimicrobial power.

The combination of all these fractions, some with bacteriostatic or antibacterial properties and others with antifungal properties, gives the *Caesalpinia bonduc* seed hull antimicrobial properties against *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans*.

The results are consistent with information received during the investigation phase, where erythema is cured when a pierced seed is attached to the hips and healing is seen when the smooth part of the shell eventually detaches from the rest of the seed. This confirms that the anti-erythematous compounds are found in the shell of the plant and that the seed contains volatile compounds (essential oils) with fungicidal, anti-infectious, bactericidal, antiseptic and anti-inflammatory properties.

The antimicrobial activity of our extracts would thus be due to the presence of tannins, flavonoids and essential oils in the seed of *Caesalpinia bonduc*.

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The antimicrobial activity of our extracts would thus be due to the presence of tannins, flavonoids and essential oils in the seed of *Caesalpinia bonduc*.

V. Conclusion

At the end of the present study aiming to verify the antimicrobial properties of the seeds of *Caesalpinia bonduc*

(L.) Roxb., we can retain that:

- all parts of *Caesalpinia bonduc* are used in traditherapy (seeds, leaves and roots);
- the groups of chemical compounds found in the seed of *Caesalpinia bonduc* have the property of tanning, waterproofing, softening and antipruritic of the skin tissues
- the seed shell has more important antimicrobial properties than the almond;
- all extracts (both hull and almond) are fungicidal on *Candida albicans*;
- the most antimicrobial extracts are the total aqueous and ethyl acetate extracts from the shell;
- all extracts are bacteriostatic on *Enterococcus faecalis* except the petroleum ether extract which is bactericidal;
- the lipid, aqueous and ethyl acetate extracts are bactericidal on *Staphyloccoccus aureus* while the other shell extracts are bacteriostatic.

Thus, dermoprotective and vasoconstrictor properties on small superficial vessels and the positive antibacterial, antifungal and anti-inflammatory activities with *Caesalpinia bonduc* (L) Roxb. justify its traditional use, especially on dermatoses (diaper rash).

VI. Acknowledgment

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