Original Research Article

IN -VITRO EVALUATION OF THE ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS OF SOME MEDICINAL PLANTS ON PHYTOPATHOGENIC STRAINS IN THE KISANGANI REGION (DRC)

ABSTRACT

This study evaluates the antifungal activity of essential oils of Curcuma longa and Eucalyptus globulus on strains of *Lasiodiplodia theobromae* and *Pseudocercospora fijiensis* in the Kisangani region (DR Congo).

The essential oils were obtained after extraction by the hydro distillation method. The isolation of strains of *L. theobromae* was made from the fragments of mesocarp taken from the diseased cocoa, previously cut with a Scarpel and then disinfected with bleach water, while the strains of *P. fijiensis* were obtained by the method of discharging ascospores on agar medium (H2O Agar) and then transplanting on PDA. In addition, inhibition of mycelial growth on a petri dish in solid medium (PDA) was used as a method to assess the sensitivity of strains to the essential oils of the medicinal plants studied.

The results obtained show that the essential oils of the rhizomes of C. longa and the leaves of E. globulus showed a low yield in EO of 0.50% and 0.48% respectively. However, the EOs of the rhizomes of *C. longa* and the leaves of *E. globulus* exhibited inhibitory action on the mycelial growth of strains of *L. theobromae* and *P. fijiensis.*

The ANOVA test applied to the mean mycelial growth values of these two strains (*L. theobromae* and *P. fijiensis)* on the HE of *C. longa* showed that the difference is not significant (p-value = 0.47). In addition, there is also no significant difference (p-value = 0.51) between the mean mycelial growth values of these two fungal strains on the essential oil of *E. globulus.*

Keywords: Antifungal activity, essential oils, medicinal plants, phytopathogenic strains and In-Vitro.

1. INTRODUCTION

For a long time, essential oils (EO) have occupied a prominent place both in the perfume industry and in the pharmaceutical, culinary and canned food fields. Currently, chemists, biologists and doctors are constantly increasing their interest in these natural substances because of their uses in the treatment of certain infectious diseases for which synthetic antibiotics are

becoming less and less active or in the preservation of food against oxidation as alternatives to synthetic chemicals (1).

Despite its age, the study of EO is still relevant. The continued development of plant biotechnologies has been a major concern of researchers in recent years. Thus, the progress of science has revolutionized the history of aromatherapy (2).

Pests including plant pathogenic fungi among others continue to wreak havoc on food and cash crops. The cultivation of banana and plantain trees is threatened by the numerous attacks of diseases caused by fungi, nematodes and weevils, viruses, bacteria ... (3).

Black Leaf Streak Disease is among the various pests that these crops face (Black Leaf Streak Disease), it is the most serious leaf disease, being the most destructive and the most expensive in terms of control methods (4).

Black ray disease (MRN) or black *cercosporiosis* of the banana and plantain is caused by the fungus *Pseudocercospora fijiensis* (former name *Mycosphaerella fijiensis*) (5).

One of the most common diseases in the species Theobroma cacao is the black rot of pods. It is caused by *Lasiodiplodia theobromae* and causes significant production losses worldwide (6).

In the Democratic Republic of Congo, particularly the Kisangani region, few studies have been conducted on the in vitro evaluation of the antifungal activity of EO on the fungal strains of cocoa and banana trees.

For this reason, this study was conducted to evaluate the effectiveness of *Cucurma longa* and *Eucalyptus globulus* EOs on *L. theobromae* and *P. fijiensis* strains to control these diseases in our region.

2. MATERIAL AND METHODS

2.1. Study Area

This study was carried out in the city of Kisangani, the capital of the province of Tshopo (Figure 1). This city is located in the Central Congolese basin, at 0° 31′ 00" North and 25° 11′ 00" East. Its average altitude is 396 m (7). Administratively, Kisangani is made up of 6 communes: Makiso, Lubunga Mangobo, Kabondo, Kisangani, and Tshopo, covering a total area of 1,910 km2 (8).

The position of Kisangani city near the Equator as well as the set of ecoclimatic data gives it an equatorial climate of the Af type in the Köppen classification. This type of climate characterizes regions where the average temperature of the coldest month is above 18 °C (9). Thus, Kisangani soils can be classified into two main groups: soils from bedrock and derived soils, growing on alluvium. These soils are generally ferritic, sandy-clay and acidic. They are deep and heavily leached by rainwater (10).

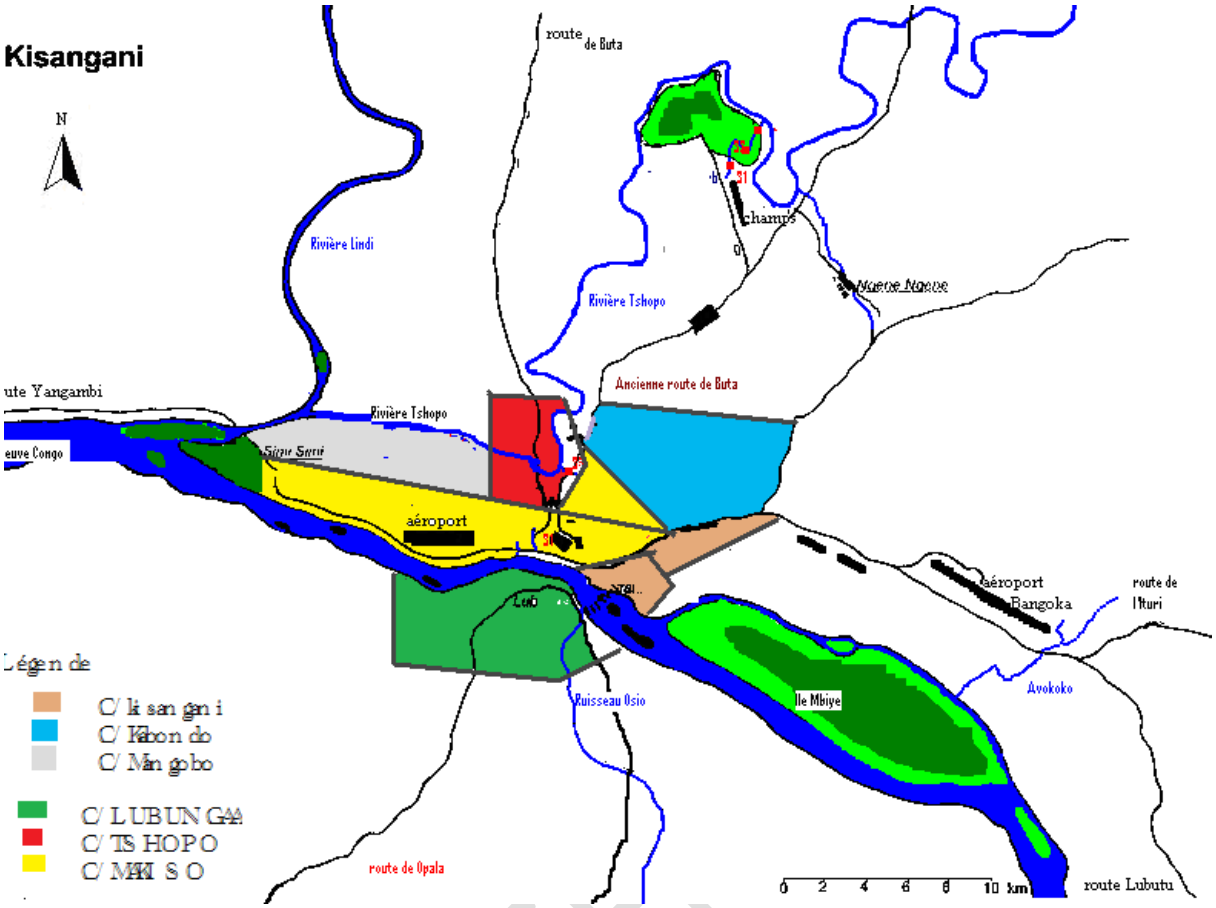

Fig. 1 KISANGANI City Map (8)

2.2. Material

2.2.1. Plants

Two medicinal plants were the subject of this study, C. longa and E. globulus. The latter were harvested in places with little traffic, in the morning in hot and dry weather. Only the healthy parts of the plant were harvested according to Debuigne (11).

1. Curcuma longa

(a) Systematic classification

C. longa is part of the family Zingiberaceae and the genus Curcuma which includes hundreds of plant species (12).

- Reign: *Plantae;*
- Division: *Magnoliophyta;*
- Class: *Liliopsida;*
- Order: *Zingiberales;*
- Family: *Zingiberaceae;*
- Genus: *Curcuma*
- Species: *C. longa.*

Native to India and South Asia, Curcuma is grown throughout Southeast Asia. It is one of the central pieces of Ayurvedic medicine are considered a symbol of prosperity and good health (13).

The plant is grown for its rhizomes, used as a dye, and spice for cooking. Chinese medicine also has a long tradition (14).

b) Botanical description

C. longa is a rhizomatous, herbaceous and perennial plant of the *Zingiberaceae* family, growing in all tropical climates, up to 1.50 m tall, with a short stem (13). The leaves are very long, having elliptical blades arranged in clumps (15).

As shown in Figure 2 below, it is a tuber plant whose flesh is orange-yellow and is both a spice, food and dye plant (16).

Figure 2: General appearance of *C. longa* rhizomes

2. Eucalyptus globulus

(a) Systematic classification

This species belongs to the *genus Eucalyptus*, family *Myrtaceae,* which includes about 90 genera with more than 600 species and varieties (17).

• Reign: *Plantae*;

• Division: *Magnoliophyta*;

• Class: *Magnolopsida*;

• Order: *Myrtales*;

• Family: *Myrtaceae*;

• Genus: *Eucalyptus*;

• Species: *E. globulus.*

It was the French botanist La Billardière who had discovered this plant in 1792, it's also called *Tasmanian Blue Gum*. This tree native to *Tasmania* (Australia) is grown today in the Mediterranean basin and in China where it's used to make paper pulp (18).

b) Botanical description

With a smooth trunk with the color varying from white to grey this tree is 30 to 60 meters high and can reach in some cases up to 100 meters. Its bark is easily detached in long strips (18). The young leaves are waxy, oval, clear, opposite and sessile. These leaves can be up to 25 centimeters long (Figure 3). They have a main rib mostly distinct on the underside and are sickle-shaped, alternate, petiolate, grey-green (18).

Figure 3: *E. globulus* Leaves

In this species, there are glands of EO secretions. These are schizogenic pockets secreting EO localized under the leaf epidermis in the palisade chlorophyll parenchyma. Their number is variable and is distributed differently in the leaves depending on the species. There is a strong correlation between secretory gland density and EO yield (17).

2.3. Methods

2.3.1. Preparation of the plants studied

The rhizomes of C. longa were used for fresh extraction while the leaves of E. globulus were harvested and dried at room temperature in the laboratory for 2 weeks.

The Sartorius AX2202 scale was used to weigh 800 grams of the C. longa sample, fresh and E. globulus, after drying. The quantity weighed was then crushed before moving on to the extraction.

2.3.2. EO extraction

The extraction of EO was done by the hydro distillation technique, according to the Levenger type C assembly (19).

This technique consists of heating the plant material in the presence of water until it boils. Thus, plant cells burst and release the odorous molecules that are driven by water vapour to the refrigerant where they condense. Then, a liquid is collected that is formed of two phases: the aqueous phase (the most abundant) and the organic phase consisting of EO (20).

The 800 g were put in a round-bottomed flask and 7 liters of water added, the whole was brought to a boil for 4 hours to obtain the distillate.

2.3.3. Calculation of yield

EO yield is defined as the ratio of the mass of EO obtained to the mass of the treated plant material (21). It is calculated by the following formula:

Rd = M'/M *100

With:

Rd: yield in EO in %;

M': mass of he in gr;

M: mass of the plant matter used in gr.

2.3.4. Obtaining the strains

• Strain of *L. theobromae*

The isolation of the strains was done from the fragments taken from the diseased cocoa from Bengamisa, previously cut with a scalpel and then disinfected with bleach for two minutes and then rinsed with sterile distilled water.

These fragments were seeded with pliers on the Potato Dextrose Agar culture medium (PDA, 39g/l) already solidified in Petri dishes (Figure 4). These boxes were taped with the parafilm and then incubated at 25 ° C under the darkness.

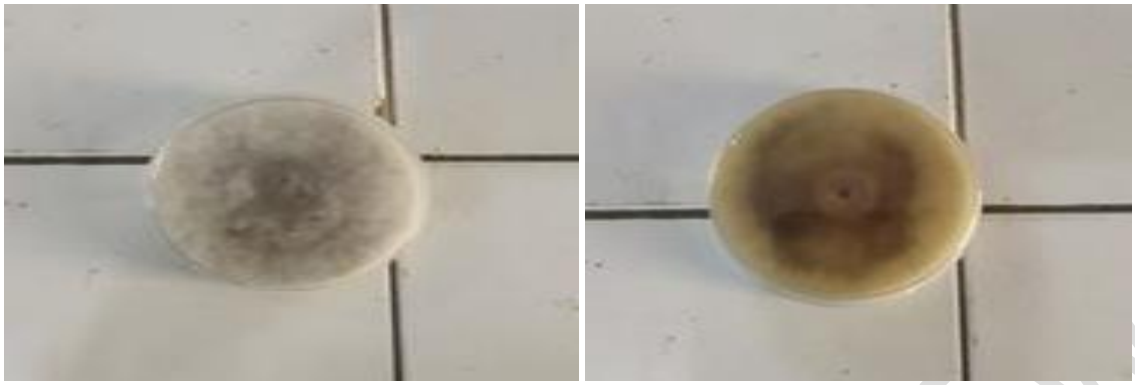

Figure 4: Strain of *L. theobromae* on a Petri dish. Upper (A) and underside (B).

Obtaining strain of *P. fijiensis*

The strains were isolated by the technique of discharging ascospores on agar medium (H2O Agar) from banana leaf samples collected at the Faculty of Sciences of the University of Kisangani, then transplanted to PDA (3).

For landfilling, the pieces of necrotic leaves were cut and soaked in sterile distilled water for 20 minutes to moisten them. Then they were placed inside the lids of Petri dishes and placed on boxes containing 3% agar, the underside of the sheet directed upwards on the culture medium. The boxes were incubated at 25°C overnight (in the dark). The next day, the ascospores unloaded on the agar were transplanted individually on PDA (39 g/l).

The transplanting was done by observation under an inverted microscope (Motic AE21), carefully recovering, one by one, the ascospores discharged using a needle. Crops were incubated at 25°C under permanent white light (22). Figure 5 below shows the strain of *P. fijiensis* on a Petri dish.

Figure 5: Strain of *P. fijiensis* on a Petri dish, upper (A) and lower (B) face.

2.3.5. Evaluation of antifungal activity

The evaluation of the in vitro antifungal activity of EO was performed in six repetitions. The mycelial growth of the strains is expressed based on the percentage of inhibition (PI). Indeed, 420 µl (0.42 ml) of the mixture consisting of EO and the 5% solution of Tween 80 (1:1 ratio) were added to 41580 µl (41.58 ml) of PDA cooled to 45 ° C after sterilization. Thus, 7000 µl (7 ml) of this homogenized mixture were poured into each petri dish of 50 mm in diameter. A 5 mm diameter mycelial explant obtained after perforation using a cookie cutter was placed on the culture medium in each petri dish. Mycelial diameter (MD) was measured every 24 hours until maximum growth. The witness was made under the same conditions, but without EO and Tween 80 (23).

The following formula was used for the calculation of IP:

IP= ((DM Tell-DM Essential Oil) X 100)/(DM Tell-tale)

Tween 80 (C64H124O26) or polyoxyethylene (80) monolaurate sorbitan is a surfactant that solubilizes essential oils in water.

A total of 24 Petri dishes were seeded with the strain of *L. theobromae* including 6 boxes with C. longa; 6 boxes with E. globulus; 6 boxes with Tween 80 and 6 control boxes. Incubation was done at 25 °C. This same procedure was repeated for the strain of *P. fijiensis.*

3. RESULTS AND DISCUSSION

3.1. EO performance

The percentage yield of EOs is presented in Table 1 below.

Table 1: EO Performance

EO	Extraction mode	**Yield in %**
C. longa	Fresh hydrodistillation	0,50
E. globulus	Hydrodistillation (drying)	**0,48**

The results of this table show that the two plants have almost the same yield of essential oils, i.e. 0.50% for *C. longa* and 0.48% for *E. globulus*. This slight difference would be due to the material subjected to distillation, one being fresh and the other dry.

The yield of C. longa obtained in this study is lower than that found by Tidjinil & Taibi (24), i.e. 0.9% and much lower than that of Gounder & Lingamallu (25) or 3.52%. Aicha & Khadidja (26) also worked on the EO of C. longa and obtained 0.86% while Soraya & Houda (27) obtained 0.6% on the polyphenolic content of *C. longa*.

In addition, the results of *E. globulus* are lower compared to Pereira et al. (28) which obtained 1.57% and Raho et al. (29) which is 1.2%.

On the other hand, our results are higher than those of Naziha & Rahma (30) or 0.07% and with those of Zakia (31) or 0.1% who worked on the EO of E. globulus.

This difference in yield is due to the extraction method, packaging and place of collection of the sample, the composition of chemical substances that may change depending on several factors including climate, environment and harvest period as well as the extraction technique.

Organoleptic parameters showed a resemblance between the two species of the plants studied. However, only the color marks the organoleptic difference between these two species. The table below illustrates the organoleptic parameters of the EO used (Table 2).

Table 2: Analysis of organoleptic parameters of EO

Parameters	***E. globulus***	***C. longa***
Appearance	Liquid	Liquid
Color	Dark- yellow	Light-yellow
Smell	Characterist	Characterist

This table reveals a color difference, either dark yellow for *E. globulus* or light yellow for *C. longa*. This difference would be due to the organ of the plant used for the extraction of EO of the species studied (leaves for *E. globulus* and rhizome for *C. longa*) and the abundance of pigments according to the plants studied.

Figure 6 below illustrates the EOs of the plants we used.

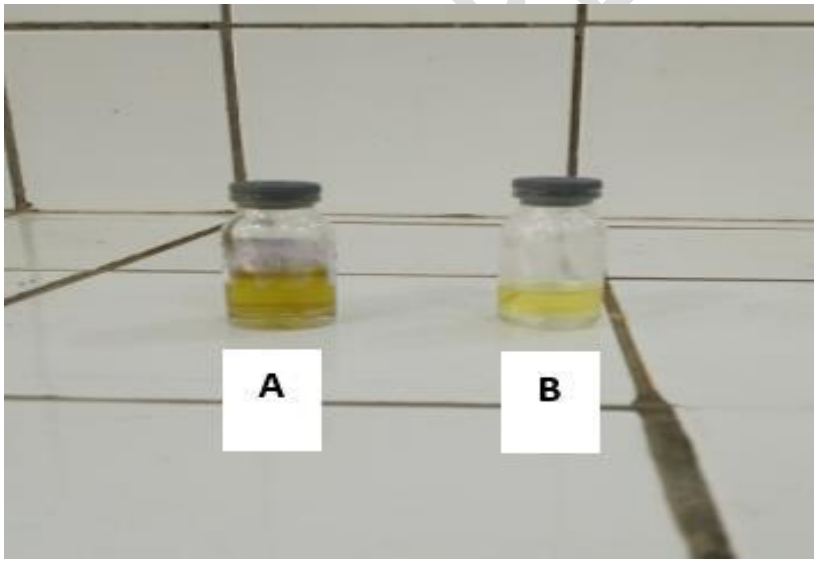

Figure 6: EO of *E. globulus* (A) and *C. longa* (B).

3.2. Mycelial growth

3.2.1. Strain of *L. theobromae*

The mycelial growth of *L. theobromae* strains on PDA added to *C. longa* EO is revealed in Figure 7 below.

The values in the graph do not take into account the initial diameter of the mycelial explant (5 mm)

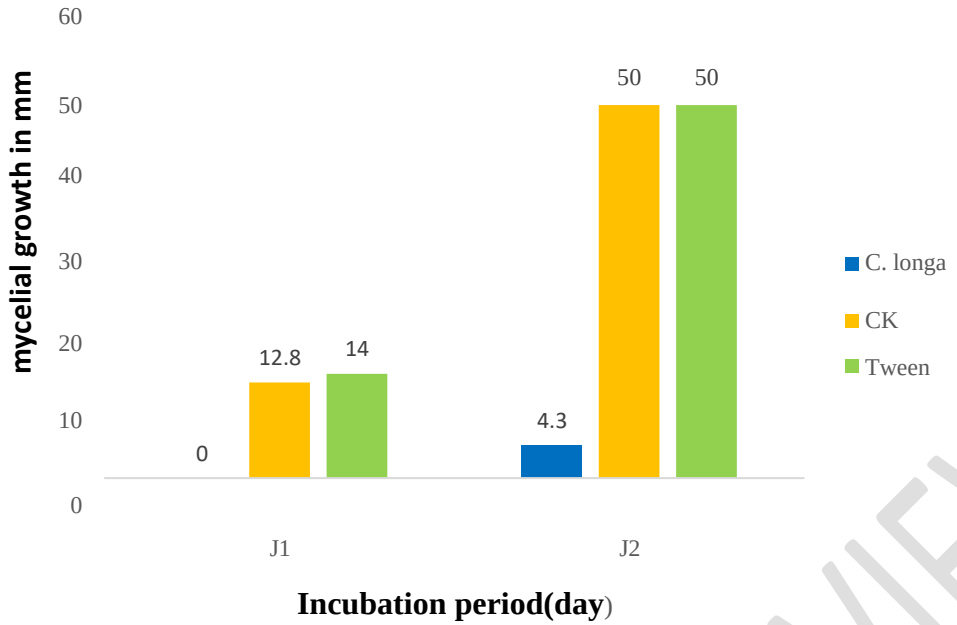

Figure 7: Mycelial growth of *L. theobromae* strain on the EO of *C. longa*.

It appears from this figure that with 5 mm of the seeded mycelial explantant, after 24 hours of incubation there is no growth while that of the control and tween 80 increased by 12.8 and 14 mm in diameter respectively. In addition, on the second day a mycelial growth of 4.3 mm in diameter on the EO was constant, while that of control and tween 80 increased further up to 50 mm in diameter.

In terms of Percentage of Inhibition (PI), on the first day of incubation we observed an PI of 100% and after 48 hours it was equal to 91.4%.

Sibatu (32) achieved 11.5 mm growth of this strain with *Ageratum conizoides* EO and 0 mm with *Cymbopogon citratus*. The difference could be explained by the plants used.

The mycelial growth of strain of *L. theobromae* on PDA added EO of *E. globulus* is illustrated by Figure 8.

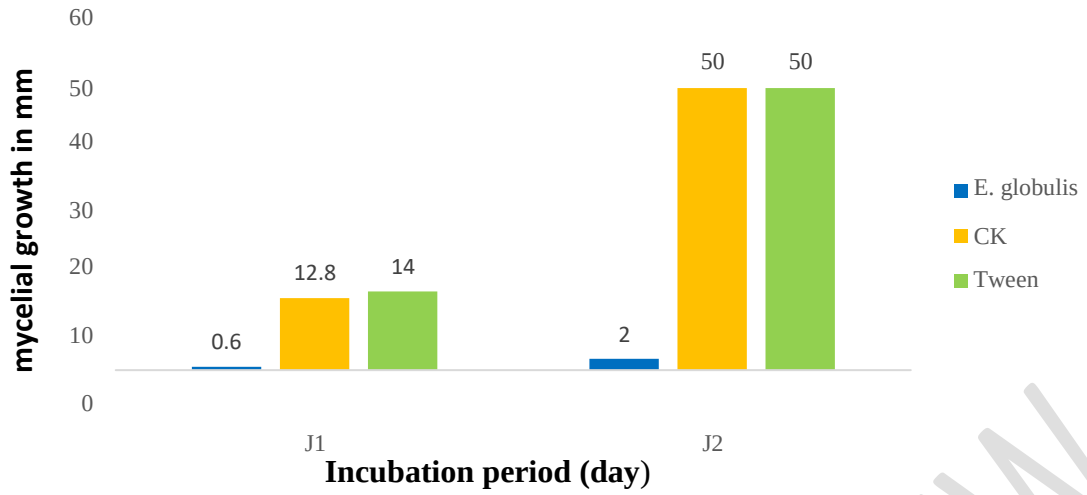

Figure 8: Mycelial growth of strain of *L. theobromae* on the EO of *E. globulus*.

It emerges from this figure that with 5 mm of the seeded mycelial explantant, the result shows that after 24 hours of incubation, the mycelial growth of the strain on the EO increased by 0.6 mm while that of control and tween 80 increased by 12.8 and 14 mm in diameter respectively. In addition, the result of the second day shows a growth on the HE of 2 mm in diameter while that of the control and tween 80 reached the maximum of 50 mm in diameter.

After 24 hours of incubation, we observed an PI of 98.8% and 48 hours later, there was a slight decrease in PI of 96%.

Anass and Rachid (33) had obtained an antibacterial activity of 10 mm with the *E.globulus* EO. The difference in strains used could explain this difference in inhibition.

Figure 9 illustrates the behaviour of the *L. theobromae* strain towards EO, control and tween 80.

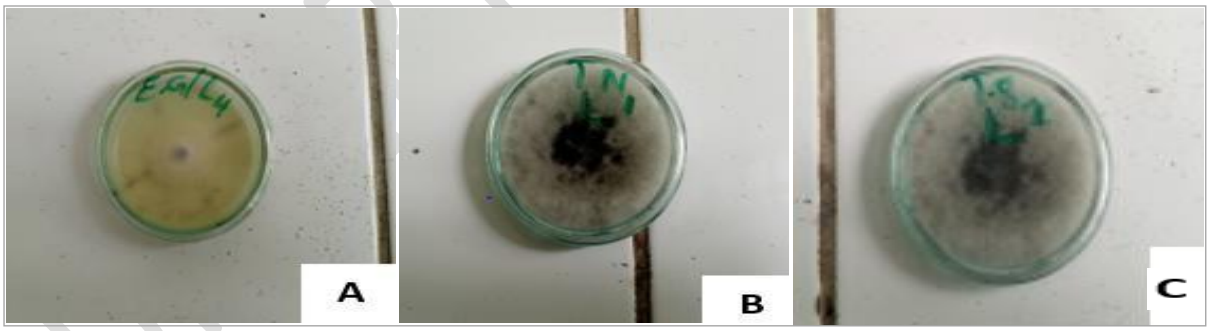

Figure 9: Mycelial growth of the strain of *L. theobromae*, the behavior of the strain EO (A), negative control (B) and control with tween 80 (C).

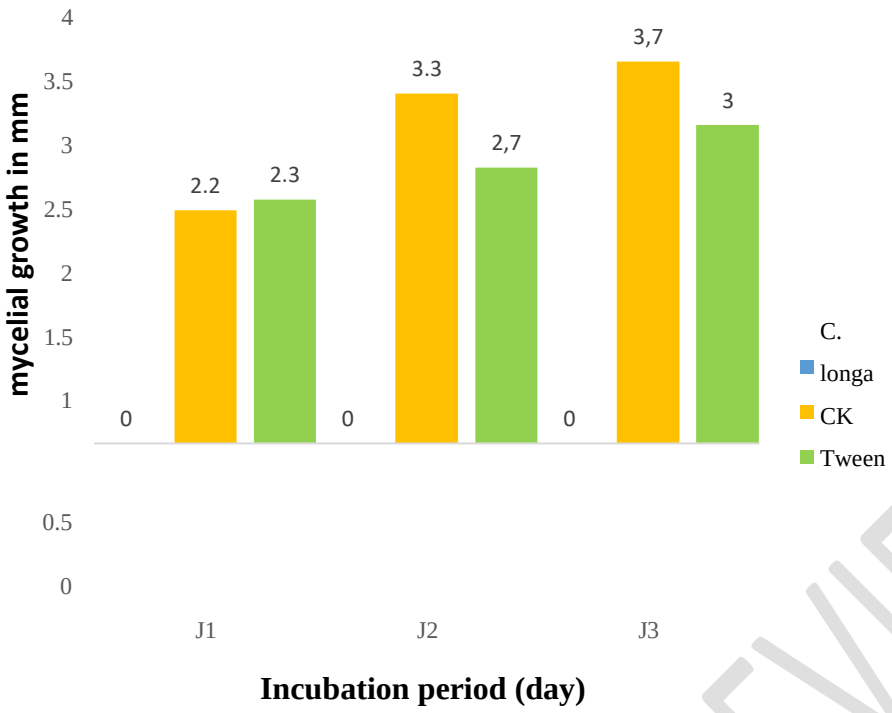

Figure 10: Mycelial growth of *P. fijiensis* strain on *C. longa* EO.

The results of this figure show that during three days of incubation there was no mycelial growth of the strain on the EO or 0 mm, moreover the mycelial growth on the control and tween 80 increased respectively 2.2 and 2.3 mm after 24 h; 3.3 and 2.6 mm after 48 hours of incubation and finally 3.6 and 3 mm in diameter after 48 hours of incubation.

The PI during the first, second and third day of incubation is maximum, i.e. 100%.

These results are similar to those obtained by Mukendi (34) with two plants (*Zingiber officinale* and *Tephrosia vogelii*) or 0 mm of mycelial growth of the strain of *P.fijiensis*.

Ludmila and Amira (35) had obtained an antimicrobial activity of 7 mm with *C.longa* EO. The difference in inhibition can be explained by the nature of the strain used.

Figure 11 illustrates the mycelial growth of *P. fijiensis* strain on PDA added *E. globulus*. EO

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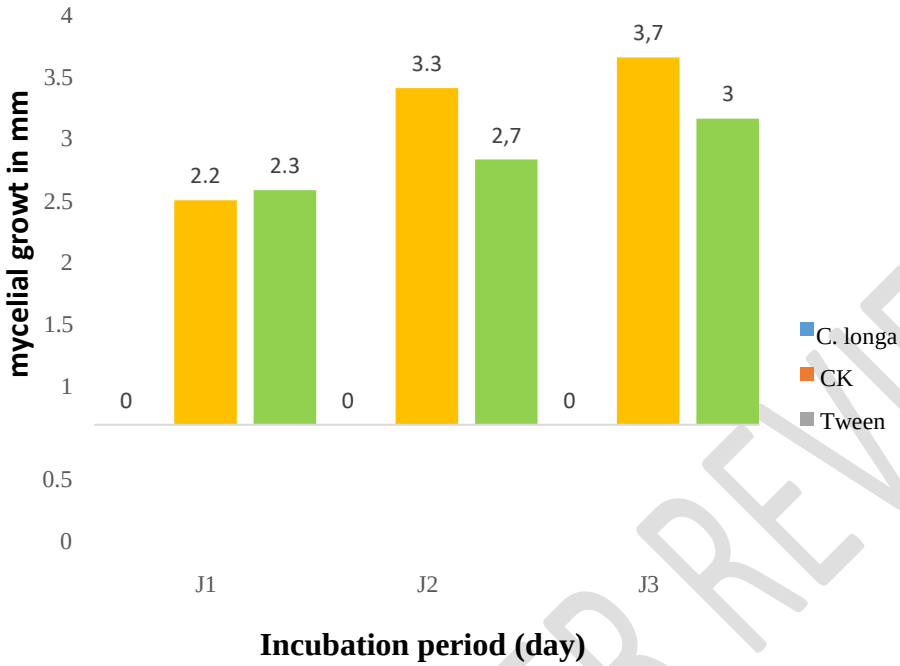

Figure 11: Mycelial growth of *P. fijiensis* strain on E. globulus.EO

It emerges from this figure that during three days of incubation, there was no mycelial growth of the strain on the EO or 0 mm. In addition, on the control and tween 80, there was a mycelial growth of 2.2 and 2.3 mm respectively after 24 h of incubation and 3.3 and 2.6 mm after 48 h and finally 3.6 and 3 mm of incubation diameter after 72 h.

Samoussa et al. (36) had obtained with the *E.globulus* EO an inhibition diameter of 10 mm on staphylococcal strains whereas for this study the PI was 100%. The explanation for this difference lies in the strains used.

4. CONCLUSION AND SUGGESTIONS

The present study focuses on the in vitro evaluation of the antifungal activity of EO of two medicinal plants including *C. longa* and *E. globulus* on strains of *L. theobromae* and *P. fijiensis* in the Kisangani region.

The essential oils were obtained after extraction by the method of hydrodistillation. The isolation of strains of *L. theobromae* was made from mesocarp fragments taken from the diseased cocoa while the strains of *P. fijiensis* were obtained by the method of discharging ascospores on agar medium (H_2O Agar) and transplanting on PDA. The method of inhibition of mycelial growth on a petri dish in a solid medium (PDA) made it possible to evaluate the sensitivity of the strains against the essential oils of the medicinal plants studied.

The main results achieved are as follows:

• The EOs of the rhizomes of *C. longa* and the leaves of *E. globulus* showed a low EO yield of

0.50% and 0.48%.

• The EOs of the rhizomes of *C. longa* and the leaves of *E. globulus* showed inhibitory actions on the mycelial growth of the strains of *L. theobromae* and *P. fijiensis*

• The PI of *C. longa* EO on the *L. theobromae* strain was 100% and 91.4% while that of *E. globulus* was 98.8% and 96% after 24 and 48 hours of incubation, respectively. For the strain of *P. fijiensis*, the PI of 100% for the essential oils of the plants used.

• The ANOVA test applied to the mean mycelial growth values of these two fungal strains on the HE of *C. longa* showed that the difference is not significant (p-value = 0.47). In addition, there is also no significant difference (p-value = 0.51) between the mean mycelial growth values of these two fungal strains on the EO of *E. globulus*.

Given the growing interest of EO, further research in this direction should be encouraged, particularly with other medicinal plants and also by diversifying extraction methods.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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