

Evaluation of the *in vitro* antifungal activity of extracts of some medicinal plants on strains of *Pseudocercospora fijiensis* in the region of Kisangani (DR Congo)

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Abstract

Summary: Due to its impact and its epidemiology, the black Sigatoka disease caused by *Pseudocercospora fijiensis* is a multidimensional problem, given the insured nutritional and economic importance by the banana and plantain in our region. The limits of existing therapies and especially the inaccessibility to the latter as well as the consequences in the environment and the health lead to the search for new sources. It is in this framework that this work has focused on the evaluation of the antifungal activity of extracts from 14 plants used in traditional medicine against fungal infections of the human resources in the Kisangani region. It has been tested by the method of inhibition of the growth of the mycelian explant on Potato Dextrose Agar (PDA) medium in a Petri dish. The main results obtained show, after the evolution of the diameters of growth of mycelian explants on media containing different extracts compared to the diameter of the light during the incubation period, that all plants tested have antifungal effects at different levels and/or moments during the incubation. And these effects have been observed either during the entire incubation period, or at the beginning of the incubation, or toward the end of the incubation, or only slowed growth in relation to the witness without as much completely inhibiting it. *Carica papaya* has been the only plant having kept its inhibitory effect complete for all forms of extracts tested.

Keywords: Antifungal activity, plant extract, *Pseudocercospora fijiensis*.

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INTRODUCTION

The Banana and the plantain constitute, in D.R. Congo, rank second as food crop and contribute greatly to the food security of the population. They constitute the commercial crop, which in the most part of cases is the third source of income for the households after cassava, rice or corn, or even after the palm oil. This shows the needs of research, training and dissemination of techniques for this crop are very significant [1]. The attacks of the crop by the species can have many negative impacts, direct and indirect impacts on nutrition and the income of farmers, with as a consequence the food insecurity and poverty [2].

The black Sigatoka disease (BSD), is the most devastating parasitic disease of banana and plantain. It is caused by an ascomycete fungus called *P. fijiensis* Morelet [3]. This disease is present in the greater part of the production areas of banana in the world [4].

The fight against the BSD is essentially chemical. However, this method of struggle has a high cost and the frequency of application leads to the appearance of resistant strains to fungicides. In addition, this struggle carried out by massive sprays and quasi-systemic fungicides causes, however, outbreaks of persistence of diseases related to the emergence of resistant strains with, as consequence, the increase in the cost of the fight, but also the increase in environmental impacts [5].

The search for new products of natural origin not polluting the environment and available at lower cost represents an important element of sustainable agriculture [6]. It is for this fact that we propose to try an approach based on medicinal plants, given that medicinal plants bring relief and/or healing when faced with the human fungal infections.

The use of plants is explained by their accessibility and availability in traditional medicine in

developing countries on the one hand, as well as the high cost and the harmfulness of side effects caused by the synthetic drugs, on the other hand [7].

MATERIALS AND METHODS

The Ground of the Study

This work has been carried out in the region of Kisangani. The chief town of the Tshopo province, in the DR Congo, in the eastern part of the Congo basin to 0° 31' North and 25° 11', at an altitude of 396 m. Its surface area is approximately 1 910 km² [8].

Plant material

Table-1: Plants used

N°	Species	Family	Vernacular name	Part used
1	<i>Euphorbia hirta</i>	Euphorbiaceae	Kabudimbu (Tshiluba), Djembolembo (Tetela), Kasa ya ndika (Lingala)	Whole the plant
2	<i>C. papaya</i> L.	Caricaceae	Payi payi (Swahili)	Leaves
3	<i>Ananas comosus</i> (L.) MERR	Bromeliaceae	Nanasi (Swahili), Ekomi (Tetela)	Fruit
4	<i>Cassia hirsuta</i>	Fabaceae	Dikake dia ndjadi (Tetela)	Leaves
5	<i>Conyza sumatrensis</i>	Asteraceae	Aloma (Kumu), Basuause (Lokele), Ofokafoka (Tetela)	Leaves
6	<i>Cyphostemma adenocaula</i>	Vitaceae	Mwandula (swahili), Lombeye (lingala), Mwandula (swahili), Lombeye (lingala)	Leaves
7	<i>Mitracarpus scaber</i>	Rubiaceae	Kafua- nkusu, (Tshiluba), Mutuale (Ngwaka), Kafua- nkusu, Kashipa- nkusu (Tshiluba), Kinde, Mutuale (Ngwaka)	Leaves and flowers
8	<i>Nicotiana tabacum</i>	Solanaceae	Luyengele (Kumu), Foka (Tetela)	Leaves
9	<i>Senna alata</i> (L)	Fabaceae	Ofofo (Kumu), Tshilongo (Tshiluba), Tshilongo (Tshiluba)	Leaves
10	<i>Solanum lycopersicum</i>	Solanaceae	Mata (Tetela)	Leaves
11	<i>Spermacoce latifolia</i>	Rubiaceae	Solola (Kumu)	Leaves and flowers
12	<i>Synedrella nodiflora</i>	Asteraceae	Kutu (Fulu), Gbinikpangbala (Ngwaka)	Leaves
13	<i>Tetradenia riparia</i>	Lamiliaceae	Mulavumba (swahili)	Leaves
14	<i>Vernonia amygdalina</i>	Asteraceae	Lembidha (Boa), Ololo kondjo (Tetela)	Leaves

Fungal Material

The fungal material used in this study is consisted of the strains of *P. fijiensis* isolated and identified by the laboratory of microbiology and phytopathology of the Faculty of Sciences of the University of Kisangani from necrotic leaves with symptoms of the black Sigatoka disease of the banana and plantain in Kisangani.

METHODS

Preparation of Concentrated Crude Extracts

The organs of the plants to be tested are crushed and the juice collected in a test tube. 10 ml of each of the prepared juice are collected and then, concentrated by evaporation (at a temperature not exceeding 50°C) up to obtaining a quantity of approximately 2 ml [10].

Preparation of the Ethanolic and Ethereal Extracts

The 95% ethanol and petroleum ether have served as extraction solvent. 50 ml of each solvent are poured in droplet series in the test tubes in which are each time drawn 10 grams of fresh and chopped plant material. The mixtures are macerated during 48 hours and then filtered. The filtrates are finally concentrated by evaporation up to 1 ml of extract in each tube [11-14].

Obtaining of Strains

The fungal strains were obtained from samples of banana and plantain sheets harvested in the collection of the Faculty of Sciences; the insulation has been achieved by the technique of discharge of ascospores on the agar medium (H₂O agar) and then by culture of ascospores discharged, on Potato Dextrose Agar (PDA) medium as described by Carlier *et al.*, [15].

Activity of Extracts of Plants to the Strains

The method of the inhibition of the mycelial growth on solid medium on a Petri dish was used to investigate the sensitivity of the Strains vis-a-vis the extracts of medicinal plants. The latter consists in centering a mycelial explantant 5mm in diameter of each Petri dish, as obtained after punching with a cookie-cutter.

The mycelial growth was followed regularly during 9 days by measuring 3 times the diameter growth of the explantant under different extracts due to 3 repeats per extract of plants studied.

The Data Treatment

The data collected from this experiment were processed under Microsoft Excel 2016, as well as the

Software past (version 2.15) which helped to check the similarity between the species of plants by extracts of medicinal plants.

The statistical analyzes have been verified under the software R (version 3.1.3) which has been used for the comparison of the averages, the test of correlation between the parameters, the analyzes of variance (ANOVA) multivariate and simple.

The SPSS software 20 allowed the analysis of the dependent variables of the growth (effect inter subject)

RESULTS AND DISCUSSION

Concentrated Crude Extracts

The antifungal activity of the concentrated crude extracts of each of the different plants tested is illustrated in Figure-1.

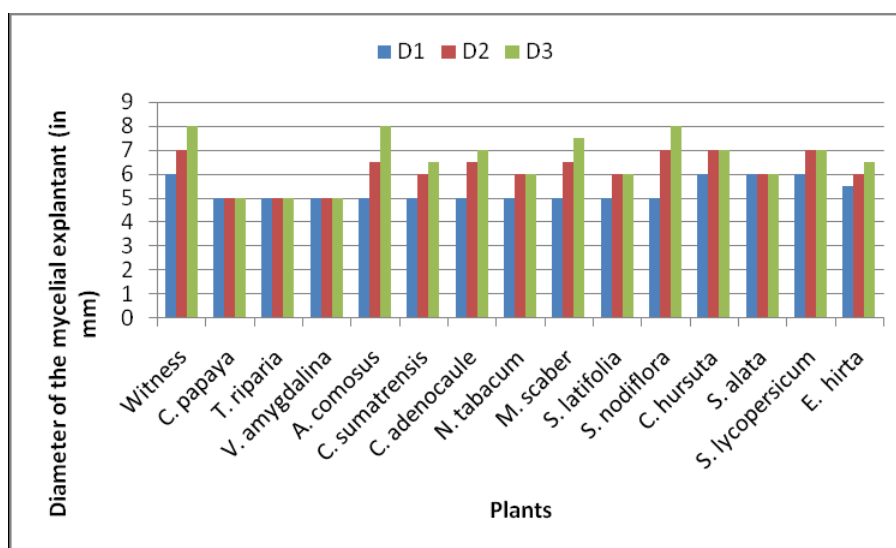


Fig-1: Diameter of the mycelial explantant strains of *P. fijiensis* on concentrated crude extracts of the plants tested. D1, D2 and D3 respectively represent the 1st, 2nd and 3rd days of observation

It emerges from the observation of this figure that the different plants tested have all antifungal activity at different levels. From the diameter of the mycelial explantant which was 5 mm, all plants tested have shown an action on the mycelial growth with regard to the witness.

Thus, the plants tested can be combined into 4 groups according to the evolution of the growth of the diameters of the mycelial explantants in environments containing the concentrated crude extracts. It is:

Plants which completely blocked the growth of the diameters of the mycelial explantants (*C. papaya*, *T. riparia* and *V. amygdalina*);

Etobo [16] has on 20 extracts tested, obtained 4 Extracts inhibitors of 7 strains on the 38 strains of staphylococci resistant to common antibiotics in Kisangani, including the crude extract of the aqueous mixture of *C. papaya* and *Psidium guajava* which inhibited to him only 3 strains on the 7 but also with the larger diameter of inhibition of 20 mm. Yet, the concentrated extract of the mixture of *C. papaya* and *Piper guineensis* reacted among the 20 extracts tested, with an activity of 50% on 21 strains of these staphylococci.

The research conducted by Etobo [16] has seen the crude extract of the aqueous mixture of *A. comosus* and *Nymphaea lotus* inhibit two strains of the 7 strains of staphylococci resistant to common antibiotics in Kisangani.

The plants having blocked the growth of the diameters of the mycelial explantants at the beginning of the incubation (*A. comosus*, *C. sumatrensis*, *C. adenocaula*, *M. scaber*, *N. tabacum*, *S. latifolia* and *S. nodiflora*);

We note that each plant has evolved according to the effectiveness of its active principle although they have reacted in a similar manner at the beginning of the incubation.

The research conducted by Etobo [16] has seen the crude extract of the aqueous mixture of *A. comosus* and *Nymphaea lotus* inhibit two strains of the 7 strains of staphylococci resistant to common antibiotics in Kisangani.

Gbaguidi [17] in his study, was able to demonstrate not only the activity of the extract of *M. scaber* but has also provided useful information for the preparation of a controlled product and less toxic that can be marketed as phytomedicament, in his study including the characterization of the chemical profile

of *M. scaber* and measurement of the in vitro effect of brake of the fractions examined on the growth of *Dermatophilus congolensis* in Benin. This study has not yet reached this stage.

Those plants blocking the growth of the diameters of the mycelial explantants later during the incubation (*Cassia hirsuta*, *Nicotiana tabacum*, *Senna alata*, *Solanum lycopersicum*, *Spermacoce latifolia*).

It therefore emerges that the concentrated crude extract of these plants would have an inhibitory effect but which is manifested with delay. This is reflected by the fact of the complete inhibition of the growth of the diameter from J2. These plants could by association with that shown the inhibition at the beginning of the incubation (*C. sumatrensis*, *C. adenocaula*, *A. comosus*, *S. nodiflora* and *M. scaber*) make an association to inhibitory effect complete. That is to say the association of plants with tardive inhibitory effect with those inhibiting at the beginning of the incubation would give the expected results.

In addition, *N. tabacum* and *S. latifolia* were particularly cumulated the merits to inhibit the growth of mycelial diameter at the beginning and at the end of the incubation of strains in the experimental conditions.

In their study based on the analysis of the *in vitro* antibacterial activity of aqueous extracts and alcoholics of *Cassia alata*, *Lantana camara* and *M. scaber* and the comparison with oxytetracycline and procaine penicillin was on a Gram-positive bacterium, *D. congolensis*, Agent of the bovine dermatophilosis in Benin, Ali-Emmanuel et al., [18] have noticed that the aqueous extract of *M. scaber* showed a antibacterial activity higher on *D. congolensis* than those of *C. alata* and *L. camara*.

In Burkina Faso, Nikiéma et al., [19] have experienced the action of SAPS from the expression of fresh leaves of *M. scaber* and *S. alata* as antimycosique in the opportunistic infections in people living with HIV.

And finally the plant not having at all completely stopped the growth of diameters, but having slowed down the latter during the duration of the incubation period (*E. hirta*).

In his study on the strains of bacteria resistant to antibiotics, Etobo [16] received no activity of crude extracts and aqueous crude extracts concentrated aqueous of *E. hirta* on the strains of staphylococci and on the strains of Enterobacteriaceae. This could be the fact that this plant has more inhibitory action on the fungi than on the bacteria (prokaryotes) or than the factor inducing the resistance that these bacteria would be non-sensitive in the principle inhibitor of the plant.

Nikiéma et al., [19] have shown the aerial parts of *E. hirta* L. and the pulp of the fruit of *Adansonia digitata* L. in diarrheal diseases among people living with HIV, in the strategy aimed to clarify the fears of the risk of drug interactions in patients PLHA with conventional treatments and more particularly the ARV drugs that meet the national standards of treatment in the offering in the cases with lower risk for the use of natural substances in the treatment of opportunistic infections, nutritional recovery and immunological.

Ethanollic Extracts

Figure-2 illustrates the action of the ethanollic extracts after measurement of the diameters of mycelial explantants during the incubation period.

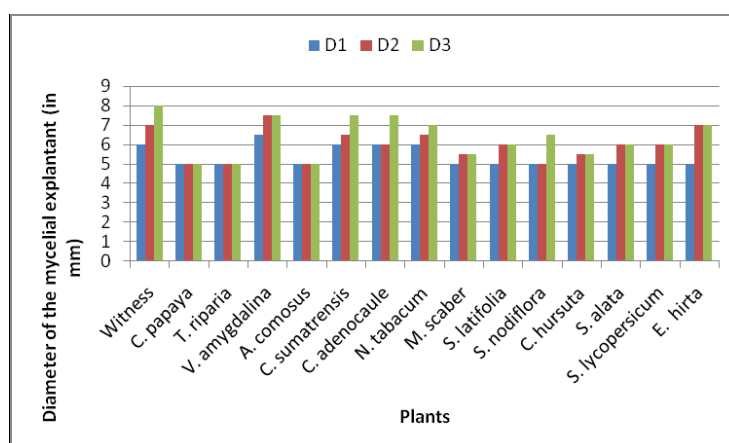


Fig-2: Mycelial growth of the strains of *P. fijiensis* on the ethanollic extracts of the plants tested. D1, D2 and D3 respectively represent the 1st, 2nd and 3rd days of observation

The grouping as a function of the evolution of the growth of the diameter of the mycelial explantants brings together the following plants: *A. comosus*, *C.*

papaya, *T. riparia* as plants having completely blocked the growth of explantants. *Tetradenia riparia* and *C. papaya* still have maintained the inhibitory action after

extraction by ethanol while *A. comosus* has seen its inhibitory action improved from the inhibitory action at the beginning of the incubation to the total inhibition during the entire period of experimentation. This would be the fact the solvent extractor (ethanol) that would have improved by concentration the antifungal action of the plant *A. comosus*. But we note a different effect for *V. amygdalina* which has lost its action view in the concentrated raw extract.

Cakupewa *et al.*, [20] have obtained that the alcoholic extract of the leaves of *T. riparia* which proved to be a drug effective against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*, in a study on the antibacterial activity of a few recipes used against infectious diseases in Kinshasa because it inhibited the three strains to the times.

These results, which seem to corroborate those of this study, suggest that the extraction solvents have not changed the active principle of this plant. Gbogbo *et al.*, [21] have obtained extracts of stem bark of *Fucus platyphylla* (Moraceae), one of the Medicinal Plants of the Togolese flora used in traditional medicine in the treatment of opportunistic diseases of AIDS that the aqueous extracts and hydro-ethanolic extracts of this plant have a fungicidal activity at low concentrations on yeast and dermatophytes involved in various conditions of human and animal.

C. hirsuta, *M. scaber*, *S. latifolia*, *S. nodiflora*, and *S. lycopersicum* as plants having blocked the growth of mycelian explantant at the beginning of the incubation. Pissang *et al.*, [22] have evaluated the antimicrobial effect of total extracts and fractionated from *C. alata* (linn) harvested in the South of Togo on of bacteria (*S. aureus*, *E. coli* and *K. oxytoca*) and of

yeasts (*C. albicans* and *C. krusei*). The results have shown that the extract ethanolic gross (EBE) sheets has been the most active on all microbial strains tested.

Ali-Emmanuel *et al.*, [18] have noticed in their study that the alcohol extracts gross of *C. alata*, *Lantana camara* and *M. scaber* have proven to be more active on *D. congolensis* than their aqueous extracts.

Kporou *et al.*, [23] have assessed *in vitro* the sensitivity of *Candida albicans* to extracts of *M. scaber* a codified Rubiaceae MISCA and have obtained that the strain tested was sensitive to the nine extracts from MISCA and that the extract X11 obtained by partition of the extract hydro-alcoholic in a mixture of solvents hexane-water (v/v) was the most active

V. amygdalina, *M. scaber*, *S. latifolia*, *C. hirsuta*, *S. alata*, *S. lycopersicum* and *E. hirta* such as plants blocking the growth of explantants late during the incubation. Etobo [16] in his study has observed that many of the ethanolic extracts have responded but their activity remains relatively low compared to that obtained with the aqueous extracts concentrated on the *Staphylococcus* (50%). It has obtained a reaction extracts of *E. hirta*, *A. comosus* and *V. amygdalina* on strains of staphylococci resistant to antibiotics.

C. sumatrensis and *N. tabacum* as those not having the all stopped the growth during the duration of the incubation

Ethereal Extracts

Figure-3 represents the action of ethereal extracts after measurement of the diameter of the mycelian explantant during the incubation period.

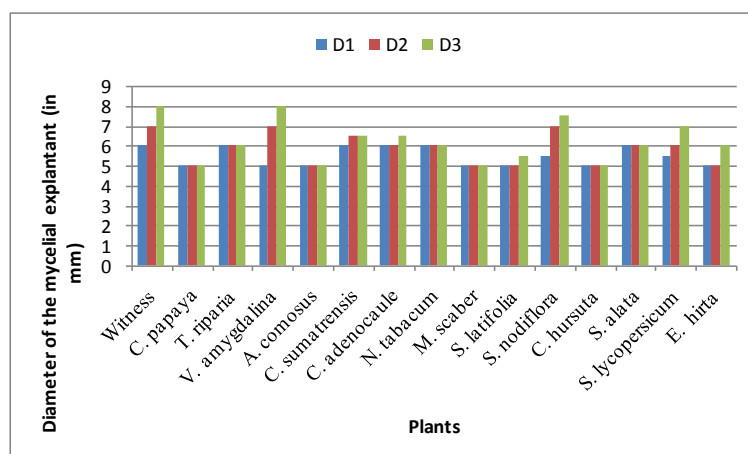


Fig-3: Mycelial growth of the strains of *P. fijiensis* on the ethereal extracts of the plants tested. D1, D2 and D3 respectively represent the 1st, 2nd and 3rd days of observation.

The observation of the evolution of the mycelial growth such as shown in the Figure 3 shows the effectiveness of ethereal extracts of plants *C. hirsuta*, *C. papaya*, *A. comosus*, *M. scaber*, because the diameters of the mycelian explantants have not

increased during the entire duration of the experiment and remained equal to 5 mm. These *status quo* diameter would prove a enabled effective.

The comparison of the activity of the Extracts ethereal, ethanolic, and crude concentrated, show as a function of diameter maximum reached for *A. comosus*, *C. hirsuta*, *M. scaber*, *S. latifolia* and *E. hirta* an increase of the antifungal activity in comparison to the crud concentrated and ethanolic extract.

V. amygdalina itself has lost its antifungal activity observed in crude extracts concentrated after extraction ethanolic and ethereal. *T. riparia* against has not lost that by extraction by the ether.

C. papaya and *S. alata* have not shown a change in the maximum diameter reached for all

extracts, except with regard to the time of the complete inhibition which has appeared.

Biabiany, [24] says that in herbal medicine and traditional search of extraction, of active molecules can vary significantly from one solvent to another: all extracts should be dissolved in the same solvent, the latter must therefore have a power of dissolution important, ranging from apolar compounds to polar compounds.

Affinity between the species of plants compared to various excerpts

The following chart gives the main distinct groups of species according to their affinities.

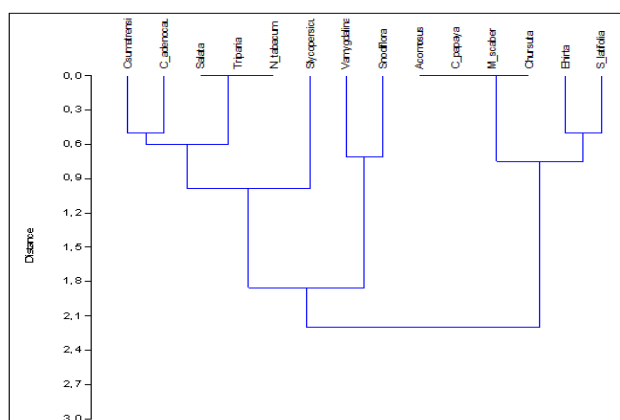


Fig-4: Similarity between the species in relation to different extracts

The most remote to the nearest, the first group includes *S. latifolia* up to *A. comosus*. However, this first group is subdivided into 2 sub-groups. 1 Sub-group relates to the species *S. latifolia* and *E. hirta* ; and the second includes species *C. hirsuta*, *M. scaber*, *C. papaya* and ends by *A. comosus*.

The 2nd main group includes species *S. nodiflora* and *V. amygdalina*.

And finally, the 3rd account the species *S. lycopersicum*, *N. tabacum*, *T. riparia*, *S. alata*, *C. adenocaule* and *C. sumatrensis*. This last group is also restarted in 3 sub-groups. The first accounts only a single species (*S. lycopersicum*), the 2nd includes *N. tabacum*, *T. riparia* and *S. alata* and finally, the last sub-group accounts the species *C. adenocaule* and *C. sumatrensis*.

Analysis of the variance of the Extracts

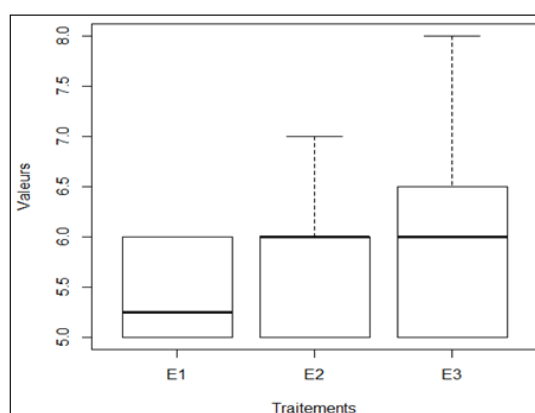


Fig-5: Influence of extracts on the treatments

Legend: E1: concentrated crude extract, E2: ethanolic extract, E3: ethereal extract

This chart compares the average observed between the 3 treatments. It is to point out that the ether extract has presented a higher average compared to those of the concentrated raw extract and the ethanolic extract. In considering the statistical analyzes, the test of the analysis of variance (ANOVA) has shown that there is not a significant difference between the treatments with ($F=2,537$, $p\text{-value}=0.09 > 0.05$).

CONCLUSION

At the end of this study scope on the evaluation of the *in vitro* antifungal activity of extracts of some medicinal plants of the region of Kisangani on strains of *P. fijiensis*, the intent is two-fold. On one hand, to select plants that could be used to combat this pathogen and to highlight an approach to fight in biological and ecological adaptable to the conditions of our region for some phytopathogenic fungi. And on the other hand, with a view to put in value the traditional medicine and to maintain in good condition the resources of biodiversity in our region on the basis of the information collected from the practitioners. The main results obtained from the action of the various types of extracts (crude concentrated, ethanolic and ethereal) show that:

- It exists in all plants tested antifungal activity in the face of the strains of *P. fijiensis*, but the extraction of the active principle can be variable depending on the solvent used extractor.
- The plants tested have shown effects antifungal at different levels and /or moments during the incubation; and these effects have been observed either during the entire period, either at the beginning of the incubation either toward the end of the incubation or slowed down only the growth by report to the witness without as much completely inhibit it.
- *C. papaya* has been the only plant having kept its inhibitory effect complete for all forms of extracts tested
- Has Taking into account the maximum diameter is reached, the extraction by the ether gave a performance of improvement of diameter of growth for 7 plants at 50%, against 2 plants of non improvement is 14.2% of the plants tested report to the diameters of the crude extracts concentrated while the extraction by ethanol gave a performance of improvement of the diameters of the growth of 5 plants or 35.7% against 4 plants in diameter non-improvement.
- The statistical analyzes have shown, however, that there is no significant difference between the different extracts of plants used.
- The settings Day (duration of incubation) and treatment (types of extract) influence the growth, but they are independent.

Based on the results of the analyzes carried out, the following analysis tracks are proposed: (1) Apply this *in situ* evaluation to confront the reality of *in vitro* field activity and possibly perform molecular identification analyzes; (2) To test the efficacy of the activity of the associations of complementary plants with respect to the incubation time; (3) To compare the effectiveness of the activity of plant extracts by the determination of the minimum inhibitory concentration (MIC) and (4) Identify the active ingredient of the tested plants.

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REFERENCES

1. Dheda, D., Swenner, R., Moango, M., & Blomme, G., (2011) : Rapport sur l'enquête diagnostique sur la culture des bananiers plantains dans les zones périphériques de la ville de Kisangani et quelques villages du district de la Tshopo (RDC), 101.
2. Soro, D., Koné, M. W., Kone, D., & Kamanzi, K. (2012). Evaluation de l'activité antifongique par bioautographie de quelques plantes médicinales de côte d'ivoire contre deux formes spéciales de *Fusarium oxysporum*. *Agronomie Africaine*, 24(1), 19-28.
3. Carlier, J., Zapater, M. F., Lapeyre, F., Jones, D. R., & Mourichon, X. (2000). Septoria leaf spot of banana: a newly discovered disease caused by *Mycosphaerella eumusae* (anamorph *Septoria eumusae*). *Phytopathology*, 90(8), 884-890.
4. Pasberg-Gaahl, F., & Jones, D. R. (2000). Distribution and economic importance in: Jones, éd. *Disease of Banana, Abaca and Enset* Wallingford, UK: CAB Publishing. 37-44.
5. Essis, B., Kobenan, K., Traoré, S., Koné, D., & Yatty, J. (2010). Sensibilité au laboratoire de *Mycosphaerella fijiensis* responsable de la cercosporiose noire des bananiers vis-à-vis de fongicides couramment utilisés dans les bananeraies ivoiriennes. *Journal of Animal & Plant Sciences*, 7(2), 822-833.
6. Rodríguez, R. S., Algora, J. P., Plous, C. V., Rodríguez, M. P., Sosa, Y. I., & Romero, F. M. (2002). Action du fongicide naturel F20 contre la cercosporiose noire (*Mycosphaerella fijiensis* Morelet) chez le bananier plantain (AAB) et le bananier (AAA). *INFOMUSA* *INFOMUSA*, 11(1):14-16.
7. Biyiti, L. F., Tamze, V., Nnanga, N., Agbor, A. G., & Gangoue-Pieboji, J. (2012). Formulation d'une pommade antibactérienne à base d'un extrait éthanolique des écorces du tronc de

- Tabernaemontana crassa Benth. *Pharmacopée et médecine traditionnelle africaine*, 16.
8. Kahindo, J. M. (2011). Potentiel en Produits Forestiers Autres que le Bois d'œuvre dans les formations forestières de la région de Kisangani. Cas des rotins *Eremospatha haullevilleana* De Wild and *Laccosperma secundiflorum* (P. Beauv.) Kuntze de la Réserve Forestière de Yoko (Province Orientale, RD Congo). *Unpublished PhD thesis, Faculty of Science, University of Kisangani*.
9. Onautshu, O., Hamadi, M., Lutala L., Lebisabo, B., Tchatchambe, N. B., D'heda, D. (2014). Dynamique de l'évolution de la cercosporiose noire du bananier dans la région de Kisangani (RD Congo). *Ann. Fac Sc. Unikis*, 16(1) 26-39.
10. Mbuyi, M., Kumbukawa, L. B., & Ambali, L. (1994). Etude comparée de l'activité antibactérienne in vitro des extraits bruts et celles des alcaloïdes totaux isolés de *Penianthus longifolius* niens (menispermaceae) et *decognauxie trilobata* coyn (cucurbitaceae) in *Ann. Fac. sciences. Unikis*, 10, 119-126.
11. Harbone, J. B. (1983). Photochemical methods. Chapman and Hall. London, 28.
12. Bourret, J. C. (1984). Le défi de la médecine par les plantes. France-Empire, Paris, 345.
13. Wagner, H., Blandt, S., Zgainski, E. M. (1984). Plant Drug Analysis. Sprig-Verlag, New York, 320.
14. Janovska, D., Kubikova, K., & Kokoska, L. (2003). Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine. *Czech journal of food sciences*, 21(3), 107-110.
15. Carlier, J., De Waele, D., & Escalant, J. V. (2002). *Evaluation globale de la résistance des bananiers à la fusariose, aux maladies foliaires causées par les Mycosphaerella spp. et aux nématodes* (Vol. 6). Bioversity International.
16. Etobo, K. (2012). Etude de l'activité antibactérienne des extraits de quelque plantes médicinales sur les souches bactériennes résistantes aux antibiotiques courants à Kisangani, Thèse inédit UNIKIS. Fac des Sciences, 142.
17. Gbaguidi. (1999). Evaluation de l'activité antimicrobienne de *M. scaber* sur *Dermatophilus congolensis*, Maîtrise en biochimie, Université d'Abouney – calari, Bénin.
18. Ali-Emmanuel, N., Moudachirou, M., Akakpo, A. J., & Leclercq, J. Q. (2002). Activités antibactériennes in vitro de *Cassia alata*, *Lantana camara* et *Mitracarpus scaber* sur *Dermatophilus congolensis* isolé au Bénin. *Revue d'élevage et de médecine vétérinaire des pays tropicaux*, 55(3), 183-187.
19. Nikiéma, J. B., Djierro, K., Simpore, J., Sia, D., Sourabié, S., Gnoula, C., & Guissou, I. P. (2009). Stratégie d'utilisation des substances naturelles dans la prise en charge des personnes vivant avec le VIH: expérience du Burkina Faso. *Ethnopharmacologia*, 43, 47-51.
20. Cakupewa, M. C., Fundiko-Mandango, M. A., & Ludiongo, N. (2000). Activité antibactérienne de quelques recettes utilisées contre les maladies infectieuses à Kinshasa (R.D. Congo) *Revue de Pédagogie Appliquée (RPA)*, IPN, KINSHASA, Vol. XVI, N° 2, 275-280
21. Gbogbo, K. A., Dourma, M., Akpavi, S., Batawila, K., & Akpagana, K. (2013). Evaluation de l'activité antifongique de *Ficus platyphylla* Del. *Moraceae*. *Eur Scient J*, 9(33), 252-60.
22. Pissang, P., Agban, A., Hoekou, Y. P., Tchacondo, T., Sadj, A. Y., Effoe, S., ... & Batawila, K. (2016). Evaluation In Vitro De L'activité Antimicrobienne Des Extraits De *Cassia Alata* Linn.(Fabaceae). *European Scientific Journal, ESJ*, 12(21), 116-129.
23. Kporou, K. E., Kra, A. K., Ouattara, S., & Guédé-Guina, F. (2009). Evaluation de la sensibilité de *Candida albicans* aux extraits de *Mitracarpus scaber* une rubiaceae codifiée MISCA. *Bulletin de la société royale des sciences de Liège*, 78, 12-23.
24. Biabiany, M. (2011). *Recherche et développement d'extraits antifongiques issus de la flore guadeloupéenne: caractérisations phytochimiques, pharmacologiques et formulation* (Doctoral dissertation, Université du Droit et de la Santé-Lille II), 174.