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Extraction Kinetics and Antifungal Activities of Essential Oil from the Aerial Parts of Gros Baume (*Hyptis suaveolens*) Against Three Fungal Strains in Côte d'Ivoire

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Abstract | **Original Research Article**

Introduction: Microscopic fungi colonize many food substrates resulting in considerable economic losses. Their development leads to an alteration in the organoleptic quality of the food, and produces mycotoxins. The aim of the present work was to evaluate the extraction kinetics and in vitro antifungal activity of the essential oil of *Hyptis suaveolens* on the growth of three toxigenic fungal strains. ***Methodology***: The essential oil was extracted by steam distillation using a Clevenger-type device. It was tested for in vitro antifungal activity against three mold strains (*Aspergillus flavus*, *Aspergillus niger* and *Fusarium sp*) using the agar dilution method. ***Results***: The results of the extraction kinetics evaluation show that in fifty minutes of distillation, almost all the essential oil contained in the aerial parts of *H. suaveolens* is recovered. In vitro antifungal tests showed a slight slowdown in mycelial growth for the three strains studied. However, the antifungal parameters determined show that these strains are sensitive to 50 µL/mL with a CMF of 100 µL/mL. This is just a simple fungistatic effect of this extract on the mycelial growth of the strains tested. Aspergillus strains seem to be slightly more affected than Fusarium strains. ***Conclusion*:** The antifungal potential of *H. suaveolens* essential oil does not offer a novel approach to the control of these three fungal strains.

Keywords: Essential Oil, *Hyptis Suaveolens*, Extraction Kinetics, Antifungal Activities.

INTRODUCTION

Filamentous fungi have a remarkable ability to colonize a wide range of food substrates, including cereals, dried fruit, peanuts and spices.

Their development on foodstuffs, through enzymatic activity, can improve the organoleptic qualities of the product. However, in the majority of cases, it leads to an alteration in the organoleptic quality of the food and produces mycotoxins (El Khoury, 2016; Mutlu-Ingok *et al*., 2020). Certain species of the genera Fusarium, Penicillium, Aspergillus and Alternaria are particularly well known for their high potential to spoil and produce various mycotoxins in food. Fungal contamination is responsible for around 30% of annual global food losses, with considerable economic losses (Saladino *et al.*, 2016).

Attempts to combat the incidence of food contamination remain predominantly chemical (Ben Miri, 2019). Yet the haphazard use of chemical fungicides leads to various health risks, environmental pollution and can cause the development of resistant strains (Souza *et al*., 2020). Biological control using natural antifungal substances can be an alternative to chemical products. This is because essential oils have a number of biological activities (Bertella, 2019). Their use as antimicrobial agents offers considerable advantages. They are natural substances whose use presents less risk of resistance development by pathogenic microorganisms and preserves the safety of the population and the environment (Tatsadjieu *et al*., 2010).

A number of research projects are focused on studying natural compounds with antifungal properties, as alternatives to the use of chemical agents (Jeršek *et al*., 2014). These extracts can extend the shelf life of foods and guarantee their quality, constituting a promising substitute for synthetic chemical additives (GOLY *et al*., 2017, Basak and Guha, 2018; Debonne *et al*., 2018; Dianez *et al*., 2018). GOLY *et al*., (2015) highlighted; the antifungal properties of savannah tea (*L. multiflora*) essential oil against *Aspergillus flavus*, *Aspergillus niger* and *Fusarium sp*, in Côte d'Ivoire.

The aim of the present study was to evaluate the antifungal properties of *Hyptis suaveolens* essential oil against *Aspergillus flavus*, *Aspergillus niger* and *Fusarium sp*.

MATERIAL

Plant Material

The plant material consists of *Hyptis suaveolens* aerial parts (Figure 1) collected in July and September 2013, in Yamoussoukro region (Côte d'Ivoire). Botanical identification was carried out in the laboratory of Botany Department of Agriculture and Agricultural and Animal Resources (ARA) at the Institut National Polytechnique Félix Houphouet-Boigny of Yamoussoukro (INP-HB).

Figure 1: Photograph of aerial parts of *Hyptis suaveolens* (Photo GOLY, 2013)

Biological Material

Three fungal strains isolated from cola samples at the Mycology and Parasitology Laboratory of the Institut Pasteur de Côte d'Ivoire (IPCI), were tested. These were: *Aspergillus Niger*, *Aspergillus flavus* and *Fusarium sp*. Antifungal tests were carried out on Sabouraud chloramphenicol agar (Bio-Rad) (France).

METHODS

1. Sampling

Fresh *Hyptis suaveolens* aerial parts, harvested early in the morning before sunrise, were immediately transferred to the laboratory (Laboratoire des Procédés Industriels de Synthèse, de l'environnement et des énergies Nouvelles) (LAPISEN) at the Institut national polytechnique Félix Houphouet-Boigny (INP-HB) in Yamoussoukro (Côte d'Ivoire). They are laid out at room temperature (27 ± 2°C), out of direct sunlight, for ten days for drying.

2. Essential Oil Extraction

The essential oil is extracted by steam distillation, using a Clevenger-type apparatus (Simard *et al*., 1988). A 300 g mass of dried leaves is brought to the boil in a pressure cooker containing distilled water. The essential oil-laden water vapour is condensed in the Clevenger coil, with the aid of a water flow. Two hours after the first drop of distillate appears, the essential oil is separated from the water.

The essential oil is dried with magnesium sulfate and stored in a sealed bottle at 4°C, protected from light. The extraction yield is determined by the ratio of the mass of oil extracted to the mass of plant material used (Ben Miri, 2019) (formula1).

$$R(\%) = 100 \times \frac{m_2}{m_1} \qquad (1)$$

R = yield (%); $\mathbf{m_1}$ = leaf mass (g) and $\mathbf{m_2}$ = oil mass (g).

3. Essential Oil Extraction Kinetics

In order to determine the extraction kinetics of the essential oil contained in plant (*Hyptis suaveolens*) part, different distillation times were used. From two (02) hours, the distillation time was increased to ten (10) minutes, in intervals of ten (10) minutes. For each distillation time, the test was repeated three times.

4. Preparation of the Fungal Inoculum

Using a platinum loop, a young colony was collected which was homogenized in 10 ml of sterile distilled water in order to obtain the mother suspension (100) concentrated at 106 cells/ml. From the suspension (100), a second suspension (10-1) is prepared by diluting 1/10 of the first in order to obtain a suspension of 105 cells/ml which constituted the fungal inoculum.

5. Inhibition of Mycelial Growth

Antifungal tests were carried out on Sabouraud chloramphenicol agar (Bio-Rad) (France). Different concentrations of essential oil obtained by the double dilution method were tested (Kra, 2001).

The agar prepared according to the prescribed instructions was distributed in seven (07) test tubes numbered 1 to 7, with 29.7 mL in tube 1 and 15 mL per tube in the other tubes (2 to 7). The contents of tube 1 are made up to 30 mL by adding 0.30 mL of a Tween/essential oil mixture (1/9; V/V). A double dilution is made from this 10% essential oil solution to tube 6 (Zihiri *et al*., 2003). Tube 7 is the control tube for fungal growth.

After homogenization by shaking, the contents of each tube are transferred to a 90 mm diameter Petri dish. After solidification, the agar is seeded by central pricking. A 5 mm diameter mycelial explant is taken from a 72-hour culture and deposited in the center of the agar using a sterile platinum loop. Petri dishes are incubated at 25 ± 2°C. Mycelial growth is recorded every 24 hours, by determining the average of two perpendicular radii passing through the center of the Petri dish (Khallil, 2001). Tests are performed three times.

6. Determination of Minimum Inhibitory Concentration (MIC) and Fungicidal Concentration (FMC)

The Minimum Inhibitory Concentration (MIC) is determined by incorporating the essential oil into the agar. The prepared agar is dispensed into seven (07) test tubes, then sterilized for 15 min at 121°C. Before solidification, the essential oil is incorporated (2 mL of a Tween/essential oil mixture (1/9; V/V) in 18 mL of agar), and a double dilution is carried out as above. After cooling the agar to room temperature in slant tubes (Zihiri *et al*., 2003), a well-isolated colony is picked and streaked on the surface of the slant agar. The MIC is determined by comparing the experimental tubes with the growth control, after 72 hours incubation at 25°C ± 2.

It corresponds to the lowest concentration above which no fungal growth is observed. Tubes in which no growth is observed are retained for FMC determination. A subculture is performed on fresh agar. The agar surface of the experimental tubes is scraped with a platinum loop, then streaked onto the surface of new agar (without plant extract) (Guede-Guina *et al*., 1997).

After 3 days of incubation, if mycelial growth is still inhibited, the extract has a fungicidal effect. A resumption of mycelial growth testifies to the fungistatic activity of the extract. The lowest concentration above which there is no resumption of fungal growth, corresponds to the Minimum Fungicidal Concentration.

Statistical Analysis

Results were analyzed by the variance method (ANOVA) using STATISTICA software version 6.0 (1-factor ANOVA treatment). Means were compared using the 5% Tukey test.

RESULTS AND DISCUSSION

1. Essential Oil Yield and Extraction Kinetics

Essential oil was extracted from *Hyptis suaveolens* leaves with a yield of 0.34 ± 0.21. This yield is identical to that determined by Saliou *et al*., (2012) in Senegal. However, it is relatively higher than that (0.23±0.0) determined by Adjou and Samanou (2013). The quality and quantity of compounds available in essential oils can be affected by several factors (Diánez *et al*., 2018). The difference in yields observed could be linked not only to the area of collection, the nature of the soil, the stage of development of the plant, the time of harvest and the method of extraction of the essential oil (Dammak *et al*., 2019), but also to the handling of the extraction equipment.

By varying the distillation time, we found that in ten (10) minutes, over 60% of the essential oil contained in the aerial parts of *Hyptis suaveolens* had already been extracted. In fifty (50) minutes, 100% of the essential oil is extracted (figure 2).

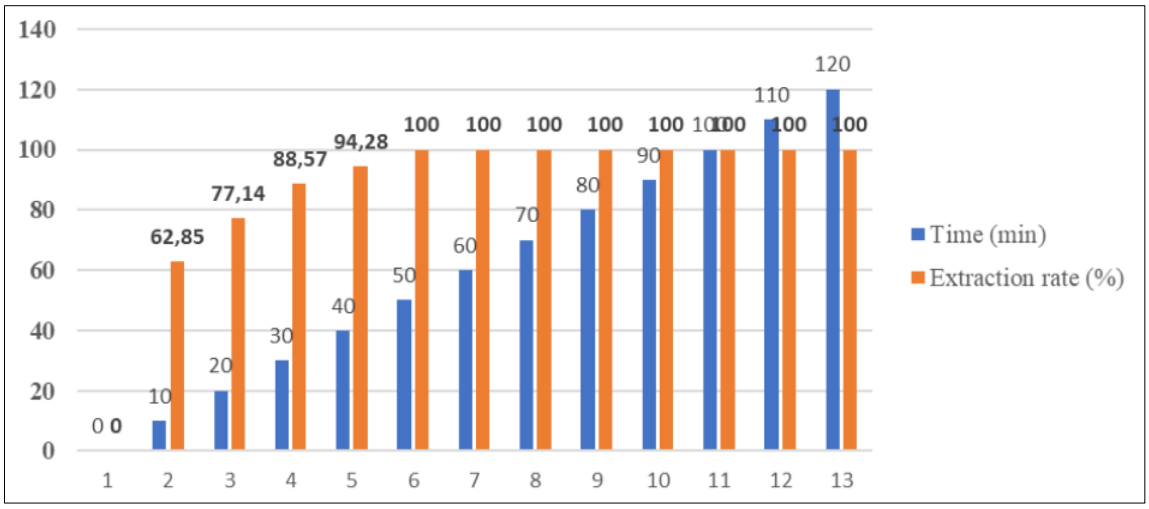

Figure 2: Extraction kinetics of *Hyptis suaveolens* essential oil

2. Antifungal Activity of *Hyptis Suaveolens* Essential Oil

Mycelial growth is slowed by increasing essential oil concentration. However, at the concentrations used in this study, *Hyptis suaveolens* essential oil appears to have no significant antifungal effects on the growth of the three strains tested. *Aspergillus niger* strain appears to be more sensitive. *Fusarium sp* is by far the most resistant to this extract.

The antifungal parameters (MIC and MFC) determined were identical for all three strains, sensitive at 50 µL/mL (Table). Similar work carried out by GOLY *et al*., (2015) with *Lippia multiflora* essential oil showed a high sensitivity of these same strains. Inhibition of fungal growth reached 100%. Clearly essential oil of *Lippia multiflora* has stronger antifungal properties than that of *Hyptis suaveolens*. Moreira *et al*., (2010) demonstrated the broad-spectrum activity of *H. suaveolens* essential oil against Aspergillus species. The antifungal parameters determined were lower than in our study. Strong antifungal activity of *H. suaveolens* leaf oil against Fusarium was reported by Malele *et al*., (2003). The antifungal parameters (MIC and MFC) determined were 500 and 1000 µg/mL, respectively. The differences observed in antifungal parameters could be explained by the composition and content of the secondary metabolites contained in the different extracts studied. This is because the activity of a plant substance depends on its active ingredient content, which is influenced by the geographical environment (NANTITANON *et al*., 2007).

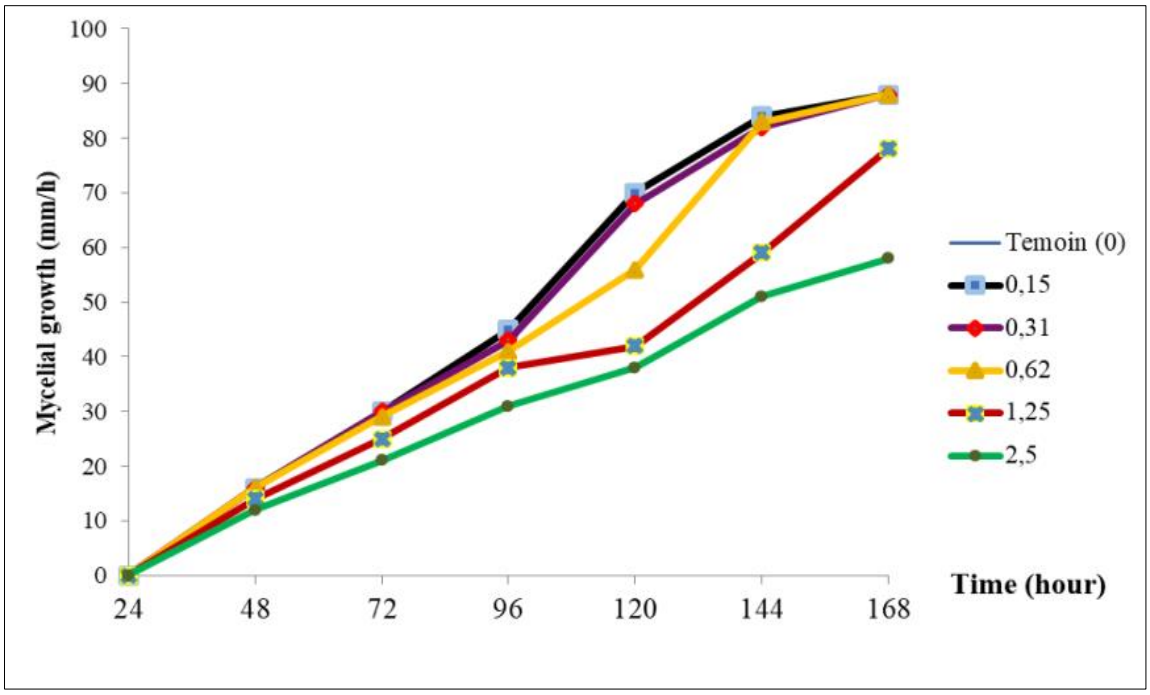

Figure 3: Evolution of mycelial growth of *Aspergillus flavus* in presence of *Hyptis suaveolens* essential oil

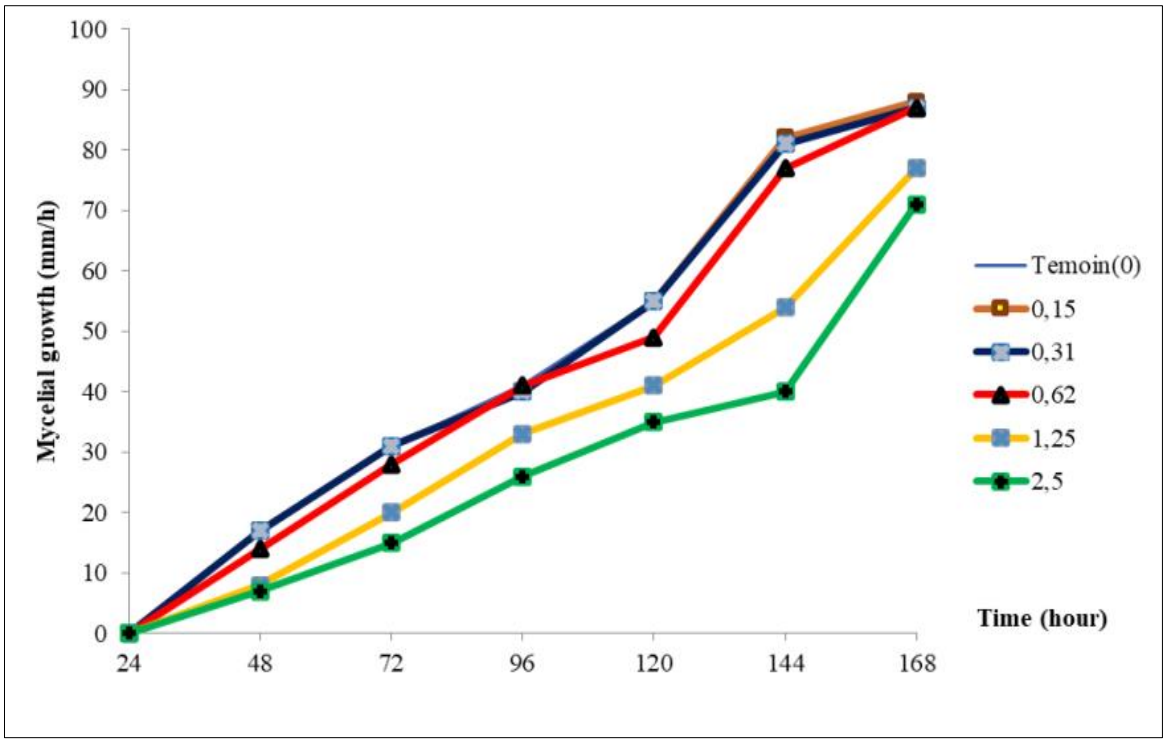

Figure 4: Evolution of mycelial growth of *Aspergillus niger* in presence of *Hyptis suaveolens* essential oil

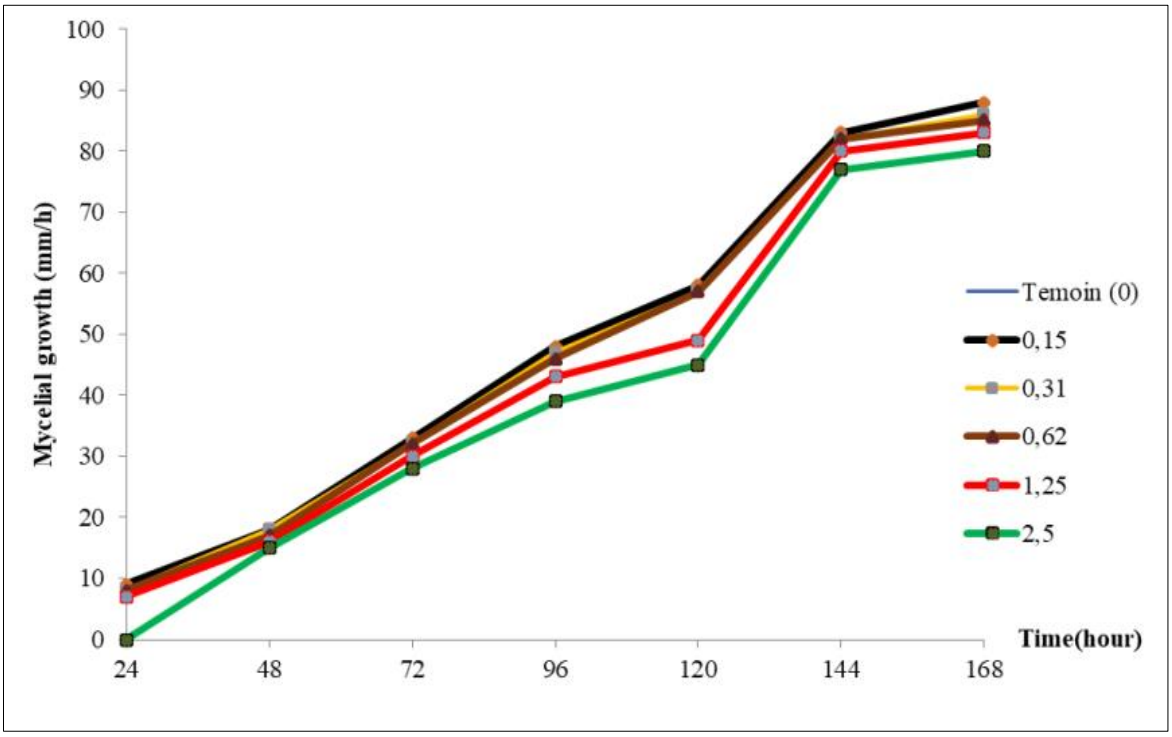

Figure 5: Evolution of mycelial growth of *Fusarium sp* in presence of *Hyptis suaveolens* essential oil

Table 1: Antifungal parameters of *Hyptis suaveolens* essential oil

Antifungal activity		
Fungal strains tested	MIC (µL/mL)	MFC (µL/mL)
Aspergillus flavus	50 ± 0	100 ± 0
Aspergillus niger	50 ± 0	100 ± 0
Fusarium sp	50 ± 0	100 ± 0

MIC: Minimum Inhibitory Concentration; CFM: Minimum Fongicidal Concentration

CONCLUSION

A maximum distillation time of one hour is sufficient to extract the essential oil from the aerial parts of *H. suaveolens*. In vitro antifungal tests have shown that essential oil has no pronounced antifungal activity at low concentrations. The antifungal potential of this extract does not offer a novel approach to combating these three fungal strains. The use of this plant, despite its antimicrobial properties, in the fight against these toxigenic fungi, would be better directed towards non-volatile extracts.

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REFERENCES

- Adjou, ES, & Aoumanou, MM (2013). Effectiveness of plant extracts in the fight against toxigenic molds isolated from post-harvest peanuts in Benin. *Journal of Applied Biosciences* , *70* , 5555-5566.
- Basak, S., & Guha, P. (2018). A review on antifungal activity and mode of action of essential oils and their delivery as nano-sized oil droplets in food system. *Journal of food science and technology*, *55*, 4701-4710.
- Ben Miri, Y. (2019). Etude du potentiel antifongique, antiaflatoxinogène et antioxydant de certaines huiles essentielles et leur efficacité dans le système alimentaire. *Université Mouloud MAMMERI de Tizi-Ouzou, Tizi-Ouzou*.
- Bertella. (2019). Etude de l'activité antimicrobienne et antioxydante des huiles essentielles d'*Artemisia herba-alba*, *Artemisia campestris* et *Rosmarinus tournefortii*. Département de Biologie, Laboratoire de Microbiologie Appliquée.
- Dammak, I., Hamdi, Z., El Euch, S. K., Zemni, H., Mliki, A., Hassouna, M., & Lasram, S. (2019). Evaluation of antifungal and anti-ochratoxigenic activities of Salvia officinalis, Lavandula dentata and Laurus nobilis essential oils and a major monoterpene constituent 1, 8-cineole against Aspergillus carbonarius. *Industrial Crops and Products*, *128*, 85-93.
- Debonne, E., Van Bockstaele, F., De Leyn, I., Devlieghere, F., & Eeckhout, M. (2018). Validation of in-vitro antifungal activity of thyme essential oil on Aspergillus niger and Penicillium paneum through application in par-baked wheat and sourdough bread. *Lwt*, *87*, 368-378.
- Diánez, F., Santos, M., Parra, C., Navarro, M. J., Blanco, R., Gea, F. J. (2018). Screening of antifungal activity of twelve essential oils against eight pathogenic fungi of vegetables and mushroom. *Letters in Appled Microbiology*, 67(4), 600-610.
- El Khoury, R. (2016). *Maîtrise du risque aflatoxique* : utilisation d'extraits naturels et mise en évidence de leurs mécanismes d'action. Université De Toulouse.
- Goly, K. R. C., Dadie, A., Soro, Y., Kouame N'zebo, Dé., Kassi, A. B. B., & DJE, M. (2017). Antimicrobial and preservative activities of *Lippia multiflora* essential oil on smoked mackerel (*Scomber scombrus*) fish. *Archives of Clinical Microbiology*, *8*(1), 1-5.
- Goly, K. R. C., Soro, Y., Dadie, A., Kassi, A. B. B., & Djé, M. (2015). Antibacterial activity of essential oils and extracts from the leaves of Hyptis suaveolens and Lippia multiflora on multi-resistant bacteria. *Rasayan Journal of Chemistry*, *8*(4), 396-403.
- Guede-Guina, F., Kra, A. M., Vangah-Manda, M., Bonga, G. M., & De Souza, C. (1997). Inhibition par MISCA-F 2 de la croissance de Aspergillus fumigatus; Candida albicans et Cryptococcus neoformans, 3 germes fongiques opportunistes du SIDA. *J. Afr. Biomed*, *2*, 11-16.
- Jeršek, B., Ulrih, N. P., Skrt, M., Gavarić, N., Božin, B., Možina, S. S. (2014). Effects of selected essential oils on the growth and production of ochratoxin A by *Penicillium verrucosum*. *Archives of Industrial Hygiene and Toxicology*, 199-208.
- Khallil, A. R. M. (2001). Phytofungitoxic properties in the aqueous extracts of some plants. *Pakistan Journal of Biological Science*, *4*(4), 392–394.
- Kra, A. K. M. (2001). Evaluation et amélioration par séquençage chromatographique d'une action antifongique de MISCA contre Aspergillus fumigatus. *UFR Biosciences*, 126.
- Malele, R. S., Mutayabarwa, C. K., Mwangi, J. W., Thoithi, G. N., Lopez, A. G., Lucini, E. I., & Zygadlo, J. A. (2003). Essential oil of Hyptis suaveolens (L.) Poit. from Tanzania: Composition and antifungal activity. *Journal of Essential Oil Research*, *15*(6), 438-440.
- Moreira, A. C. P., Lima, E. D. O., Wanderley, P. A., Carmo, E. S., & Souza, E. L. D. (2010). Chemical composition and antifungal activity of Hyptis suaveolens (L.) poit leaves essential oil against Aspergillus species. *Brazilian Journal of Microbiology*, *41*, 28-33.
- Mutlu-Ingok, A., Devecioglu, D., Dikmetas, D. N., Karbancioglu-Guler, F., & Capanoglu, E. (2020). Antibacterial, antifungal, antimycotoxigenic, and antioxidant activities of essential oils: An updated review. *Molecules*, *25*(20), 4711.
- Nantitanon, W., Chowwana, P. S., & Okonogi, S. (2007). Antioxidant and antimicrobial activity of *Hyptis suaveolens* essential oil. *Sci. pharm*, *75*, 35-36.
- Ngom, S., Faye, F. D., Diop, M., Kornprobst, J. M., & Samb, A. (2012). Composition chimique et propriétés physico-chimiques des huiles essentielles

d'Ocimum basilicum et d'Hyptis suaveolens (L.) Poit. récoltés dans la région de Dakar au Sénégal. *Bulletin de la Société Royale des Sciences de Liège*.

- Saladino, F., Luz, C., Manyes, L., Fernández-Franzón, M., & Meca, G. (2016). In vitro antifungal activity of lactic acid bacteria against mycotoxigenic fungi and their application in loaf bread shelf-life improvement. *Food Control*. 67, 273-277
- Simard, S., Hachey, J. M., & Collin, G. J. (1988). The variations of essential oil composition during the extraction process. The case of Thuja occidentalis L. and Abies balsamea (L.) Mill. *Journal of wood chemistry and technology*, *8*(4), 561-573.
- Souza, D. P., Pimentel, R. B., Santos, A. S., Albuquerque, P. M., Fernandes, A. V., Junior, S. D., ... & Goncalves, J. F. (2020). Fungicidal properties and insights on the mechanisms of the action of volatile oils from Amazonian Aniba trees. *Industrial Crops and Products*, *143*, 111914.
- Tatsadjieu, N., Jazet, M., Ngassoum, M. B., Etoa, X., & Mbofung, M. F. (2010). Investigations on the essential oil of *Lippia rugosa* from Cameroon for its potential use as antifungal agent against *Aspergillus flavus* Link ex. Fries. *Food Control*, *5*, 161–166.
- Zihiri, G. N., Kra, A. M., & Guédé-Guina, F. (2003). Evaluation de l'activité antifongique de *Microglossa pyrifolia* (LAMARCK) O. KUNZE (Asteraceae) « PYMI » sur la croissance in vitro de *Candida albicans*. *Revue de Médecine et Pharmacie Africqine*, *17*, 11- 18.