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TRIPHYTOCHEMISTRY AND ANTIBACTERIAL ACTIVITY OF 70% ETHANOLIC AND AQUEOUS EXTRACTS OF THE ROOTS OF *UAPACA GUINEENSIS* MÜLL. ARG. (EUPHORBIACEAE)

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ABSTRACT
The aim of this study was to scientifically validate the therapeutic virtues attributed to the roots of *Uapaca guineensis* müll. arg. (Euphorbiaceae), A plant used in the treatment of gastro-enteritis and male infertility in the department of Man (western Côte d'Ivoire). The aim was to carry out a phytochemical study and evaluate the antibacterial activity of aqueous (EAq) and ethanolic 70% (EEth70%) extracts of the plant's roots on four strains. the best performance was observed with EEth70%. Phytochemical analysis of the roots revealed the presence of most of the compounds of interest. However, alkaloids and gall tannins were absent from both extracts. In terms of antibacterial activity, inhibition diameters ranged from 06 to 20 mm for the ethanolic extract and from 06 to 15 mm for the aqueous extract. The ethanolic extract gave better inhibitory activity on the growth of the strains tested, especially on *Staphylococcus aureus* and *Escherichia coli*, with the highest inhibition diameters. Both extracts were less active on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. In view of the results obtained, the plant could be used against various infectious pathologies.

KEYWORDS: *Uapaca guineensis*, Antibacterial, Triphytochemistry, Côte d'Ivoire.

INTRODUCTION
To improve patient care, the WHO is encouraging intensified research into treatments, including those based on traditional herbal remedies.[1] The infectious treatments often proposed by modern medicine are too expensive, inaccessible, of questionable efficacy and sometimes have numerous side-effects.[2] Medicinal plants thus become the ultimate means for rural populations to solve their therapeutic problems.[3] Such is the case with *Uapaca guineensis* Müll. Arg. is a plant belonging to the Euphorbiaceae family, known for its various therapeutic virtues. In Côte d'Ivoire, the plant is used for its aphrodisiac, anti-abortive, laxative, tonic, sexual asthenia, sexual impotence and gastrointestinal properties.[4,5] **(**In Central Africa, root bark decoction is prescribed as a drink or enema for edema, verminosis and gastrointestinal disorders.[4] The aim of the present study was to verify the antibacterial properties of extracts from the root of *Uapaca guineensis*, a plant used in western Côte d'Ivoire to treat various pathologies including gastroenteritis and male infertility.

MATERIALS AND METHODS
Plant Material
The plant material used for this study consisted of *Uapaca guineensis* (Euphorbiaceae) roots harvested at the Université de Man site, located opposite the village of Kassiapleu in western Côte d'Ivoire. The plant was identified by the Centre National de Floristique (CNF) of the Université Félix Houphouët Boigny de Cocody (Abidjan).

Microorganisms
The bacterial support used in this study comes from the strain bank of the Institut Pasteur de Côte d'Ivoire (IPCI). It comprises four (4) clinical strains isolated from

biological products: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Preparation of plant extracts

Preparation of aqueous extract

100 g powder of the root of *Uapaca guineensis* were macerated for 24 hours in 1L of distilled water.[6] The macerate has been wrung into a square of sterile tissue, filtered successively on cotton wool and one-fold on filter paper (Whatman paper® 2 mm). The filtrate was dried slowly in the stove at 50° C. The powder obtained was stored in a hermetically sealed jar and refrigerated at 4 °C.[7]

Preparation of ethanolic 70% extract

It was carried out using modified method.[8] A mass of 100 g of plant powder was added in 1L of ethanol 70% and subjected to maceration for 72 hours. The macerate was treated according to the same procedure like the aqueous extract.

Phytochemical screening

Test for sterols and polyterpenes (reaction LIEBERMANN)

After evaporation to dryness 5mL of each solution in a capsule on a sand bath without charring, the residue was dissolved in hot acetic anhydride and 1 mL in a test tube, we poured cautiously with 0.5 mL of concentrated sulfuric acid along the tube wall to the solution. The applications to the interphase of a purple or purple ring, turning blue to green, indicate a positive reaction.[9]

Test for alkaloids (reactions Dragendorff and Bouchardat)

Six milliliters of plant extract were evaporated. The residue was taken up in six milliliters of alcohol at 60 ° and the alcoholic solution thus obtained was divided into two test tubes.

In the first tube was added two drops of Dragendorff reagent. The appearance of a precipitate or an orange color indicated the presence of alkaloids.

In the second tube was added two drops of reagent Bouchardat. The appearance of a reddish-brown color indicated a positive reaction to the presence of alkaloids.[10]

Test for polyphenols

Two milliliter of extract was added a drop of alcoholic solution of ferric chloride at 2%. The appearance of a dark green or lighter or darker blue color indicated the presence of polyphenolic derivatives.[11]

Test for flavonoids

For this research, two milliliter of the extract was evaporated to dryness in a porcelain dish on a sand bath. The residue was taken after cooling in five milliliter hydrochloric alcohol half. The successive addition of three magnesium shavings and three drops of isoamylic alcohol showed an intense pink or violet in the presence of flavonoids.[8]

Test for saponosides

A volume of two milliliters of each extract was evaporated and taken up in five milliliters of water. After vigorous stirring, the foaming of more than one centimeter, stable and persistent high for 30 minutes indicated the presence of saponins.[10]

Test for catechol or condensed tannins (reaction Stiasny)

A volume of five milliliter of each extract was evaporated and an amount of 10 ml of a reagent solution Stiasny was added to the residue. This mixture was placed in a water bath at 80 ° C for 30 minutes and was cooled to room temperature. Positive feedback had resulted in the formation of large flakes brown clear or dirty precipitates.[9]

Quinonic substances research

For this research, 2 mL of each extract solution is first evaporated to dryness in a sand-bath capsule without charring, then the residue is triturated in 5 mL of 1: 5 hydrochloric acid. Then the solution obtained is brought to the boiling water bath for half an hour. Finally, after cooling on a current of cold water, the hydrolyzate is extracted with 20 ml of chloroform and the chloroform phase is collected in another test tube supplemented with 0.5 ml of ammonia diluted by half. The appearance of a color ranging from red to purple indicates the presence of quinones.[9]

Search anthocyanins

The presence of anthocyanins in an extract solution is indicated by a red color which increases with the addition of dilute HCl and turns purplish-blue-green by the addition of ammonia.[9]

Test for Gallic tannins

The above solution was saturated and one or two drops of alcoholic solution of iron chloride to 2% have been added. The positive response has led to the appearance of blue-black coloration characteristic intense tannins Gallic.[9]

Preparation of bacterial inoculum

Two isolated colonies from each bacterial culture for 18 hours were homogenized in 10 mL of Muller-Hinton broth and incubated for 3 hours at 37° C for preculture. A levy of 0.1 mL of the preculture broth was diluted in a tube containing 10 mL of Mueller-Hinton (MH). This bacterial suspension was made consisting of 10^0 dilution of bacterial inoculum so as to obtain a bacterial load estimated to 10^6 Unit Format colonies per milliliter (CFU / mL).

Preparation of extracts concentration ranges

A range of concentration of each extract was prepared with a series of ten vice tubes through the method of double dilution an in medium liquid. This range of concentration is 200 mg/mL to 0.39 mg/mL numbered T1 to T10. For this, 10 mL of a mixture solution of DMSO / sterile distilled water (V / V) were placed in the tubes T1 and 5mL in all the other tubes. Two grams (2g) of each extract were dissolved in the tubes T1 to obtain a concentration of 200 mg/mL. A 5 mL volume of the tubes T1 was transferred into the tubes T2 and then homogenized. This operation was repeated until T10 tubes where 5 mL of T10 tubes are rejected. All tubes are kept refrigerated at 4 °C.[12]

Determination of growth inhibition zones

The method of holes punch in the MH agar described has been accepted by.[13] Each pit or holes of 6 mm diameter was filled with 80 µL of extract concentrations of 200 and 100 mg / mL, taking care to separate two holes of at least 20 mm. A negative control wells was performed for each bacterial strain with 80 µL of the mixture of DMSO / sterile distilled water solution (V/V). After a pre-release of 45 minutes at laboratory temperature to 16° C, all the Petri dishes were incubated in an incubator at 37° C for 18-24h. Meanwhile, Ceftriaxone (CRO 30µg) for Enterobacteriaceae and oxacillin (OX 5µg) for staphylococci were used as positive controls. After incubation, the activities of the extracts were assessed by measurement of a growth inhibition area around the wells using a caliper. According to a strain is called insensitive or resistant, sensitive and very sensitive if the diameters of inhibition are respectively less than 8 mm, between 9 and 14 mm and between 15 and 19 mm.[14]

Determination of Minimum Inhibitory Concentration (MIC)

The macro dilution method in liquid medium described by was used to determine these antimicrobials parameters.[15] Thus, in a series of 10 hemolysis tubes numbered C1 to C10 for each extract was introduced 1 mL of the bacterial inoculum. Then 1 mL of each extract concentration well known by the range of prepared concentration was added in the same tubes. This distribution of plant extract is made so that 1 ml of plant extract of 200 mg/mL was transferred in the tube C1, that of 100 mg/mL in the tube so C2 to C9 tube receive 1mL plant extract of 0.78 mg/mL. C10 has been tube, received instead of plant extract, 1 mL of DMSO / Sterile distilled water (V/V), was used as a control. This distribution of plant extract concentration is well known in each tube already containing 1 mL of inoculum reduced the concentration of plant extract in medium at its half. Tube and the concentration of C1 increased from 200 mg/mL to 100 mg/mL. 100 mg/mL to 50 mg/mL for C2 so on until a concentration of 0.39 mg/mL for T9. This experiment was performed identically for each sample tested. The first nine (9) tubes (C1 to C9) are called "experimental tubes" and the last tube (C10) is rated "growth control tube or TC." The loaded tubes were incubated at 37° C for 24 h. The MIC is the concentration of the first tube where it finds no trouble visible to the naked eye.

Determination of Minimum Bactericidal Concentration (MBC)

From the MIC, the lowest concentration that leaves no more than 0.01% survival of bacteria suspended starting 24 hours corresponds to the CMB. It is determined by plating by a streak on Mueller-Hinton agar by streaking 5 cm using a loop, beginning with the first and incubated undisturbed at 37° C for 24 h tube.

Antibacterial activity of the extracts tested

The antibacterial effect of different extracts tested was considered bactericidal or bacteriostatic depending on the MBC / MIC ratio. According when this ratio is greater than 4, the extract has bacteriostatic and bactericidal, if the ratio is less than or equal to 4.[16]

RESULTS

Yields

Yield values were calculated in relation to the initial mass of *Uapaca guineensis* powder for one trial. Plant extraction yields are 5.33% for the ethanolic extract (EEth) and 5% for the aqueous extract (EAq). Both extracts have a powdery appearance. In terms of color, the extracts have the same coloration (Table I).

Table I: Yield of aqueous and ethanolic extracts of *Uapaca guineensis* roots.

Extracts	Weight (g)	Yield (%)	Color	Aspect
EAq	7,5	5,00	Maroon	Powder
EEth	8	5,33	Maroon	Powder

EAq : Aqueous extract EEth : Ethanolic extract

Triphytochemistry

Table II shows the main groups of chemical families contained in the two *Uapaca guineensis* extracts. Screening of the chemical constituents of the various *Uapaca guineensis* extracts revealed the presence of polyphenols, tannins, flavonoids, saponosides, anthocyanins, sterols and polyterpenes, alkaloids and quinones in the roots. As for saponosides, alkaloids, gallic tannins and quinones, they are only slightly present in EEth. The same applies to alkaloids and gallic tannins in EAq. However, alkaloids and gall tannins are totally absent from both extracts.

Table II: Phytochemical analysis of ethanolic and aqueous extracts of *Uapaca guineensis* roots.

Extracts		EEth	EAq
alkaloids	B	-	-
	D	-	-
Saponosides		-	++
anthocyanins		+++	+
Tannins	Cat	+++	++
	Gal	-	-
flavonoids		++	+++
Polyphenols		+++	++
Quinones		-	+++
Sterols and polyterpenes		+	+++

- : Absence +: Presence ++: Strong presence +++: Very strong presence EAq: Aqueous extract; EEth: Ethanolic extract; Gal: Gallic; Cat: Catéchiques; B: Bouchardâf; D: Dragendoff

Antimicrobial activity

Table III shows the different diameters of the inhibition zones of the two *Uapaca guineensis* extracts tested on four bacterial strains. All the microorganisms tested responded to the extracts. Inhibition diameters ranged from 06 to 20 mm for both extracts. However, the highest inhibition diameters were observed with EEth extracts in *E. coli* (20 mm) followed by *S. aureus* (10 mm). With regard to EAq, the greatest sensitivity was observed with the *S. aureus* strain (15 mm). However, *K. pneumoniae* and *P. aeruginosa* were resistant on EEth and *P. aeruginosa* on EAq.

Table III: Diameters of inhibition zones (mm) of ethanolic and aqueous extracts of *Uapaca guineensis* and Ceftriaxone on tested strains.

extracts	Strains tested	Concentrations of extracts (mg/mL)				Ceftriaxone (CRO)
		200	100	50	25	
EEth	*E. coli*	20	15	08	06	12
	S. aureus	10	08	06	06	10
	K. pneumoniae	06	06	06	06	08
	P. aeruginosa	06	06	06	06	08
EAq	*E. coli*	10	08	06	06	12
	S. aureus	15	12	06	06	10
	K. pneumoniae	10	06	06	06	08
	P. aeruginosa	06	06	06	06	08

Where: Ts = T = 0: Sterility control including well diameter (6 mm) with DMSO/Water (0.5: 0.5; V/V); CRO = Ceftriaxone, EEth: Ethanolic extract; EAq: Aqueous extract.

DISCUSSION

The aim of this work was to contribute to a better understanding of *Uapaca guineensis* Müll. Arg in order to scientifically verify its therapeutic and antibacterial properties. The extraction method used was maceration of *Uapaca guineensis* roots with two solvents, one inorganic (distilled water) and the other organic (70% ethanol), as described by.[7] Extraction revealed that the ethanolic extract of the plant roots had a yield of 5.33%, while that of the aqueous extract was 5%. Calculating yields enables us to quantitatively assess the extracts that can be obtained from each species.

These yields also make it possible to consider the quantity of organs to be harvested, should the need arise for a similar study, which would make the use of medicinal plants more rational and therefore sustainable for the species concerned. The quantity of solvent must be appropriate to the quantity of plant material to be extracted. This also implies that the choice of extraction solvents influences the yields of secondary metabolites in the extracts.

The extracts were then subjected to qualitative phytochemical analysis. The presence of the majority of the desired metabolites was detected in the inorganic extract. This could be explained by the fact that water concentrates the compounds better than ethanol. All the extracts studied in the present work showed a low presence of alkaloids and gall tannins. Our results differ from those of.[17] This difference or absence of a compound could be explained by the nature of the solvent used, the study area or the soil quality. Also, explained that the extraction method carried out at a certain temperature makes it possible to extract the maximum number of compounds and prevent their probable denaturation or modification due to the high temperatures used in other extraction methods.[18]

In addition, the antibacterial activities of the two extracts (EEth, EAq) from *Uapaca guineensis* roots gave inhibition diameters on the growth of certain germs tested, namely *Escherichia coli* and *S. aureus*. According to, an extract is considered antibacterial when it induces a zone of inhibition greater than or equal to 10 mm.[14]

The ethanolic extract showed good inhibitory activity on the growth of the strains tested, compared with EAq. This is because inhibition diameters are generally between 06 and 20 mm for ethanolic extract and between 06 and 15 mm for aqueous extract.

With regard to EAq, the greatest sensitivity was observed with the *Staphylococcus aureus* strain (15 mm). However, the Pseudomonas strain gave no inhibition diameter. With EAq, *K. pneumoniae* and *P. aeruginosa* strains are sensitive to the 200 mg/mL concentration. However, it should be noted that the antimicrobial activities of secondary plant metabolites depend on a number of factors, including the origin of the plant, extraction methods, the nature of the solvent, the concentration of active compounds, the nature of the tests applied and the strains tested.[19]

Overall, the ethanolic extract showed the greatest sensitivity to strains of *Staphylococcus aureus* and *E. coli*. It is difficult to compare our results with those in the bibliography, as no similar scientific studies have been carried out on this plant organ. Antibacterial activity may depend on the composition of the culture medium.[20] Organic matter in the culture medium may reduce the efficacy of an antibacterial agent by combining with it to form inactive compounds, by absorbing it and reducing its concentration, or by precipitating it and eliminating it altogether. Other factors influencing activity include harvesting period, climate, extraction method and resting time.[21]

CONCLUSION

This work has enabled us to identify the presence of various chemical compounds and to evaluate the antibacterial activity of the aqueous and ethanolic extracts of Uapaca guineensis roots.

Triphytochemistry showed that the aqueous extract was richer in chemical compounds than the ethanolic extract. It revealed the presence of polyphenols, flavonoids, saponosides, tannins, anthocyanins, sterols and polyterpenes in the aqueous extract, but relatively few alkaloids.

Antibacterial activity, on the other hand, showed that the ethanolic extract of the plant's roots was more active on most of the strains tested, particularly *S. aureus* and *E. coli*. However, the aqueous and ethanolic extracts were less effective on *P. aeruginosa* and *K pneumoniae*. This sensitivity is dose-dependent and varies according to the germs and the presence of chemical compounds.

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