



Research Article

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Evaluation of the antibacterial activities of the aqueous extract, alkaloids and flavonoids from *Abrus precatorius* Linn, (Fabaceae)

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ABSTRACT

Antibacterial activities of aqueous total extract, alkaloids and flavonoids of *Abrus precatorius*, the Ivorian herbal pharmacopoeia, were evaluated in vitro growth of *Salmonella Typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*, germs that are involved in gastroenteritis in humans. The antibacterial tests were performed by the methods of diffusion in solid and broth dilution coupled with the spread on agar plates. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) obtained showed significant antimicrobial activities of different extracts of seeds tested. However, alkaloids and flavonoids were less active against bacteria compared to the total aqueous extract (ETA). The strong activity of ETA could be explained by the presence among other alkaloids and flavonoids with antibacterial properties have been proven in this study.

Key Words: *Abrus precatorius*, alkaloids, flavonoids, antibacterial activity, Côte d'Ivoire.

INTRODUCTION

Cultural habits and socio-economic difficulties faced by developing countries, especially african countries are that people do not always have access to modern medicinal products. To remedy this situation, the traditional therapeutic methods that were considered were identified on the african continent about 50,000 species of plants with medicinal properties proved [1] including nearly 761 species in Côte d'Ivoire [2]. In addition, the emergence of multidrug-resistant pathogens, due to misuse and inappropriate use of antibiotics [3, 4] is currently a public health problem of particular concern. Indeed, the resistance of bacteria to antibiotics sometimes makes the therapy ineffective [5, 6, 7, 8] and puts the practitioner in delicate situations, especially when the patient's life is at stake.

The solution to this problem is so urgent and requires the search for new antimicrobial agents. Therefore the use of medicinal plants with antimicrobial properties constitutes an interesting alternative to explore. [9]. It is in this context that we are interested in the study of the antimicrobial activity of seeds of *Abrus precatorius* plant known for its use in traditional medicine and its therapeutic diverse. Indeed, it has antitussive properties, abortive, antidiarrheal, antimicrobial, diuretic, laxative, antipyretic, purgative, anticancer, anthelmintic [10, 11,12, 13, 14]. Several studies have already been carried out on this plant, including Traoré [15], Bolu [16] and Kone [17] showed that *Abrus precatorius* contain substances with antibacterial properties.

Moreover, these authors stated that the 70% ethanolic extract was more active than the ETA. The overall objective of this study is to investigate the antibacterial activities of ETA, alkaloids and flavonoids extracted from the seeds of *Abrus precatorius* Linn (Fabaceae) on bacterial strains involved in gastroenteritis in humans.

EXPERIMENTAL SECTION

Plant material

The plant material is made from the seeds of *Abrus precatorius* collected in the botanical garden of the University of Felix Houphouët-Boigny of Cocody-Abidjan (Côte d'Ivoire). These seeds were dried in sunlight and roasted for 15 minutes before being reduced to a fine powder by grinding. From the powder obtained after spray, the different extracts tested (ETA, alkaloids and flavonoids) were prepared.

Bacterial strains

Four hospital bacterial strains were used in this study. Referenced as shown below, they were kindly provided by the Institut Pasteur de Côte d'Ivoire: 1107c/10 *Klebsiella pneumoniae*, *Staphylococcus aureus* 1046y/10, BA05798/10 *Salmonella Typhi*, *Escherichia coli* 1104c/10.

Extract preparation

1-The aqueous total extract

The aqueous total extract was prepared according to the method of Zirihi et al. [18]. Thus, using a blender Mammomlex, 100 g of the plant powder were stirred vigorously in 1L of distilled water. The homogenate obtained was first spun in a square of white cloth and then filtered twice in succession on cotton wool and once on paper Whatman 3 mm. The resulting filtrate was then evaporated in an oven at 55 °C Venticel for giving the ETA.

2-Alkaloids and flavonoids

The extraction of alkaloids was done according to the method of Zirihi, [19] which is to vigorously shake 300 g powder plant in 1.5 L of ethanol and then allowed to stand at Bain-Marie at 65°C for 45 minutes. The homogenate obtained was first spun in a square of white cloth and then filtered successively on cotton wool and Whatman paper 3 mm. The filtrate obtained is concentrated to reduce the amount of alcohol. Then, after adding water 10 times the volume of the filtrate, the filtrate is shaken vigorously. After decantation, the aqueous phase is obtained which is precipitated in the total alkaloids. The evaporation in an oven at 55°C of the aqueous phase results in the obtaining of the total alkaloids. Dragendorff reagent was used to confirm the alkaloids. For the extraction of flavonoids, 100 g powder *Abrus precatorius* were homogenized in distilled water 1L and 20 mL acetic acid for 20 minutes. The homogenate obtained was first spun in a square of white cloth, and then undergoes a double filtration over cotton wool and Whatman paper 3 mm. The filtrate was added 300 mL of chloroform. Then, the mixture was stirred vigorously. After decantation, the aqueous phase telling all flavonoids is obtained. This aqueous phase undergoes evaporation in a rotary evaporator followed by lyophilization to give the total flavonoids that have been confirmed by the test of cyanidin.

Antibacterial tests

1- Sensitivity tests

By the method of diffusion in solid [20, 21] the sensitivity of germs was evaluated. For each extract, discs of Whatman paper N°1 of 9 mm diameter are first impregnated with 50 µL to 200 mg/µL. These disks are then deposited on the surface of an agar medium inoculated Mueller-Hinton with a bacterial suspension of 18 to 24 hours. A common antibiotic, gentamicin (500 mg) was used under the same conditions for its broad spectrum of action. After 30 minutes of pre-release, the plates are incubated for 24 hours at 37°C and the diameters of the inhibition zones seen any around the disks were measured.

2- Determination of MIC and MBC

To determine the parameters of inhibition of bacterial growth (MIC and MBC) of the various extracts, the macrométhode dilution liquid medium described by Delarras [22] was used.

- **Minimum Inhibitory Concentration:** A concentration range from 200 to 3.125 mg/mL was prepared for each sample by the method of double dilution. Each experimental tube is inoculated with a standardized inoculum of 8.10^5 cells. After incubation for 18 hours at 37°C, the MIC of the extract tested is determined. It corresponds to the concentration of the first tube of the series in which there is no trouble due to the growth compared to the control.

- **Minimum bactericidal concentration:** The nutrient agar poured into Petri dishes seeded streak is 5 cm long by 0.1 mL of the contents of tube having a concentration greater than or equal to the MIC in the previous series of tubes. The MBC was determined after incubation for 24 hours at 37°C. This is the lowest concentration that completely inhibited growth. The experiment was repeated 3 times.

Screening of phytochemical extract total aqueous

We characterized the different chemical groups with reference to the technicals described by Békro et al. [23], Savithramma et al. [24] and Shivakumar et al. [25].

RESULTS AND DISCUSSION

Table 1 shows the main chemical groups encountered in aqueous total extract. We denote the one hand, the presence of alkaloids, saponins, flavonoids and tannins and other parts, the absence of anthraquinones, sterols and glycosides.

Table 1: Chemical composition of aqueous total extract of *Abrus precatorius*

Chemical groups	Presence or absence
alkaloids	+
Glycosides	-
tannins	+
flavonoids	+
saponins	+
Anthraquinones	-
Sterols	-

- = Absence ; + = Presence

The diameters of the inhibition zones obtained with different extracts and efficacy of these extracts compared to gentamicin are summarized in Table 2. We note that ETA has produced zones of inhibition greater than those alkaloids and flavonoids. But overall, *S. aureus* was more sensitive to the three extracts with 15.2, 14.5 and 14.6 mm respectively for ETA, alkaloids and flavonoids. The greatest resistance was observed against *E. coli* with 11.1 mm in order to flavonoids, 11.2 mm and 12.2 mm alkaloids for ETA. Moreover, with the exception of the strain of *E. coli* for which no zone of inhibition was observed (0 mm) and *K. pneumoniae* (6 mm) diameters induced by extracts are inferior to those of the reference antibiotic, gentamicin, with diameters of inhibition varied between 25 and 29 mm.

Table 2: Diameter of growth inhibition of the tested germs and effectiveness of various extracts and antibiotic usual

Bacterial strains	Diameter of IC (mm)				Effectiveness			
	ETA	ALC	FLA	GEN	ETA	ALC	FLA	GEN
<i>K. pneumoniae</i>	14,1	12,3	12,8	6	+	+	+	-
<i>S. aureus</i>	15,2	14,5	14,6	29	+	+	+	+
<i>S. Typhi</i>	14,7	12,4	12,9	25	+	+	+	+
<i>E. coli</i>	12,2	11,1	11,2	0	+	+	+	-

ETA: Total Aqueous Extract; ALC: Alkaloids; FLA: Flavonoids; GEN: Gentamicin

On the basis of the diameters of inhibition, the results show a sensitivity of all germs tested at various extracts. According Biyiti et al. [26], an extract is considered active if it causes a zone of inhibition greater than or equal to 10 mm. Under these conditions, with diameters between 12.2 and 15.2 mm, ETA appears as the most active extract. And with respect to this extract, the strain of *S. aureus* was the most sensitive while the highest resistance was observed against *E. coli*. As for the secondary metabolites that are alkaloids and flavonoids, they exhibit nearly identical variations in their inhibition diameters (11 to 15 mm) over the whole of the tested germs. Moreover, the evolution of the sensitivity of the germs studied with respect to our plant extract is almost comparable to that observed with the reference antibiotic, gentamicin 500 mg for which *S. aureus* (29 mm) has the highest sensitivity followed by *S. typhi* (25 mm). *K. pneumoniae* and *E. coli* were resistant with respectively 6 and 0 mm inhibition.

Table 3: Parameter Values antibacterial and reports MBC/MIC of various extracts of *Abrus precatorius* Linn

Extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	bacterial strains
ETA	12,5	12,5	1	<i>K. pneumoniae</i>
ALC	50	100	2	
FLA	50	100	2	
ETA	6,25	12,5	2	<i>S. aureus</i>
ALC	100	200	2	
FLA	100	200	2	
ETA	6,25	25	4	<i>S. typhi</i>
ALC	50	200	4	
FLA	50	100	2	
ETA	12,5	50	4	<i>E. coli</i>
ALC	50	200	4	
FLA	50	200	4	

ETA: Total Aqueous Extract, ALC: Alkaloids; FLA: Flavonoids MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration

Table 3 presents the antibacterial parameter values for the different extracts tested

Regarding alkaloids and flavonoids, their activities were similar when tested to the germs studied their MIC and MBC are respectively between 50 and 100 mg/mL and 100 to 200 mg/mL. Moreover, if we consider the efficiency ratio MBC/MIC remains less than or equal to 4, the activity of each extract is called bactericidal.

Taking into account the MIC, we note that ETA has induced the largest inhibition zone has the lowest MIC values of the corresponding germs. The same observation was done for the recorded values of the MBC. This means that the ETA is the most active of the three extracts studied. These results are in agreement with those of Bolu et al. [27] on the same strains tested. Considering both the MIC and MBC, the activities of both secondary metabolites are also similar as before. In addition, they were less active compared to the ETA. And, the activity report, MBC / MIC for each sample on a given germ is less than or equal to 4. We then coated according Berche et al. [28] that all extracts are bactericidal on selected germs. The observed activities of our extracts find their explanation in the analysis of the results of phytochemical screening revealed that the ETA, the most active extract, contains saponins, alkaloids, tannins and flavonoids, phytochemicals whose antimicrobial properties have already been demonstrated [29, 30, 31, 32]. This work has allowed us to confirm the antibacterial activities of alkaloids and flavonoids found in *A. Precatorius*. But their low activity compared to the ETA when tested lonely could be justified by a synergistic action between the various constituents of the plant. The possibility of the existence of antimicrobial agents belonging to other molecular families is not ruled out. This assertion is supported by the work of Yeo [33] who worked on improving the activity of *A. precatorius* on a certain number of bacteria including *S. typhi* and *K. oxytoca*.

In view of all the results, we can note that the sensitivity of germs tested at our various extracts could justify the use of the plant in the traditional treatment of diarrhea. Moreover, in the specific cases of *E. coli* and *K. pneumoniae* who have shown resistance to gentamicin, their inhibition by our extracts is important insofar as it might advocate the use of *A. precatorius* for the treatment of both diarrhea and pneumonia and this effectively.

CONCLUSION

This study allowed us to evaluate the antibacterial properties of aqueous total extract, alkaloids and flavonoids of *Abrus precatorius*. The concentrations at which these extracts have been shown to be active and their action spectra broad enough leads us to say that this plant is useful in the treatment of gastroenteritis. Furthermore, alkaloids and flavonoids that have antibacterial activities highlighted in the present work, the presence of saponins and tannins were noted. In isolation, these alkaloids and flavonoids were less active than the ETA. It would be interesting to undertake further studies to better understand the contribution of all major chemical groups encountered at the plant in the development of an optimal synergistic.

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REFERENCES

- [1] E.J. Adjanohoun, **1990**, *Bull. Med. Trad. Pharm. Afri. Paris*, 1:59-63.
- [2] L. Ake-Assi. **1991**, *Bull. Med. Trad. Pharm. Afri. Paris*, 2:203-205.
- [3] J.Breuil, A. Brisabois, L. Arnaud, S. Fremy and E. Collatz, **2000**, *J. of antimicrobial Chem.*, 46 (6), 965-971
- [4] K. Ouattara, A. Coulibaly, J.D. N'guessan, Y. J. M'baïasbe, F. Guede-Guina, A.J. Djaman, **2007**, *Rev. de Méd. et Pharm. afr.*, (20), 1-8
- [5] K.C. Akoua, N. Guessend, V. Gbonon, A.Y.H. Faye-kette and M. Dosso, **2004**, *Med. Mal. Infect.*, 34(3): 132-36
- [6] D. Soro, M.W. Kone et A.K. Kamanzi, **2010**, *Europ. J. Sci. Res.*, 40, 307-317.
- [7] K.J. Golly, S. Siaka, N. Guessennd, Y. Soro, A.J. Djama, M. Dosso, **2012**, *J. of Chem. and Pharm. Res.*, 4(5), 2490-2494
- [8]. C. Tarik, A.B. Fawzia, H. Farah, B. Zahia, **2012**, *J. of Chem. and Pharm. Res.*, 4(1):216-220
- [9]. A. Djamila, **2010**, Synthesis, physico-chemical and preformulation derivative pyrido [3,2 g] quinoline trimethyl (in French) Thesis of doctorate in chemistry, Faculty of Pharmacy, University of Aix-Marseille II, 183p
- [10] J. Kerharo et A. Bouquet, **1950**, *Medecinal plant and toxic of Côte d'Ivoire and Haute-Volta, mission study native pharmacopoeia AOF* (in French). Editor Vigot frère, Paris, 300 p.
- [11] A. O. Prakash and R. Mathur, **1976**, *Indian J. Exp. Biol.*, 14, 623-626.
- [12] B. N. Dhawan, G. K. Patnaik, R. P. Rastogi, K.K. Singh and J.S. Tandon, **(1977)**, *Indian. J. Exp. Biol.*, 15, 208-219.
- [13] O.F.C. Nwodo and E.O. Alumanah, **1991**, *J. Ethnopharmacol.*, 3, 395-398.

- [14] A.M. Ibrahim, **1992**, *Phytother. Res.*; 6, 155-157.
- [15] Z Traore, **2008**, Evaluation of the antibacterial activity of ethanolic extract of *Abrus precatorius* (Fabaceae) on *Salmonella typhi* and other bacteria responsible for infections nosocomial (in French). DEA Biotechnology: Option Pharmacology of Natural Substances. UFR Biosciences University of Cocody-Abidjan, Côte d'Ivoire, 30p
- [16]. G.E.K. Bolou, **2006**, Study of antimicrobial activity of *Abrus precatorius* on *Salmonella Typhi* and *Staphylococcus aureus*. DEA Biotechnology: Option Pharmacology of Natural Substances (in French). UFR Biosciences University of Cocody-Abidjan, Côte d'Ivoire, 30p. [17] M. Kone, **2010**, Evaluation of the antibacterial activity of *Abrus precatorius* (Fabaceae) on three multiresistant strains: *Salmonella typhi*, *Salmonella enteritidis* and *Staphylococcus aureus* involved in gastro-enteritis (in French). DEA Biotechnology: Option Pharmacologie des substances naturelles, UFR Biosciences Université Cocody-Abidjan, Côte d'Ivoire, 32p.
- [18] G.N. Zirihi, A.K.M. Kra, F. Guede-Guina, **2003**, *Rév. Méd. Pharm. Afr.*, 17, 11-19.
- [19] G.N. Zirihi, **2006**, Botany, Phytochemical and Pharmacological study of some antimalarial and immunogens medicinal plants used by Bete, people from Issia Department, in western Côte d'Ivoire (in French). Thesis of doctorate in Botany University of Cocody-Abidjan, Côte d'Ivoire, 181p.
- [20] M. Yusha'u, D.W. Taura, B. Bello and N. Abdullahi, **2011**, *Int. Res. of Pharm. and Pharmacol.* 1(19), 237-241.
- [21] C. Delarras, **1998**, *Gaétan Morien Ed.*: 169-178.
- [22] R. Kavitha, P. Kamalakannan, T. Deepa, R. Elamathi, S. Sridhar K.J. Suresh, **2011**, *J. Chem. Pharm. Res.*, 3(6):115-121
- [23] Y.A. Békro, J.A.M. Békro, B.B. Boua, B.F.H. Tra and E.E. Ehilé, **2007**, *Rev. Sci. Nat.*, 4 (2), 217-225.
- [24] N. Savithramma, M.R. Linga and G. Bhumi, **2011**, *J. Chem. Pharm. Res.*, 3(5):28-34
- [25] B.S. Shivakumar, M. Ramaiah, M.R. Hema, V.P. Vaidya and B. Abida, **2011**, *J. Chem. Pharm. Res.*, 3(5):420-424
- [26] L.F. Biyiti, D.J.L. Meko'o, V. Tamzc et Z.P.H. Amvam, **2004**, *Pharm. Méd. Trad. Afr.* 13, 11-20
- [27] G.E.K. Bolou, I. Bagre K. Ouattara and A.J. Djaman, **2011**, *T. J. of Pharm. Res.* 10 (3), 335-340
- [28] P.Berche, J.L. Gaillard et M. Simonet, **1991**, Bacteria of human infections (in French). Editor Flammarion, Médecine et Sciences, 660p
- [29] D M. Nguyen, **1983**, *Rev. Fran Med Trad Chinoise.* 100: 303-312
- [30] A. Scalbert, **1991**, *Phytochemistry*, 30, 3875-83
- [31] Z.P.H. Amvam, L. Biviti., F. Tchoumbovngang, C. Menut, G. Lamatv and P H. Bouchet, **1998**, *Flavour and Fragrance J.* 13, 107-114.
- [32] M.M. Cowan, **1999**, *Clin. Microbiol. Rev.* 4 (12), 564-582.
- [33] S.O. Yeo, **2008**, In vitro evaluation of the activity of the ethanolic extract of *Abrus precatorius* 70% on some bacterial strains that cause infectious diarrhea in humans (in French) DEA Biotechnology: DEA Biotechnology: Option Pharmacology of Natural Substances. UFR Biosciences University of Cocody-Abidjan, Côte d'Ivoire, 33p.