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Revue Congolaise des Sciences & Technologies

ISSN: 2959-202X (Online); 2960-2629 (Print)

<https://www.csnrdc.net/>REVUE
CONGOLAISE
DES SCIENCES
ET TECHNOLOGIES

Evaluation of the antibacterial activity of *Mitragyna stipulosa* (DC.) O. (Kuntze, 1891) on *Streptococcus pneumoniae* (Klein, 1884) Chester, causative agent of Pneumonia

[Évaluation de l'activité antibactérienne de *Mitragyna stipulosa* (DC.) O. (Kuntze, 1891) sur *Streptococcus pneumoniae* (Klein, 1884) Chester, agent causal des Pneumonies]

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Résumé

La pneumonie est l'une des principales causes de décès chez les enfants de moins de cinq ans et une cause majeure de mortalité infantile dans toutes les régions du monde, la plupart des décès survenant en Afrique subsaharienne et en Asie du Sud. L'agent principal ayant la prévalence la plus élevée dans les pneumonies graves est, comme son nom l'indique, la bactérie *Streptococcus pneumoniae*. L'objectif général de cette étude est d'évaluer l'activité antibactérienne de la décoction des feuilles de *Mitragyna stipulosa* sur la bactérie *S. pneumoniae* par le test de l'antibiogramme, de déterminer la concentration minimale inhibitrice et de comparer l'effet antibactérien de la décoction par rapport à l'infusion et à la macération, ainsi qu'à l'antibiotique de référence utilisé. Ce travail s'inscrit dans le cadre de la promotion et de la valorisation de la pharmacopée africaine en général et de la pharmacopée congolaise en particulier, pour venir à bout d'un problème de santé publique avéré et important.

Mots clés : *Mitragyna stipulosa*, *Streptococcus pneumoniae*, antibiogramme, concentration minimale inhibitrice, criblage chimique.

Abstract

Pneumonia is one of the leading causes of death in children under five and, a major cause of infant mortality in all regions of the world, most deaths occur in sub-Saharan Africa and South Asia. The main agent with the highest prevalence in severe pneumonia is, as the name suggests the bacterium *Streptococcus pneumoniae*. The general objective of this study is to evaluate the antibacterial activity of the decoction of the leaves of *Mitragyna stipulosa* on the bacterium *S. pneumoniae* by the antibiogram test, to determine the minimum inhibitory concentration and, to compare the antibacterial effect of the decoction compared to infusion and maceration, as well as the reference antibiotic used. This work is part of the promotion and enhancement of the African pharmacopoeia in general and the Congolese pharmacopoeia in particular, to overcome a proven and significant public health problem.

Keywords: *Mitragyna stipulosa*, *Streptococcus pneumoniae*, antibiogram, minimum inhibitory concentration, chemical screening.

1. Introduction

Plants contain chemical compounds that are just as potent as those found in prescription drugs. (Shaki and Bencheick, 2008). Since medicinal plants are pharmacologically active, they can be responsible for harmful, dangerous or even fatal effects requiring continuous vigilance (Shaki and Bencheick, 2008). According to the World Health Organization (WHO), traditional medicine is defined as the set of all practical

knowledge, explicable or not, to diagnose or eliminate a physical or mental imbalance, relying exclusively on lived experience and the observation, transmitted from generation to generation (orally or in writing) (Adjanooun et al., 2001).

Compounds found in plants are of many types, but most belong to four major biochemical classes, alkaloids, glycosides, polyphenols and terpenes (Sexana et al., 2013).

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Reçu le 26/04/2024; Révisé le 29/05/2024 ; Accepté le 18/06/2024

DOI: <https://doi.org/10.59228/rcst.024.v3.i2.88>

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In addition, according to the WHO, nearly 6377 species of plants are used in Africa, of which more than 400 are medicinal plants which constitute 90% of traditional medicine. In 2004, nearly 75% of the African population used the plants around them to heal themselves and did not have access to so-called modern medicines (WHO, 2002).

Pneumonia is one of the leading causes of death in children under five and is a leading cause of infant mortality in all regions of the world, with most deaths occurring in sub-Saharan Africa and South Asia (Wardlaw et al., 2006).

The principal agent with the highest prevalence in cases of severe pneumonia is, as its name suggests: the bacterium *Streptococcus pneumoniae* (Wardlaw et al., 2006).

Medicinal plants have special value and importance in ensuring the health status of society in terms of treatment and prevention of human diseases (Lozano et al., 2013).

Antimicrobials derived from plant extracts are effective in treating infectious diseases without several side effects typically associated with synthetic antimicrobials (Lozano et al., 2013).

The side of the leaves of *Hallea stipulosa* (DC) J-F Leroy (1975) renamed *Mitragyna stipulosa* (DC) Kuntze (1891), is used in the treatment of pneumonia (Konda et al., 2014).

In previous studies, *M. stipulosa* demonstrated medicinal properties: antivenomous (Fatima et al., 2002), anticonvulsant, anxiolytic and sedative (Fageyinbo et al., 2018), antibacterial (Aboaba et al., 2006).

Pharmacological investigations have also demonstrated that plants of the genus *Mitragyna* possess extensive pharmacological effects; antitumor, antibacterial, and against cardiovascular diseases (Gong et al., 2012).

Our research question is therefore whether pneumonia caused by *S. pneumoniae* can be effectively treated by aqueous extracts of *M. Stipulosa* leaves.

The general objective of this study was to evaluate the antibacterial activity of *M. Stipulosa* leaf decoction on *S. pneumoniae* bacteria using the antibiogram test.

2. Materials and methods

2.1. Area

The study was carried out at the Mention Sciences de la Vie, Faculty of Sciences and

Technologies, of the University of Kinshasa. The analyzes were carried out at the Bacteriology Laboratory of the University Clinics of Kinshasa.

2.2. Materials

2.2.1. Vegetal material

The biological material consists of leaves of *M. stipulosa* (DC) O. Kuntze, 1891 (Synonym: *Hallea stipulosa* (DC) J.-F. Leroy), harvested in the Lac de Ma Vallée concession, south-west of Kinshasa. This species belongs to the Rubiaceae family.

2.2.2. Bacterial strains

The bacterial biological material consists of *S. pneumoniae*, a Gram-positive diplococcus with a lanceolate appearance belonging to the phylum Firmicutes, the class Bacilli, the order Lactobacillales and the family Streptococcaceae (Janoir, 2014). It is a commensal bacterium of the human upper airways with a nasopharyngeal prevalence of 60% in children (Cohen et al., 2012) and 10% in adults (Orihuela & Tuomanen, 2006).

2.3. Methods

2.3.1. Preparation of aqueous extracts of fresh leaves.

a) Maceration

- Weigh 185 g of well-cleaned fresh leaves (Balance BERKEL),
- Chop the leaves and grind them in a blender (New Jumbo III, Model DA3000A),
- Add 555mL of distilled water to obtain a 1/3 (m/v) ratio.
- Macerate the leaf paste for 10 minutes.
- Leave the macerated to rest then filter with filter paper (Qualitative filter paper, Jiao jie) under a vacuum pump. The extract is stored in a 500mL flask in a refrigerator (Whirepool).

b) Decoction

- Weigh 185 g of fresh leaves (BERKEL balance),
- Roughly cut with a knife,
- Put in a pot mounted on a hot plate (WiseStir),
- Add 555mL of distilled water to have a ratio of 1/3 (m/v),
- Boil for 15 minutes, then let cool to room temperature,
- Filter the decoction with a filter paper (Qualitative filter paper, Jiao jie) under a vacuum pump,
- The extract is stored in a 500 mL glass jar in a refrigerator (Whirpool)

c) *Infusion*

- Weigh 160 g of fresh leaves, coarsely cut with a knife,
- Heat distilled water in a pot on a hot plate (480 mL) to have a ratio of 1/3 (m/v),
- Put the leaves in boiling water, then let cool to room temperature,
- Filter the infused with filter paper (Qualitative filter paper, Jiao jie) under a vacuum pump,
- Store the decoction in a 500mL glass jar in a refrigerator (Whirpool).

2.3.2. *Preparation of aqueous extracts of dried leaves*a) *Maceration*

- Dry the leaves in the shade, and grind them in a blender (New Jumbo III, Model DA3000A) to obtain a fine powder,
- Weigh 100 g of powder (Metler Toledo analytical balance),
- Put in a glass jar, and add 300 mL of distilled water to have a ratio of 1/3 (m/v),
- Leave to macerate for 1 hour,
- Filter the macerated with a filter paper (Qualitative paper, Jiao jie) under a vacuum pump,
- Store the extract in a 500 mL glass jar in the refrigerator.

b) *Decoction*

- Dry the leaves in the shade, grind in a mixer (New Jumbo III, Model DA3000A) to obtain a fine powder,
- Weigh 100 g of powder (Metler Toledo analytical balance),
- Add 400 mL of distilled water in a jar, to have a ratio of ¼ (m/v),
- Mix everything and boil for 15 minutes on a hot plate (WiseStir),
- Let cool to room temperature,
- Filter the decoction through filter paper (Qualitative filter paper Jiao, jie) under a vacuum pump,
- Store the extract in a 500 mL glass jar in the refrigerator (Whirpool).

c) *Infusion*

- Dry the leaves in the shade,
- Grind the dried leaves in a blender (New Jumbo III, Model DA30000A) to obtain a fine powder. Weigh 100 g of powder (Metler Toledo analytical balance),
- Heat 300mL of distilled water until boiling (Ratio 1/3: m/v),

- Add powder to boiling water and let cool to room temperature.
- Filter the infused with a filter paper (Qualitative filter paper, Jiao jie) under a vacuum pump.
- Store the extract in a 500 mL glass flask in a refrigerator (Whirpool).

2.3.3. *Preparation of daughter solutions*

Prepare 3 daughter solutions for all the extracts, by dilution according to the relationship $C1V1=C2V2$. From the stock solution, we prepared a first daughter solution of 1mg/mL which is a standard concentration used during analyzes of antimicrobial activity of plant extracts according to [Sandeep et al., \(2010\)](#); [Govindappa et al., \(2011\)](#); [Linthoingambi & Mutum, \(2013\)](#) and [Marimuthu et al., \(2014\)](#). The first daughter solution was then diluted to ½ to obtain the second daughter solution (500 µg/mL). The second daughter solution was then diluted to ½ to obtain the third daughter solution (250 µg/mL). The daughter solutions were stored in 28 mL glass vials of aromatic essence, then kept cool in a refrigerator (Whirpool). The volumes of distilled water and extract solutions were sampled using a 100 µL micropipette and a 2 mL pipette.

2.3.4. *Bacteriological tests*a) *Pharyngeal carriage*

The strain of *S. pneumoniae* was obtained from a pharyngeal carriage. Pharyngeal carriage was done using a sterile swab, followed by direct inoculation in streaks on COLUMBIA BLOOD AGAR BASE culture medium. Pharyngeal carriage was performed on 5 children whose age varied between 3 and 15 years. The bacteriological manipulations took place in the Bacteriology Laboratory of the Faculty of Medicine and that of the Clinical Biology Service of the University Clinics of Kinshasa (CUK), of the University of Kinshasa.

b) *Preparation of culture media*

We prepared the COLUMBIA BLOOD AGAR BASE culture medium according to the manufacturer's recommendations. Weigh 39 g of powder (OHAUS-PIONEER analytical balance) and dissolve in one liter of distilled water; Prepare Mueller Hinton II Agar medium, for the determination of the inhibitory concentration. Weigh 38 g of powder (OHAUS-PIONEER analytical balance) and dissolve in one liter of distilled water. The media were then sterilized in an autoclave (LDZX-75B) at 120 °C for 15 minutes, and allowed to cool to room temperature; The media were

then poured into Petri dishes and kept cool in a refrigerator (SAMSUNG).

c) Culture

The inoculated medium was placed in an incubator (MEMMERT) at 37° C. and 5% CO₂ for 24 h. After 24 hours of incubation, the formation of a greenish halo could be observed in certain places in the medium, testifying to an α -hemolytic activity which is one of the characteristics of *S. pneumoniae*.

d) Macroscopic test and sensitivity test

Confirmation of the identity of the bacterium was made by the optochin sensitivity test, an antibiotic commonly used in the diagnosis of pneumococcal infections. *S. pneumoniae* is susceptible to optochin. However, 5% of pneumococcal strains are resistant to this antibiotic and very rare strains of oral streptococci have been described as sensitive to optochin (Freney, 2007; Kellog, 2001).

Around an alcohol lamp we transplanted a visible colony located in the greenish part of the medium, to inoculate it in a healthy medium (COLUMBIA BLOOD AGAR BASE);

Then we placed an optochin disc loaded with 5 μ g, then incubated at 37°C for 24 hours. Reading the results gave us a zone of inhibition of 14.5 mm, thus validating our test according to Burckhardt (2017).

e) Biological tests

1° Antibiogram

- We used amikacin discs loaded at 30 μ g as the reference antibiotic and positive control;
- Then, using a perforator, we perforated blotting paper to obtain discs to be soaked in our different daughter solution extracts;
- Wearing gloves and using a Bunsen burner, we transplanted a colony of *S. pneumoniae* onto a healthy culture medium (COLUMBIA BLOOD AGAR BASE), then using tweezers we placed the reference antibiotic in the centre of the medium and the discs soaked in the daughter solution extracts all around, taking care not to place them too close together to avoid overlapping the inhibition zones and placing them far enough from the edge of the Petri dishes. The whole set was then incubated at 37°C for 24 hours, each disc having a code number written with a marker on the bottom of the Petri dishes.

2° Test for determining the minimum inhibitory concentration (MIC)

The MIC determination test was carried out in test tubes each containing 9 mL of physiological saline.

- Put using a 5 cc syringe, 9mL of physiological saline in 49 test tubes,
- Make 1/10 dilutions up to a dilution factor of 10⁻⁸,
- The test tubes were then plugged with cotton wool and then incubated at 37°C for 48 hours,
- After 48 hours, the reading of the MIC was done by direct observation of the turbidity of the medium on a white background,
- The MIC was calculated by the following formula:

$$\text{MIC} = \text{Cs} \times \text{fd}$$

Where Cs = concentration of the extract solution used; fd = dilution factor.

2.3.6. Data analysis

The data were encoded in Excel 2016 and R software was used for visualize our data.

3. Results and Discussion

3.1. Antibiogram

Table 1 presents the overall values obtained during the antibiogram test carried out with the daughter solutions.

Table 1. Overall result of the antibiogram test

Diluted solutions	Concentration (μ g/mL)	DZI (mm)
Distilled water	-	-
A1	1000	13
A2	500	10
A3	250	-
B1	1000	14
B2	500	14
B3	250	13
C1	1000	14
C2	500	13
C3	250	15
D1	1000	14
D2	500	14
D3	250	13
E1	1000	11
E2	500	11
E3	250	11
F1	1000	14
F2	500	12
F3	250	10
Amikacine	30	24

Legend: Distilled water = Negative control; A1 = first daughter maceration solution with fresh leaves; A2 = second daughter solution of maceration with fresh leaves; A3 = third daughter solution of maceration with fresh leaves; B1 = first daughter solution of decoction with fresh leaves; B2 = second daughter solution of decoction with fresh leaves; B3 = third daughter solution of decoction with fresh leaves; C1 = first infusion daughter solution with fresh leaves;

C2 = second infusion daughter solution with fresh leaves; C3 = third infusion daughter solution with fresh leaves; D1 = first daughter maceration solution with dry leaves; D2 = second daughter solution of maceration with dry leaves; D3 = third daughter solution of maceration with dry leaves; E1 = first infusion daughter solution with dry leaves; E2 = second infusion daughter solution with dry leaves; E3 = third infusion daughter solution with dry leaves; F1 = first daughter solution of decoction with dry leaves; F2 = second daughter solution of decoction with dry leaves; F3 = third daughter solution of decoction with dry leaves; Amikacin = positive control; DZI = Diameter of the Inhibition Zone

From [table 1](#) we can see that the antibacterial activity of the daughter solutions follows a lower or equal step progression, i.e. from the first daughter solution to the third daughter solution, the activity is lower than or equal to the previous daughter solution. The daughter solutions in extract C are an exception to this pattern, as C3 gave a higher DZI than C1 and C2. As we proceeded by direct measurement without repetition, it could be that a parameter in the manipulation influenced this result, just as this result could be justified by the unique phytochemical profile of the extract, knowing that the molecules interact and that these interactions can reinforce, diminish or even cancel out the biological activity.

According to CASFM recommendations ([Soussy et al., 2013](#)), three clinical categories have been retained for the interpretation of in vitro susceptibility tests: Susceptible (S), Resistant (R) and Intermediate (I).

S strains are those for which the probability of therapeutic success is high in the case of systemic treatment with the dosage recommended in the summary of product characteristics (SPC), drawn up by the French Agency for Sanitary Safety of Products of Health (AFSSAPS).

R strains are those for which there is a high probability of therapeutic failure regardless of the type of treatment and the dose of antibiotic used. I strains are those for which therapeutic success is unpredictable. The intermediate category is also a buffer zone that takes into account technical and biological uncertainties ([Soussy et al., 2013](#)).

The reference antibiotic used in this study is Amikacin, which is an aminoglycoside antibiotic similar to gentamicin, discovered in 1972 by Japanese researchers ([Kumazawa & Yagisawa, 2002](#)). According to CASFM recommendations ([Soussy et al., 2013](#)), the standard critical diameters of Amikacin during the antibiogram test are presented in [table 2](#).

Table 2. Standard critical diameters of Amikacin

Antibiotic	Disk load	Critic diameters (mm)	
		S	R
Amikacine	30 µg	≥ 17	< 15

Legend: S = sensible; R = resistant

According to this determination scale, only extract 9 (C3), third infusion daughter solution with fresh leaves, 250 µg/mL, is in the intermediate zone. All the other extracts that produced DZIs strictly below 15 mm had no effect on the bacteria, which proved to be resistant.

In the intermediate zone, the strains may present a resistance mechanism whose expression is not sufficient to justify classification in the R category, but weak enough to hope for a therapeutic effect under certain conditions (high local concentrations or increased dosages) ([Soussy et al., 2013](#)).

It should also be noted that various abiotic factors influence qualitatively and quantitatively the production of secondary metabolites. A wide range of environmental stresses (high and low temperature, drought, alkalinity, salinity, UV stress, nutrient deficit and pathogen infection) are potentially harmful to plants, and determine the quantity and quality of secondary metabolites produced ([Akula, 2011](#)).

The extracts having given a DZI of between 10 mm – 14 mm are therefore not devoid of antibacterial activity. Indeed, if there was no activity, this would be observed by anarchic colonization of the bacteria and no halo formation.

When preparing extracts or phyto-therapeutic solutions in the villages, the notion of concentration is generally not taken into account.

While herbal medicine practitioners are often comfortable with the application of these medicines and are also widely convinced by the results they achieve with patients, the scientific validity of herbal medicines in research and the development are still often questioned. This is partly due to the lack of quality control, identification, and standardization of chemical compounds and drug formulations, as well as the challenges of applying the same methodologies for different drugs ([Karbwang et al., 2019](#)).

Although according to the interpretation of the antibiogram according to CASFM indicates that almost all of our extracts are in the resistant category, the effectiveness of the aqueous extract of *Mitragyna stipulosa* on *S. pneumoniae* could also reside in high concentrations (doses) and increased dosage.

Treating an infection with an antibiotic declared active by an antibiogram does not guarantee

therapeutic success, while using an antibiotic to which the bacteria is resistant is synonymous with failure (Jehl, 2015). The antibiotic or the extract in the intermediate zone is between these two extremes, the probabilities of success and failure of the treatment will therefore depend on additional parameters that may be linked to the patient, the environment or the strain. bacteria itself.

Clinical breakpoints are subject to regular revision, but although the trend is towards standardization between European countries, and even with other regions of the world (Australia, for example), there are nevertheless differences. These are often consecutive to the very method of establishing these critical concentrations (Jehl, 2015).

There are few clinical studies linking treatment failures/successes with the MICs of antibiotics against the target bacteria. The data remains fragmentary, therefore, as often in such cases; divergent (Jehl, 2015).

These discrepancies can be seen, for example, in the fact that with regard to the critical concentrations and diameters of amikacin and the other aminoglycosides, unlike CASFM, EUCAST does not propose critical concentrations and diameters (The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0, 2021. <http://www.eucast.org>)

Based on the discussion above, it would be appropriate to reconsider all the extracts having given an inhibition halo between 10 mm ~ 14 mm no longer as being in the category of inactivity (resistant (R)), but rather "suboptimal" activity compared to amikacin.

However, interpreting the results of the DZI measurements by the scale used by (Ponce et al., 2003) and (Celikel and Kavaz, 2008), indicating that the bacteria are resistant to DZI less than 8 mm, sensitive for DZI from 9 to 14 mm, very sensitive for DZI from 15 to 19 mm and extremely sensitive for DZI greater than 20 mm (Dalmarco et al., 2010), we can formulate new conclusions on the activity of our extracts.

Indeed, according to this scale, all our extracts have a positive antibacterial activity on *S. pneumoniae*. The daughter solutions having given a DZI ranging from 10 mm ~ 14 mm are found in the sensitive category, and the daughter solution C3 having given a DZI of 15 mm is found in the very sensitive category.

Although having a positive antibacterial activity on *S. pneumoniae*, none of our extracts was more effective than the positive control used as reference antibiotic, Amikacin.

3.2. Minimum inhibitory concentration (MIC)

Figure 1 presents the results of the determination of the inhibitory concentration.

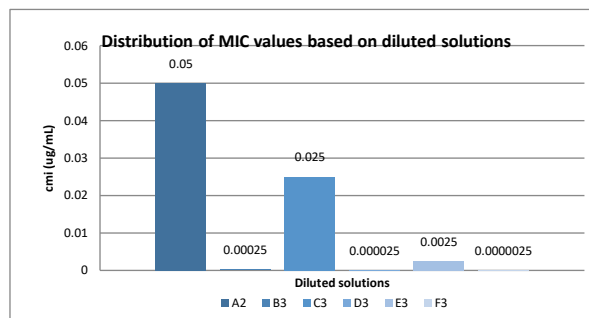


Figure 1. Distribution of MIC values based on diluted solutions

Legend: A2 = second daughter maceration solution with fresh leaves; B3 = third daughter solution of decoction with fresh leaves; C3 = third infusion daughter solution with fresh leaves; D3 = third daughter solution of maceration with dry leaves; E3 = third infusion daughter solution with dry leaves; F3 = third daughter solution of decoction with dry leaves.

The data in figure 1 shows that the extract from solution A2 has a high MIC value (0.05g/mL), while the C3 extract has a medium MIC value (0.025g/mL) while E3 extract has very low MIC value (0.0025 g/mL).

However, figure 2 reveals that the extracts from the dry leaves have MIC values that are not very distributed compared to the MIC values of the extracts from the fresh leaves, which cover a wide range of values. This tells us that, although all the extracts have a positive MIC, the variation in antibacterial activity is much greater in the extracts from the fresh leaves.

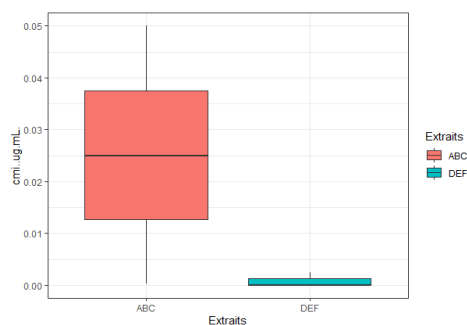


Figure 2. Visualization of the MIC according to the state of the leaves

According to CASFM recommendations (Soussy et al., 2013), the critical MIC concentrations recommended for Amikacin are presented in table 3.

Table 3. MIC Breakpoints for Amikacin

Antibiotic	Disk load	Concentration (mg/L)	
		S	R
Amikacine	30 µg	≤ 8	>16

Antibiotics belonging to the beta-lactam family, of which the most popular representative is Penicillin, were practically the first to be used against *S. pneumoniae*, and continue to be prescribed although the first resistances have also appeared. In this family.

Studies such as those of Rossi (2012) indicate a lower value of the critical concentrations for the MIC. Isolates were classified as susceptible, intermediate, and resistant to parenteral penicillin if the MIC was ≤ 2.0 µg/mL, 4 µg/mL, and ≥ 8.0 µg/mL, respectively. For Moxifloxacin, isolates were classified as susceptible, intermediate, and resistant if the MIC was ≤ 1.0 µg/mL, 2 µg/mL, and ≥ 4.0 µg/mL, respectively.

By comparing our MIC results with the breakpoints established by CASFM and Rossi (2012),

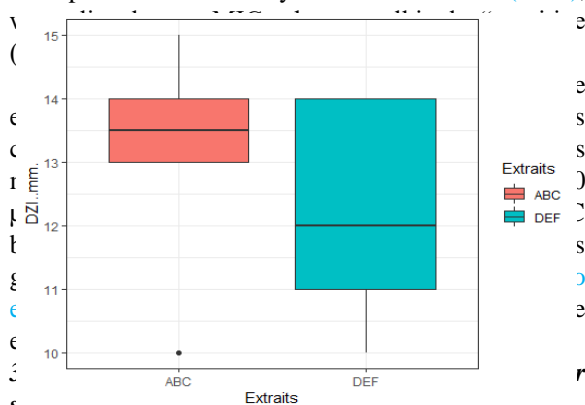


Figure 3 presents the distribution of DZI values for each daughter solution.

Figure 3. DZI values of extracts.

The data in figure 3 show us that the antibacterial activity of the daughter solutions follows a progression in a lower or equal level, that is to say that starting from the first daughter solution to the third daughter solution, the activity is less than or equal to the previous child solution. The daughter solutions of extract C are an exception to this pattern, because C3 gave a higher DZI than C1 and C2.

Figure 4 presents the distribution of the diameters of the zones of inhibition according to the state of the leaves.

Figure 4: DZI as a function of leaf condition

ABC = extracts from fresh leaves | DEF = extract from the dry leaves

The data in figure 4 shows that overall the extracts prepared from the fresh leaves exhibit the best antibacterial activity against *S. pneumoniae*. Indeed, the extracts prepared from the fresh leaves (ABC) have a minimum DZI value of 13 mm and a maximum DZI value of 15 mm, while the extracts from the dry leaves have on the whole a minimum value of DZI of 10 mm and a maximum value of DZI of 14 mm. Also, the median of ABC extracts is higher than that of DEF extracts.

The presence of aberrant data (outlier) in the ABC extracts could be due to an error during measurement, or have a specific meaning that can be explored by data analysis methods on a larger sample. It is for this last reason that we have decided not to ignore these values. An outlier is a value that contrasts greatly with all the others. Mathematically it is defined by:

$$\text{Outliers} < Q1 - 1.5 (\text{EIQ}) \text{ or } > Q3 + 1.5 (\text{EIQ})$$

This indicates that with respect to the condition of the leaves at preparation, it is more effective to use fresh leaves when the plant is to be used against *S. pneumoniae*.

The preparation based on fresh leaves is more effective than that based on dry leaves. This could be due to a degradation or a loss of bioactive potential of the group of secondary metabolites which would be involved in the biological activity evaluated, during drying.

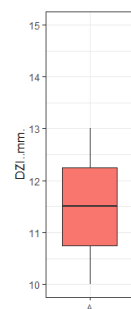
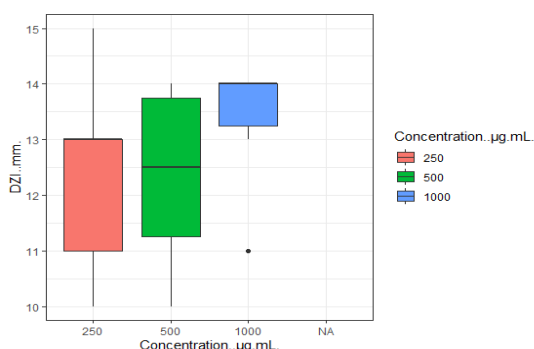


Figure 5 shows a visualization of DZIs as a function of concentration.

Figure 5. DZI as a function of concentration

We note from figure 5 that the DZI values change proportionally with the concentration of the extracts. Even if some locks reach high DZI values for relatively low concentrations, the distribution of values in the third quartile remains in an increasing level of concentration. It can be concluded that there is an a priori correlation between the concentration and the effect of the extract.

However, although the values of the third quartile are increasing, we notice that the median of



the second concentration (500 µg/mL) is lower than that of the third concentration (250 µg/mL). This is explained by the fact that the daughter solution A3 (250 µg/mL) did not respond during the test, which reduces the sample on which the median is calculated. Once again we observe the presence of an aberrant measurement (outlier) at the level of the first concentration (1000 µg/mL), we keep this value for the same reasons mentioned above.

It is worth drawing the reader's attention to the fact that contrary to what one might have expected intuitively, the value obtained by C3 is not considered as an outlier when evaluating the whole series. Concentration of the daughter solutions of 250 µg/mL, which means that this value is less likely to be an error but that it would really have a meaning that deserves a thorough attention in later studies.

Figure 6 presents visualization of the DZI according to the method of preparation of the extracts and the state of the leaves.

Figure 6: DZI according to method of preparation and state of leaves

The data in figure 6 are polysemous, which means that we derive several pieces of information from it. The first information is that according to the type of preparation of the extracts, in the category of extracts from fresh leaves, the infusion is the type of

preparation giving the extract with the most effective activity, then there is the decoction and finally the maceration. In terms of extracts from dry leaves, maceration is the type of preparation giving the extract with the most effective activity, then there is the decoction and finally the infusion. Although the maceration and the decoction from the dry leaves reach the same maximum value, for the maceration the maximum value is confused with the median and the third quartile, which means that more daughter solutions reached this DZI value.

The second information is the comparison of homologous preparations. We note that the maceration based on fresh leaves is less effective than the maceration based on dry leaves, that the decoction based on fresh leaves is more effective than the decoction based on dry leaves, and that the infusion based on fresh leaves is more effective than an infusion made from the dry leaves.

The third information is that the preparation of extracts by infusion of the fresh leaves has the highest efficiency compared to all other types of preparation.

4. Conclusion

The Democratic Republic of Congo is a country rich in biodiversity which requires that it be valued, in order to achieve a Congolese Pharmacopoeia. This study has just demonstrated once again that our immediate environment is full of resources that we can intelligently exploit in order to become independent in terms of health, and increase the life expectancy of our populations while reducing the infant mortality rate due to diseases like pneumonia and others.

This study confirms our hypothesis and our research question by validating the antibacterial activity of *M. Stipulosa* on the bacterium *S. pneumoniae*, and by showing that an effective treatment based on these extracts was possible. Indeed, this work has just given the minimum inhibitory concentrations (MIC) of the extracts of the leaves which vary from 5×10^{-2} to 2.5×10^{-6} ug/mL. The DZIs from 10 to 15mm for the extracts of the daughter solutions tested. However, no treated solution gave a DZI equal to or greater than the DZI of the reference antibiotic (24mm) for Amikacin.

We have demonstrated through this work that extracts from fresh leaves have significantly more effective results than extracts from dry leaves. We have also demonstrated that concentration increases the efficacy of extracts, indicating that dosage and

concentration are essential parameters for effective treatment.

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Annex



Leaves of *Mitragyna stipulosa* (Photo Kyong Shin, 20021)