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BIOLOGICAL ACTIVITIES OF ARREDOUL JAUNE, A PHYTOMEDICINE BASED ETHANOL EXTRACT FROM FRESH ROOTS OF *PENTADIPLANDRA BRAZZEANA* BAILL. (PENTADIPLANDACEAE) USED AS AN ANTIDIARRHOEAL DRUG IN KISANGANI-DEMOCRATIC REPUBLIC OF CONGO

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ABSTRACT
Arredoul jaune, a phytomedicine based ethanol extract from the roots of *Pentadiplandra brazzeana* (Pentadiplandraceae), its soluble fractions, crude saponins and total alkaloids extract were submitted to a broad biological screening including antimicrobial, antiamoebic and spasmolytic activities which can justify its use as an antidiarrhoeal drug. Results indicated that all samples were significantly active in each assay with varying magnitudes. Arredoul jaune exhibited good antibacterial and bactericidal activities against all selected bacteria including *Bacillus subtilis*, *Escherichia coli*, *Salmonella entertidis*, *Shigella dysenteria*, *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella thyhimurium* with MIC and MBC values < 100 µg/ml. It also displayed strong antiamoebic activity with minimal amoebicidal concentration (MAC) of 5.32 ± 0.02 µg/ml and produced more than 75% inhibition of acetylcholine (Ach) and depolarising solution rich in KCl-induced guinea-pig ileum contractions. Chroroform and ethylacetate soluble fractions from the partition of Arredoul jaune exhibited good antibacterial and bactericidal activities with MIC and MBC < 100 µg/ml, good and strong antiamoebic activity with MAC value of 15.21 ± 0.07 and 8.25± 0.12 µg/ml respectively. They produced more than 70% inhibition of both agonists- induced contractions of ileum guinea-pig. *n*-butanol fraction showed good antibacterial activity against *Salmonella enteritidis*, *Shigella dysenria* and *S. flexneri* (MIC and MBC < 100 µg/ml) while the residual aqueous phase exhibited moderate or low activity against selected bacteria according to the case (MIC or MBC = 15 or 250 µg/ml). They exhibited moderate antiamoebic activity (MAC > 10 µg/ml) and produced more than 64% inhibitions of both agonists-induced guinea-pig ileum contractions. Total alkaloids extract displayed good antibacterial and bactericidal activities with MIC and MBC < 100 µg/ml, strong antiamoebic activity with MAC value of 3.57 ± 0.05, µg/ml and produced more than 76% inhibition of both agonists-induced guinea-pig ileum contractions. Crude saponins showed only weak antimicrobial activity, moderate antiamoebic activity (MAC = 12.47 ± 0.47 µg/ml and produced more than 60% inhibition of both agonist-induced guinea-pig ileum contractions. These results can partly justify and support the use of Arredoul jaune in courant live for the treatment of diarrhoea whatever the origin of the disease.

KEYWORDS: *Pentadiplandra brazzeana*, root, Arredoul jaune, diarrhoea, antimicrobial, antiamoebic and spasmolytic activities.

INTRODUCTION
Medicinal plants represent a rich source of antimicrobial, antispasmodic and antiamoebic agents and are used as a source of many potential and powerful remedies in several countries in the world.[1,2] These plants contain many bioactive natural substances which possess various biological activities and can lead to the discovery of new drug for animal and human.[3]

Infectious diseases are a prominent cause of morbidity and mortality worldwide, despite technical and scientific advances due to the ability of microorganisms of

resistance to many antibiotic substances.[4,5] But, many infectious diseases have been known to be treated with herbal extracts.[6] Moreover, a number of microorganism such as *Escherichia coli*, *Salmonella tiphymurium*, *Shigella flexneri*, *Staphylococcus aureus*, *Campylobacter* are responsible in the production of diarrhoea.[7] In addition, many natural metabolites with different chemical structures isolated from various medicinal plants have been proven to possess antimicrobial properties against Gram positive and negative bacteria *in vitro* and *in vivo* tests[8] and these medicinal plants can be used in part, to treat diarrhoea and other infectious diseases according to the case.

The diarrhoea is an expulsion of a quantity of bowel in an important volume than normal (> 300 g/day) and with great frequency (> 3 bowels /day). Bowels are generally wet, but in some cases soft, accompanied of abdominal pains, phlem or blood and variable symptoms depending of the cause of diarrhoea.[9] It is caused by some bacteria and virus. It is treated by application of a treatment on causal affection, slowing transit, regime without residue, to fight dehydration, faecal bacteriotherapy, the use of active or non-active charcoal and some drugs with antispasmodic, antibacterial and antiamoebic properties,[10] but also with various medicinal plants.[11] The disease is one of major health problem in tropical and subtropical countries and is responsible of about 5 to 8 million deaths per year mainly among children under five year of age particularly in developing countries.[12-14] The disease continues to be a major health problem through the world, especially causing malnutrition in children under five years old. Diarrhoea involves increased gastrointestinal mobility, secretion and decreased absorption of electrolytes and water.[15] In developing countries, the principal causes of diarrhoea is associated with enterotoxins that are produced and secreted from bacterial microorganisms like *Escherichia coli*, *Salmonella thyphi*, *Shigella flexneri*, *Staphylococcus aureus* and *Vibro cholera*.[16] On the other hand, many medicinal plants are used in traditional medicine as macerate and decoction to treat diarrhoea in many developing countries and people find some reliefs. Some of them are scientifically investigated to prove their effectiveness in animal model.[11,15,16,17-21] Thus it could be interesting to find some medicinal plants with antimicrobial, antispasmodic and antiamoebic activities against a specific type of diarrhoea.

The amoebiasis caused by the parasite *Entamoeba histolytica* is also another infectious disease which cause several problem in developing countries on children and adults. It also cause diarrhoea. It is treated using synthetic drugs which have more side effects. These all infections are treated in traditional medicine with various medicinal belonging to different botanical families since some of them have been reported to have antibacterial[2,5,6,21-26] spasmolytic,[23,27-28] and antiamoebic activity.[22,23,29]

Pentadipladra brazzeana is a shrub or climbing plant reaching 20 m long. The leaves are alternes, simples, limbs elliptic to oblanceolated reaching 15 x 5 cm. It has two basic forms. In can be much-branched shrub that can grow up 5 m tall, or climbing plant with stems up to 20 m long. Inflorescences in axillar grapes or terminal reaching 2 cm long, hermaphrodite flowers, unissued, pentameres, sepal elliptics, to lanceolated, green, purple on edges, free petals, lanceolated to oblacenceolated, whites or yellow. The fruit is a globulous berry of 2-4 cm diameter, orange, spotted of white to maturity. In Africa, the plant is found from Nigeria to Centrafrica and toward South until Democratic Republic of Congo (DRCongo) and Angola. It is found currently at the edge of rivers and savannah, and in secondary forests The plant commonly in upland primary forest dominated by *Scorodopleus zenkeri*, also commonoly occurs on river banks and in secondary forests. In some countries such as Cameroun, the plant is mainly found on the edges of forests bordering savannah. It is nowhere gregarious. A syrup made from the bark is also sold. It is the alone monotypic tree in Pentadiplandaceae family and relict genre in a family separated on the basis of evolution of Brassicales and having a strong affinity with the American genre *Tovaria* (Tovariaceae). It is also different on viewpoint of its chimiotaxanomy with other medicinal plants.[30,31]

***Pentadiplandra brazzeana* Baill. (Pentadiplandaceae)**

Root of *P. brazzeana* is commercialized in many African countries for medicinal uses. The root bark is used as an ingredient of African whisky in bag, cheap, but dangerous. Root smells aspirin and is hanged on top house entry or put under heights to move away snakes. Rooks are consumed par some populations as vegetable and spices, its fruit is an important source of sucrose searched by European and American laboratories working on healthfood. The moeders in breast-feeding use *P. brazzeana* fruit to wean their children: after consumption of fruit, the maternal milk becomes less sweet and insipid, provocating a refusal of breast by the child. The fruit has also aphrodisiac properties and is used as fish poison. The plant is largely commercialized in markets in Congo-Brazaville, Kisangani and Beni in Democratic Republic of Congo and others many African countries.[31]

P. brazzeana is a medicinal plant largely used in traditional medicine in some African countries to treat various ailments and known many therapeutic indications[32-37] presented in Table 1 below:

Table 1: Medicinal uses of *Pentadiplandra brazzeana*.

Plant part	Indications
Root mixed with fruit *Piper guineensis*	As a purgative, to treat lumbar pains, abdominal pains, constipation, cough, intestinal ulcers, diarrhoea, amoebiasis,
Root	kidney pains and haemorrhoids. This mixture is used as a tonic, aphrodisiac, antiblennorragic, antalgic, antienteralgic, antitussive and antisyphilitic. To treat thoracic pains, epilepsy, and also to deal with range of skin problems, prevent post-partum haemorragy, cutaneous infections, cough, malaria, itching, antiseptic for wounds, ulcers, boils, colic, gonorrhoea and other urogenital infections, used as laxative, purgative, cathartic, hardworking on abdomen, against oedema, provoke the abortion (stimulate uterine contractions), mixed to *Kalanchoe crenata* for nasal instillation to stop epilepsy crisis, emmenagogue and kwashiorkor.
Root bark	Excellent aphrodisiac. To stimulate lactation for women. Treatment of chest pains, lumbago, tooth pains, rheumatism, haemorrhoids, intercostal and abdominal pains and malaria.
Leaf mixed leaf of *Morinda morindoides*	Febrifuge and malaria.
Leaf	To treat scabies
Tubers	To treat gonorrhoea and other urogenital infections, intestinal problems such as dysentery, colic uretitis, mixed with its leaf as anthelmintic and antimalarial.

The roots of this medicinal plant is a vegetal material a very popular traditional medicine in central Africa, where it is commonly harvested from the wild for local use and for trade. The plant is also provided as an edible fruit, which has attached wider attention because of the very sweet-tasting protein that it contains. The root are sold for medicinal purposes in local markets in Africa and internationally via internet [37]. It contain alkaloids named difeuine I, II and III isolated in 1970 by [38], but their chemical structure are not yet elucidated until now. Others isolated compounds include carbamates (thio-uretane), thiocarbamates (methyl-benzylthiocarbamates, methyl-N-methoxybenzylthiocarbamates, ethyl N-methoxybenzylthiocarbamates), isothiocyanates, and thioures possessing antibacterial activity against *Bacillus subtilis, Escherichia colis, Salmonella enteritis, Shigella dysenteria* and *Staphylococcus aureus* and antifongic activity against *Candida albicans*.[39] Isopropyl-N-methoxybenzythiocarbamate, dibenzythioure and methylated derivatives.[30] and a thermostable protein named brazzein or pentadin with a strong commercial interest were also isolated from the root of *P. brazzeana*.[40-42] The sweetness profile of brazzein does not diminish after incubation at 100°C for 4 hours. It is also stable over a wide pH range and is the most water-soluble protein sweetener discovered so far. This protein is 500 and 2000 times sweet than saccharose depending on the method of measuring. It is also an edulcorant more used in alimentary industries. It was originally extracted from fruit pulps and is actually converted in edulcorant poor in calory destined to alimentary industries. Its commercial interest is strong. The technology which allows to extract this protein from fruit pulps as well as technologies intended to produce the protein brazzein in transferring the gene coding for brazzein to others organisms and was breveted without intention to divide advantages.[30]

The crude extracts of tubers were been reported to exhibit moderate antiplasmodial activity *in vitro*. The oral administration of these extracts to rats increased the weight of testicules and their prostate as well as the level of testosterone.[43] Root is also rich in glucosinolates which revitalise collagene and restore skin tonicity.[30] Roots and stem bark contain alkaloids, saponins, tannins. Flavonoids, anthraqiunones, steroids and terpenes were not detected in all plant parts.[34]

Fresh roots were used by the Sorgeri society in Kisangani, Democratic Republic of Congo to prepare an ameliorated galenic form (syrup) named Arredoul jaune used for the treatment of diarrhoea.

Some clinical studies were conducted on humans using Arredoul jaune. The study conducted by Dr Ngana in rural health zone of Kimpese (Bas-Zaire, actual Kongo-Central in RDCongo) on 12 patients older than 8 months and 35 years including females and males, with acute diarrhoea were submitted to a treatment with this phythomedicine. Results have indicated that 10 cases of diarrhoea were stopped without any modification and represent 83% of good results. In a treatment of symptomatique diarrhoea, 100% of good results and 0% of effectiveness against diarrhoea provocated by

Schitosoma manosoni were obtained. The last result suggered that Arredul jaune had no effect on the growth of this parasite and was thus devoid of schitosomicidal activity.

These preliminary clinical results indicated that Arredoul jaune is effective in the treatment of diarrhoea, but no scientific experiments was made to confirm its antidiarrhoeal activity seen in humans. Thus, the present study was conducted to evaluated antimicrobial, antispasmodic and antiamoebic activity of Arredoul jaune and its fractions, proprieties which can partly explain its claimed antidiarrhoeal activity.

MATERIALS AND METHODS

Plant material

Roots of *Pentadiplandra brazzeana* Baill. (Pentadiplandaceae) were collected in Kisangani-Zaire actual democratic Republic of Congo by the Sorgeri Society in Kisangani-Democratic Republic of Congo. The plant was authentified by Mr Nlandu Lukebiabo B. of the Institut National d'Etudes et de Recherche en Agronomie (INERA), Department of Biology, Faculty of Sciences, University Of Kinshasa. A voucher specimen NLBPDR 201510 of the plant was deposited in the herbarium of this institute. (Following test to be deleted).

Preparation of Arredoul jaune

1Kg of crushing were macerate with sufficient volume of ethanol (5000) for 2 days. After filtration, the filtrate was diluted with distilled water (1:2) to reduce the hot savior of alcohol and divided in sterile plastic bottles of 60 ml. It has a yellow colour and a faint hot savior. It is taken in drops: 30 to 50 drops for adults and 10 to 15 drops for children 2 times per day each.

Qualitative phytochemical screening

The qualitative phytochemical screening was carried out by TLC using silica gel plates (thickness layer 0.25 mm, Merck, Germany) and using different mobile phases described the literature for the identification of major phytochemical groups such as alkaloids, anthocyanes, anthraquinones, flavonoids, aminated compounds, coumarines, steroids, terpenes, reducing sugars, saponins and tannins (catechic, gallic and proanthocyanidins).[44]

Fractionation of Arredoul jaune, obtention of crude saponins and alkaloids

200 ml of Arredoul jaune were submitted to successive extraction with solvent of different polarities: chloroform, ethylacetate, *n*-butanol. All fraction were evaporated *in vacuo* yielding corresponding dried extract denoted as F1 (2.58 g) for chloroform, F2 (4.58 g) for ethylacetate F3 (3.15 g) for *n*-butanol. The residual aqueous phase was treated as described above giving dried extract denoted as F4 (5.84 g).

For saponins, 100 ml of Arredoul jaune was evaporated *in vacuo*. The resulting dried extract (6.89 g) was dissolved in 200 ml methanol and boiled to back-ward surge for 2 hours. After cooling, the methanol solution was concentration to small volume (50 ml) and an excess of diethylether (200 ml) was added giving an abundant precipitate. The precipitate was recuperated by filtration and dried. It give an abundant foam as a positive test for saponins. On the other hand, alkaloids were obtained by acid/base method described in literature[44] from 100 ml of Arredoul jaune (dried alkaloids extract: 4.25 g).

IN VITRO ANTIBACTERIAL TESTING

The following microorganisms were used: *Bacillus cereus*, *Escherichia coli*, *Salmonella enteritidis*, *Salmonella tiphymurium*, *Staphylococcus aureus*, *Shigella dysenteria and Shigella flexneri*. These microorganisms were clinical isolates obtained from patients at the Laboratory of Microbiology, Cliniques Universitaires du Mont Amba, University of Kinshasa, diagnosed with infections, mainly diarrhoea.

The antibacterial activity of samples was evaluated by the dilution method as previously described by[23,45,46] Briefly, microorganisms were cultured overnight at 37°C in agar base (Difco) in sterile tubes. Colonies were directly suspended into a small volume 0.9% saline. 5 ml of this suspension was added to 100 µl Muller-Hinton medium (Difco) together with the separated sample dilution tests (0.1-100 µg/ml) from Arredoul jaune and its fractions. On the other hand, a sterile tube containing only bacterial suspension and distilled water was used as a negative control, an other tube containing bacterial suspension and Ampicillin and Tetracycline (0.1 to 25 µg/ml) was considered as a positive control.

The lowest concentration of the test sample that inhibited the bacterial growth after incubation was taken as the MIC value. A volume of 10 µl from each test tube was placed on new culture medium in order to determine the MBC which was defined as the lowest concentration yielding negative subcultures or only one colony. All samples were tested in triplicate.

IN VITRO ANTIAMOEBIC TESTING

Entamoeba histolytic used in the present study is a laboratory isolated strain from patients with acute dysentery diagnosed in the Tropical Medicine Institute, Faculty of Medicine, University of Kinshasa. The evaluation of activity was performed using the method previously described by.[22,29]

Briefly, the parasite was grown and cultured in sterile tubes containing 9 ml of diphasic medium (medium N of Pasteur Institute) called Dobbell and Laidlaw medium. The mixture was stirred and incubated for one week at 37°C. The daily examination and counting of amoebae through a optic microscope with the aid of Neubauer's cells were performed in order to monitor the parasitic growth and to detect possible contamination.

Uncontaminated tubes containing an average number of 2.5×10^6 amoebae/ml culture medium were selected as

test tubes. 10 mg of each test sample was dissolved in 10 ml hydroethanol solution (eau-ethanol :9:1) to have corresponding stock solutions of 1 mg/ml. These last solutions were submitted to two fold dilutions to give a series of test solutions of 500 to 0.1 µg/ml. Next, 1 ml of the test solution with a known concentration was added to a separated test tubes containing parasites (1 ml). On the other hand, two tubes were used as controls, one containing parasites in hydroethanol (1:9) without test sample as negative control and an other containing test tubes with parasites and Metronidazole or Dehydroemetine as positive controls tested at concentrations from 10 to 0.1 µg/ml.

All tubes were plugged with sterile cotton, vigorously stirred and incubated at 37°C for 6 days. The daily counting of dead and living amoebae was done as described above. The test was considered as positive when the vegetative or kystic forms of amoebae was not microscopically observed. The minimum amoebicidal concentration (MAC) for each tested sample was determined by using linear-courbes doses-responses. The test for each sample was done in duplicate.

SPASMOLYTIC ACTIVITY TESTING ON ISOLATED GUINEA-PIG ILEUM

The spasmolytic activity of the aqueous extracts, ethanol extracts and their respective fractions from Arredoul jaune and its fractions were evaluated according to the procedure previously described by.[27,29] For this, male guinea-pig (200-300 g body weight) were killed by blow to the head. The ileum was removed and washed before with distilled water and after with Tyrode solution composed with: (mM): KCl:2.2, $MgCl_2$: 0.11, $NaH_2PO_4.2H_2O$: 0.42, $CaCl_2$: 1.8, NaCl: 137, $NaHCO_3$: 11 and glucose: 5.6. Lengths of approximately 2.5 cm of the guinea-pig ileum were cut from the middle region of the ileum and transferred into an organ bath containing 50 ml of Tyrode's solution maintained at 37°C under carbogen (95% O2 + 5% CO2) bubbling and connected to a Palmer kymograph transducer to record the longitudinal muscle contraction of the ileum.

The guinea-pig ileum strips was allowed to stabilize during 30 min. 2 ml of Tyrode's solution was removed in organ bath and the ileum stimulated every 5 min either with 2 ml of acetylcholine solution (5 x 10^{-5}g/l) or with 2 ml of the depolarized solution rich in KCl ((mM): NaCl: 2.7, KCl: 100,$NaHCO_3$: 15, $CaCl_2$: 1.25, $MgCl_2$: 12.5 and glucose: 11) to obtain maximal contraction of the ileum. 10 mg of of extract and fractions were dissolved in 10 ml distilled water or in ethanol/ water 1/9 to have respective stock solution of 1 mg/ml After obtaining three consecutive maximal contractions of the ileum in the presence the agonist, the ileum was washed with Tyrode's solution, 2 ml of the Tyrode's solution was removed and then 2 ml of the respective stock solution in distilled water (1 mg/ml) was added in the organ (representing 40 µg/ml of the test sample in organ bath). The guinea-pig ileum was maintained in contact with the test sample for 30 min before the next stimulation with the respective agonist. Atropine and Papaverine (5 µg/ml in organ bath) were used as a reference antispasmodic products (n = 3).

The inhibition of ileum contraction by sample in the presence of each agonist was expressed as percentage of mean ± S.D from three experiments and was calculated using the following formula: % Inhibition = (A-B) x 100/A were A is the amplitude of the ileum concentration (cm) induced by the agonist and B the amplitude of the ileum contraction induced by the test sample in the presence of the agonist.[23,27,29]

STATISTICAL ANALYSIS

All data collected were summarized as mean ± sem. Significant differences were determined using Student-*t* test and the difference were considered as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

Results of qualitative phytochemical analysis of Arredoul jaune revealed the presence of alkaloids, saponins, aminated compounds and catechic tannins. Other phytochemical groups such as cardiotonic heterosids, steroids, terpenes, anthraquinones, flavonoids, gallic tannins, proanthocyanidins, coumarines and organic acids were not detected in our experimental conditions (Table 1). Our results are in good agreement with.[34]

***In vitro* antimicrobial activity**

The selected test microorganisms in this study were sensitive to Ampicillin and Tetracycline at 1.95 µg/ml. For a good interpretation of the results, following criteria were adopted: MIC or MBC ≤ 10 µg/ strong activity,10 < MIC or MBC ≤ 100 µg/ml: good activity, 100 < MIC or MBC ≤ 250: moderate activity, 250 < MIC or MBC ≤ weak activity, MIC or MBC > 500 µg/ml: inactive. The antibacterial activity of test concentrations of Arredoul jaune, its fractions, (Following test to be deleted), crude saponins and total alkaloids extract is show in Table 2.

Results revealed that all tested samples showed significant antibacterial activity with varying magnitudes. Among them, Arredoul jaune exhibited good antimicrobial activity against six bacteria with MIC values < 100 µg/ml [45, 47] and moderate activity against *E. coli* (MIC = 125 µg/ml).

Table 2: Antimicrobial activity of Arredoul jaune, its fractions, crude saponins and total alkaloids extract (MIC, µg/ml.

Echantillons	*B. s*	*E.c*	*S.e*	*S.d*	*S.a*	*S. f*	*S.t*
Arredoul jaune	62.50	125	62.50	62.50	31.25	34.25	34.25
F1	125	62.50	62.50	250	31.25	31.25	62.50
F2	31.25	62.50	125	31.25	31.25	62.50	31.25
F3	250	125	250	250	62.50	62.50	62.50
F4	250	250	500	500	125	125	125
Crude de saponins	250	>500	>500	>500	>500	>500	125
Total alkaloids extract	31.50	62.50	62.50	62.50	62.5	62.50	31.25
Ampicillin	1.95	3.90	3.90	3.90	1.95	1.95	1.95
Tetracycline	3.90	3.90	3.90	3.90	1.95	1.95	1.95

B.s: bacillus subtilis, E.C. Escherichia coli,:S.e: Salmonella enteritidis, S.a : Staphylococcus aureus, S.d. Shigella dysenteria, S.f : Shigella flexneri, S.t : salmonella tiphymurium. F1, F2, F3 and F4 : chloroform, ethylacetate, *n*-butanol and residual aqueous phase respectively from the partition of Arredoul jaune.

Chloroform F1 soluble fraction rich in steroids and terpenes showed good antimicrobial and bactericidal activity against 5 selected microorganisms (MIC or MBC values < 100 µg/ml),[45,47] with moderate activity against *B. subtilis* and *S. dysenteria* with MIC values of 125 and 250 µg/ml respectively.

Ethylacetate F2 soluble fraction rich in phenolic compounds (different to that seen in residual aqueous phase F4 by TLC) displayed good antimicrobial and bactericidal activities against all selected bacteria (Table 2 and 3). *n*-butanol F3 rich in saponins showed good activity against 3 bacteria [45, 47] and moderate activity against 4 bacteria (2). It *exhibited* good bactericidal activity against *S. flexneri* and *S. thiphymurium* with MBC values of 62.5 µg respectively and moderate or week bactericidal activity against other selected bacteria (Table 3). The residual aqueous F4 soluble fractions rich in phenolic compounds display moderate antibacterial activity against all selected bacteria (Table 2); moderate and weak activity or was inactive against some selected bacteria (Table 3).

Crude saponins were inactive against 5 selected bacteria (MC > 500 µg/ml (Table 2) and displayed moderate activity against *B. subtilis* and *S. tiphymurium* with MIC values of 250 and 125 µg/ml respectively while total alkaloids extract displayed good antimicrobial activity against all selected bacteria (MIC < 100 µg/ml)[45,47] (following test to be deleted).

Concerning their bactericidal activity, it was observed that Arredoul jaune showed good activity against 4 selected bacteria and moderate activity against *B. subtilis, E. coli* and *S. dysenteria*. Chloroform F1 soluble fraction displayed good bactericidal activity against 3 selected bacteria (Table 3)[45,47] and moderate activity against *B. subtilis, S. enteritis, S. dysenteria* and *S. tiphymurium* (MIC = 125 or 250 µg/ml). Ethylacetate soluble fraction displayed good bactericidal activity against a large number of selected bacteria with MBC < 100 µg/ml[45,47] and moderate activity only against S. *enteritidis and S. dysenteria* (Table 3). *n*-butanol fraction showed low activity against *B. subtilis, E, coli* and *S. dysenteria* (MBC = 500 µg/ml, moderate activity against *E. coli* an *S. aureus* (MBC = 125 µg/ml), and good activity against *S. flexneri* and *S. tiphymurium* (MBC = 62.50 µ/ml respectively). The residual aqueous F4 soluble fraction showed weak activity against *B. subtilis* and *E. colis*, inactive against *S. enteritidis* and *S. dysenteria* and exhibited moderate activity against *S. aureus, S. flexneri* and *S. tiphymurium* (Table 3).

Table 3: Bactericidal activity of Arredoul jaune, its fractions, crude saponins an total alkaloids extract (MBC, µg/ml).

Samples	*B. s*	*E.c*	*S.e*	*S.d*	*S.a*	*S. f*	*S.t*
Arredoul jaune	125	125	62.50	125	62.50	62.50	62.50
F1	250	62.50	125	500	62.50	31.25	125
F2	31.25	62.50	250	250	62.50	62.50	62.50
F3	500	125	500	500	125	62.50	62.50
F4	500	500	> 500	>500	125	250	125
Crude de saponins	500	>500	>500	>500	>500	>500	125
Total alkaloids extract	31.50	62.50	125	125	62.5	62.50	31.25
Ampicillin	3.90	3.90	7.80	7.80	3.90	1.95	1.95
Tetracycline	3.90	3.90	7.80	7.80	3.90	1.95	3.90

See Table 2

Crude saponins did not show interesting bactericidal activity since they were inactive against 5 bacteria and showed moderate activity against *S. tiphymurium* and weak bactericidal effect against *B. subtilis* (Table 3). Total alkaloids extract exhibited good bactericidal activity against all selected bacteria (MBC < 100 µg/ml).

Regarding the level of antimicrobial of fractions from Arredoul jaune, it was concluded that this activity was located in ethylacetate fraction which exhibited high activity compared to other fractions and to some extends to the total alkaloids extract.

The literature has disclosed that the responsible antimicrobial constituents from some medicinal plants include flavonoids,[48-51] alkaloids,[46,51-53] steroids, terpenes, tannins and saponins.[54] The presence of some of these phytochemical groups in Arredoul jaune may account for the observed antimicrobial activity. The antimicrobial activity of all tested samples was weaker compared to Tetracycline and Ampicillin used as antimicrobial reference products (Tables 2 and 3).

***In vitro* antiamoebic activity**

The antiamoebic activity of Arredoul jaune, its fractions, crude saponins and the total alkaloids expressed in minimal amoebicidal concentration (MAC) is presented in Table 4. The criteria adopted for antimicrobial activity are also valid for antiamoebic activity. Thus, Arredul jaune, chloroform F1 and ethylacetate F2 soluble fractions as well the total alkaloids extract exhibited strong antiamoebic activity with MAC values of 4.32, 8.21, 4.25 and 3.57 ± 0.05 µg/ml respectively.

Table 4: Antiamoebic activity of Arredul jaune, its fractions, crude saponins and total alkaloids.

Samples	MAC (µg/ml)
Arredoul jaune	4.32 ± 0.02
F1	8.21 ± 0.07
F2	4.25 ± 0.12
F3	17.35 ± 0.22
F4	10.04 ± 0.14
Crude saponin	12.47 ± 0.74
Total alkaloids extract	3.57 ±0.05
Metronidazole	0.05 ±0.01
Pyracantel	0.07±0.02

ee Table 2, MAC: minimal amoebicidal concentration

n-butanol F3, residual aqueous F4 soluble fractions and crude saponins showed good antiamoebic activity with MAC values of 17.35 ±0.22, 10. 04 ±0.14 and 12.47 ±0.74 µg/ml. The activity of ethylacetate F2 fraction was higher activity ($p < 0.05$) compared to other soluble fractions and crude saponins ($p < 0.05$), weaker activity compared to other tested samples ($p < 0.05$) (Table 4). The literature have reported the antiamoebic activity of some secondary metabolites including flavonoids[55,56] and alkaloids.[57-59] Thus, the observed antiamoebic activity of this phytomedicine may be due to the presence of phenolic compounds and alkaloids content. The antiamoebic of all tested samples was weaker compared to Metronidazole and Pyracantel used as antiamoebic reference products (Table 4).

Spasmolytic activity on isolated guinea-pig ileum

The spasmolytic activity of Arredoul jaune, its fractions, crude saponins and total alkaloids extract is presented in Table 5. Results indicated that Arrredoul jaune exhibited high spasmolytic activity since it inhibited acetylcholine and depolarising solution rich in KCl-induced guinea-pig ileum contractions by 81.21 ±0.03 and 78.54 ±0.12% respectively when tested at 40 µg/ml in organ bath.

Table 5: Spasmolytic activity of Arredoul jaune, its fractions crude saponins and total alkaloids: % inhibition of acetylcholine and depolarising solution rich in KCl induced- contraction of guinea-pig ileum.

Samples	Acetylcholine	Depolarising solution rich in KCl
Arredoul jaune	81.21 ±0.03	78.54 ±0.12
F1	74.11 ±0.05	71.54 ±0.14
F2	76.24 ±0.08	73.69 ±0.04
F3	68.54 ±0.01	64.21 ±0.06
F4	72.45 ±0.15	69.78 ± 0.11
Crude de saponines	67.54 ± 0.04	62.57 ±0.08
Total alkaloids extract	78.54 ±0.14	76.87 ±0.17
Papaveriine	99.47 ±0.07	97.87 ±0.05
Atropine	100.00 ±0.00	-

See Table 2

Chloroform F1 and ethylacetate F2 soluble fraction rich in steroids and terpenes, and flavonoids respectively inhibited the contractions induced by both agonists more than 70%. *n*-butanol F3 and residual aqueous F4 soluble fractions showed spasmolytic activity by producing more than 65% of both agonists-induced guinea-pig ileum contractions with with the activity of F4 higher than F3 (Table 5). The activity of fraction F2 was higher compared to other fractions ($p < 0.05$). Crude saponins exhibited good spasmolytic activity since it produced more than 60% inhibition of both agonists-induced guinea-pig ileum contractions while the total alkaloids

extract produced 78.54±0.14 and 76.87±0.17% inhibition of both agonists respectively (Table 5). Regarding the effects of Arredoul jaune, its fractions, crude saponins and total alkaloids extract on the action of both agonists on isolated guinea-pig ileum, it was concluded that these samples possessed papaverine like effects. The spasmolytic activity of tested samples from Arredoul jaune was weaker compared to papaverine and atropine used as spasmolytic reference products (Table 5).

Spasmolytic constituents isolated from some medicinal plants are reported in the literature. It concerns flavonoids isolated form *Morinda morindoides* (Rubiaceae),[29] *Alchornea cordifolia* (Euphorbiacea)[60] and *Psydium guyava* (Myrtaceae),[61-64] and alkaloids.[65] Thus, the observed spasmolytic activity in Arredoul jaune may be due to the presence of other phenolic compounds and mainly to the alkaloids content.

In conclusion, it is well known that herbal based phytomedicine plant part have large therapeutic applications since they can treat some diseases and have less side effects when compared with synthetic substances. In the present study, the reported biological activities antimicrobial, spasmolytic and antiamoebic activities can partly justify and support the claimed antidiarrhoeal property attributed to the phytomedicine Arredoul jaune based ethanol extract from fresh roots of *P. brazzeana* largely used in Kisangani and other provinces of Democratic Republic of Congo in courant live to treat diarrhoea in children as well as in adults. These biological activities of Arredoul jaune may be due to the presence of some secondary metabolites such as phenolic compounds such as tannins and mainly alkaloids identified in this phytomedicine. Due to the great interest in the use of phytomedicine based medicinal plant parts, results of this study have importance in determining the beneficial effects of the studied product and may be used as new antidiarrhoeal drug without significant side effects in human.

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