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Antioxidant, antiradical power of *Syzygium aromaticum* essential oil, and its antidermatophytic activity against *Epidermophyton floccosum* and *Trichophyton soudanense*

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Abstract

In order to provide an effective alternative for efficient management of dermatophytosis, we evaluate *in vitro* the antioxidant and antiradical potentials of the essential oil of the dry flower buds of *Syzygium aromaticum* and its antidermatophytic activity against *Epidermophyton floccosum* and *Trichophyton soudanense*. For this purpose, the essential oil was obtained by hydrodistillation through a Clevenger apparatus and the antioxidant and antiradical potentials were evaluated by the ferric reducing antioxidant power (FRAP) and the trapping of the ABTS^{•+} radical methods, respectively. The evaluation of the antidermatophytic activity was made by the agar incorporation method. The results showed that the essential oil reduced ferric iron (Fe³⁺) to ferrous iron (Fe²⁺) in a more significant manner than BHT (reference antioxidant). Moreover, the antiradical power of the essential oil was twice greater than that of BHT. Furthermore, the essential oil inhibited the mycelial growth of the two dermatophytes, with 500 ppm and 1000 ppm of minimal inhibitory concentrations against *Epidermophyton floccosum* and *Trichophyton soudanense*, respectively. This activity was greater than that of griseofulvin whose minimal inhibitory concentration was greater than 4000 ppm against both studied germs. These findings show that, within the framework of safeguarding human lives and protecting the environment, the essential oil from the dry flower buds of *Syzygium aromaticum* appeared as reliable alternative for the treatment of dermatophytosis caused by *Epidermophyton floccosum* and *Trichophyton soudanense*. Our results lay scientific foundation toward the promotion and development of Cameroonian biodiversity in treatments of dermatophytosis.

Keywords: Antioxidant; Antiradical; Antidermatophytic; *Syzygium aromaticum*; *Epidermophyton floccosum*; *Trichophyton soudanense*

1. Introduction

Mycosis are infections caused by microscopic fungi, which location can be deep, subcutaneous or superficial [1]. When they are superficial, they affect the skin, integuments (nails, body hair and hair) and mucous membranes. They are not fatal but can be very contagious, embarrassing and unsightly. Among the agents responsible for these superficial mycoses, three groups of fungi are isolated with a prevalence of (5.3 %) for molds, (36.7 %) for yeasts and (58 %) for

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dermatophytes [2, 3]. Dermatophytes are a group of filamentous fungi able of digesting keratin and responsible of dermatophytosis [4]. They affect 20 % to 25 % of the world's population [5]. They most affected are tropical and subtropical countries because of the presence of factors such as heat, high humidity, lack of adequate hygiene of life and promiscuity [6]. In Cameroon for instance, dermatophytosis constitutes a major cause of consultation in dermatology with a prevalence of 40.61 % for *tinea capitis* and 57.7 % for onychomycosis [7]. The management of dermatophytosis in modern medicine requires antifungals drugs such as terbinafine, itraconazole, ketoconazole and griseofulvin [8]. The latter although effective, present risks of toxicity, undesirable side effects and are sometimes inaccessible for low income people [9]. These synthetic drugs have reduced activity spectra as a result of their single active constituent and that lead to regular treatment failures. The situation is even more worrying because of the appearance of multi-resistant strains of microorganisms [9,10]. In addition, these microorganisms are the source of inflammatory reactions, resulting in an overproduction of free radicals that can induce oxidative stress, a source of numerous damages [11]. Thus, the use of antioxydant agents together with antifungal drugs could be helpful in both, regressing and shortening the duration of disease. Increasingly, people around the world, mainly those in developing countries, are turning to herbal remedies for their healing because they are accessible and affordable. In fact, plants contain many bioactive substances and may offer a natural and novel source of usable antimicrobial agents [12]. Among them, we can mention the clove tree, scientifically called *Syzygium aromaticum*. It is a medicinal plant native to the Moluccan Islands and traditionally used for its richness in secondary metabolites. Many biological properties of this plant have already been demonstrated by various authors [13-15]. That's why our interest in this study was to evaluate the antioxidant, antiradical and antidermatophytic activities of the dry flower buds of *Syzygium aromaticum* and contribute in the research of alternative treatments for dermatophytosis.

2. Material and methods

2.1. Plant material

Dry flower buds of *Syzygium aromaticum* were collected in Penja in the Littoral region of Cameroon (4°38'11" N and 9°40'55" E). The botanical identification of the plant was made at the National Herbarium of Cameroon under the reference: 2008SKR/CAM.

2.2. Pathogens

Two dermatophytes isolates: *Epidermophyton floccosum* and *Trichophyton soudanense* were used. They were respectively collected at the Microbiology and antimicrobial substances Laboratory of the University of Dschang (Cameroon), and at the Mycology Laboratory of the Gyneco-Obstetric and Pediatric Hospital of Douala (Cameroon).

2.3. Extraction of the essential oil

The extraction of the essential oil (EO) was done by hydrodistillation using a Clevenger type apparatus [16]. For this purpose, 500 g of dry flower buds were immersed in 2 L of water inside a reactor and the hydrodistillation was carried out for 8 hours. Two phases were obtained: an organic phase, which was the essential oil, and a water-soluble phase with a milky appearance, constituted by water and essential oil. The essential oil was separated from water by a liquid-liquid extraction [17], using hexane in proportions of 1/2 (150 mL of aqueous phase for 300 mL of hexane). The hexane was evaporated through a rotary evaporator at 70 °C, and the purified essential oil was added to the previous organic phase. The essential oil obtained was dried using anhydrous sodium sulfate and kept in a refrigerator at 4 °C.

2.4. Evaluation of the antioxidant and antiradical activities of the essential oil

The *in vitro* antioxidant and antiradical activities of *Syzygium aromaticum* essential oil were carried out according to the FRAP and ABTS methods, in comparison with a reference synthetic antioxidant: the Butylhydroxytoluene (BHT).

2.4.1. Antioxidant activity of the essential oil: the ferric reducing antioxidant power method (FRAP)

The ability of essential oil to reduce ferric iron (Fe³⁺) to ferrous iron (Fe²⁺) was evaluated according to the protocol described by Oyaizu [18]. Briefly, 250 µL of EO in gradual concentrations (25 ppm, 50 ppm, 100 ppm, 200 ppm, 400 ppm) were mixed with 625 µL of a phosphate buffer solution (0.2 M, pH = 6.6) and 625 µL of a 1 % solution of potassium ferricyanide. The whole mixture was incubated in water bath set at 50 °C for 20 min, then 625 µL of 10 % trichloroacetic acid were added to stop the reaction. The mixture was then centrifuged at 3500 rpm for 10 min using a SIGMA brand centrifuge. Then 625 µL of the supernatant were collected and mixed with 625 µL of distilled water and 125 µL of a freshly prepared 0.1 % solution of iron III chloride (FeCl₃). The blank was similarly prepared by replacing the samples with 250 µL of methanol to calibrate the instrument. The absorbances of the reaction medium were read at 700 nm.

The results expression was done qualitatively as presented by Kanoun [19], by comparing the curve of the essential oil's absorbances versus concentration with that of BHT: the substance whose curve was above had the best reducing power of iron (Fe^{3+}).

2.4.2. Antiradical activity of the essential oil: the 2,2-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) scavenging method

The ability of essential oil to trap the cationic radical $\text{ABTS}^{•+}$ was evaluated according to the protocol described by Re *et al.*, 1999 [20]. For this purpose, the $\text{ABTS}^{•+}$ cation radical was generated by mixing in equal volume a solution of potassium persulfate $\text{K}_2\text{S}_2\text{O}_8$ at 2.45 mM and a stock solution of ABTS at 7 mM. The mixture was then stored away from light, at room temperature for 15 hours. The solution obtained was diluted 20 times with distilled water. The tests were done in triplicate as followed: 1000 μL of the latter were mixed with 10 μL of the essential oil or the BHT at the concentrations of 25 ppm, 50 ppm, 100 ppm, 200 ppm, 400 ppm. The negative control was similarly prepared by replacing the essential oil with 10 μL of methanol. After 30 minutes at room temperature, the optical densities were read at 734 nm using a spectrophotometer. The following parameters were determined:

- The SC_{50} (50 % Scavenging Concentration) was determined graphically: it is the concentration required for 50 % $\text{ABTS}^{•+}$ reduction [21].

$$\% \text{ SC} = \frac{A_{\text{ref}} - A_{\text{mes}}}{A_{\text{ref}} - A_{100}} \times 100$$

A_{ref} : Absorbance of the negative control at $t = 30$ minutes; A_{mes} : Absorbance of the solution containing the essential oil at $t = 30$ minutes; A_{100} : Absorbance at the end of the reaction (close to 0) for total trapping.

- The EC_{50} (Effective Concentration 50), in grams of extract per mol of ABTS.

$$\text{EC}_{50} = \frac{\text{SC}_{50}}{C_{\text{ABTS}}}$$

C_{ABTS} : Molar Concentration of ABTS.

- The AP (Antiradical Power): expresses the antioxidant as the highest and most effective.

$$\text{AP} = \frac{1}{\text{EC}_{50}}$$

2.5. Evaluation of the antidermatophytic activity of the essential oil

The antidermatophytic activity of the essential oil was evaluated by the method of incorporation in agar medium according to the protocol described by Lahlou *et al.*, 2004 [22]. A stock solution was prepared by mixing the essential oil with Dimethylsulfoxide (DMSO) in the proportions 1/9 (1 volume of essential oil / 9 volumes DMSO). Different volumes of this solution were taken and incorporated into the liquid culture medium, so as to obtain different concentrations of the essential oil and a final volume equal to 10 ml. Each solution was then homogenized and poured into a 90 mm petri dish. For each concentration, three tests were carried out. After solidification of the culture medium, a 3 mm mycelial disc taken from a 4 days pre-culture was placed in the center of the Petri dishes. The whole was incubated at room temperature and the mycelial growth was followed by measuring the diameter of the mycelial disc daily for 10 days (*E. floccosum*) and 14 days (*T. soudanense*). The inhibition percentages were calculated according to the following formula:

$$\% \text{ I} = \frac{\text{Dt} - \text{De}}{\text{Dt}} \times 100$$

Dt = diameter of the fungal growth in the negative control Petri dish; De = diameter of the fungal growth in the test Petri dish.

Griseofulvin was used as positive control.

2.6. Minimal inhibitory concentration (MIC) and Minimal fungicidal concentration (MFC)

The MIC of the essential oil was determined as the smallest concentration of essential oil which totally inhibited the visible growth of the dermatophyte until the end of the test.

The MFC was determined by transferring the mycelial discs from the Petri dishes where the growth inhibition was complete, into new dishes containing the SDA medium not supplemented with essential oil. The Petri dishes were incubated for 10 days (*E. floccosum*) and 14 days (*T. soudanense*). Thus, the sample was said to be fungistatic when there wasn't dermatophyte growth, and fungicidal if not.

2.7. Statistical analysis of data

All tests were performed in triplicate. The results were expressed as a mean plus or minus standard deviation. Analysis of variance and testing of multiple ranges were performed to highlight significant differences between the means. Pearson's correlations were performed to test the significance link between percentages and concentration of essential oils and positive controls. The analyzes were all carried out using StatGraphics Centurium software 17.1.8 version, and the significance level was set at 0.05.

3. Results

3.1. Antioxidant activity: Ferric reducing antioxidant power

The reduction of ferric iron (Fe^{3+}) showed a significant increase ($p < 0.05$) in absorbance at 700 nm with the concentration of the essential oil and BHT (figure 1).

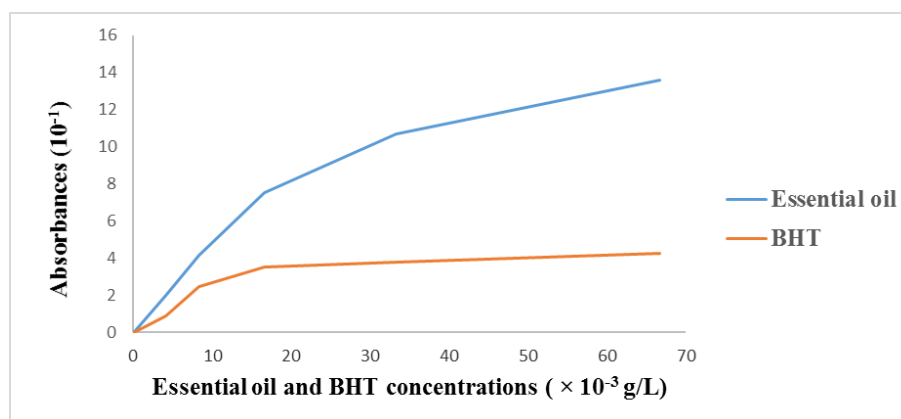


Figure 1 Variation of absorbance with concentration of the essential oil and BHT

It emerges from figure 1 that the curve for ferric iron reduction by the essential oil is above that of BHT, meaning the essential oil had a higher ferric reducing power than the BHT.

3.2. Antiradical activity: trapping of $\text{ABTS}^{\bullet+}$ radical

The absorbances values read on the spectrophotometer at 734 nm were used to calculate the scavenging percentages of the $\text{ABTS}^{\bullet+}$ radical by the essential oil (EO) and BHT, which were graphically used to determine the SC_{50} (figure 2).

Figure 2 shows that the SC_{50} values of the EO and BHT are respectively 0.8×10^{-3} g/L and 1.55×10^{-3} g/L. The 50 % Effective Concentration (EC_{50}) and the antiradical power (AP) of both substances were calculated using the SC_{50} corresponding values, and were resumed in table 1. The values in this table showed that the antiradical power of the essential oil of dry flower buds of *Syzygium aromaticum* (9.09 mol/g) is twice greater than that of the BHT (4.55 mol/g).

Table 1 SC_{50} , EC_{50} , and AP values for each tested sample

| Tested Substances | SC_{50} (g/L) | EC_{50} (g/mol) | AP (mol/g) |
|-------------------|------------------------|--------------------------|------------|
| Essential oil | 0.8×10^{-3} | 0.11 | 9.09 |
| BHT | 1.55×10^{-3} | 0.22 | 4.55 |

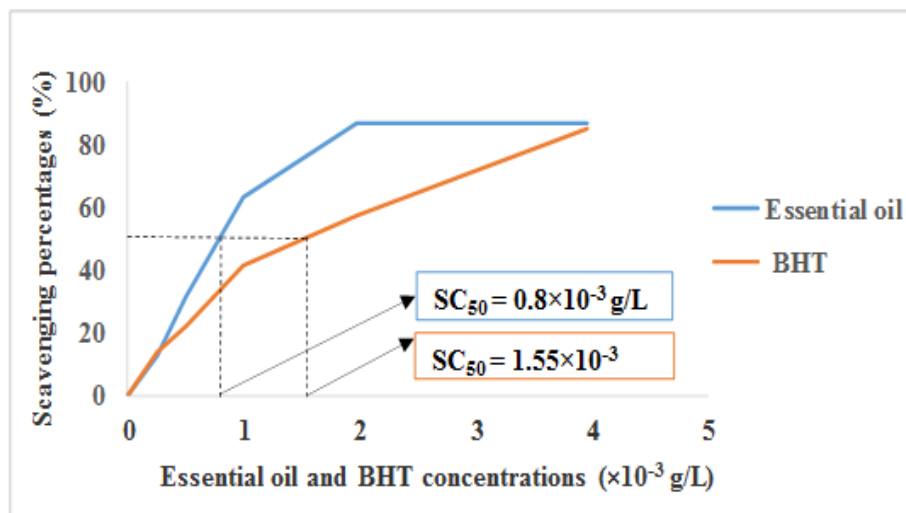


Figure 2 Variation of scavenging percentage with the EO and BHT concentrations

3.3. Antidermatophytic activity of the essential oil

The inhibition percentages of the two dermatophytes mycelial growth increased significantly ($p < 0.05$) with the concentration of the essential oil of *S. aromaticum*.

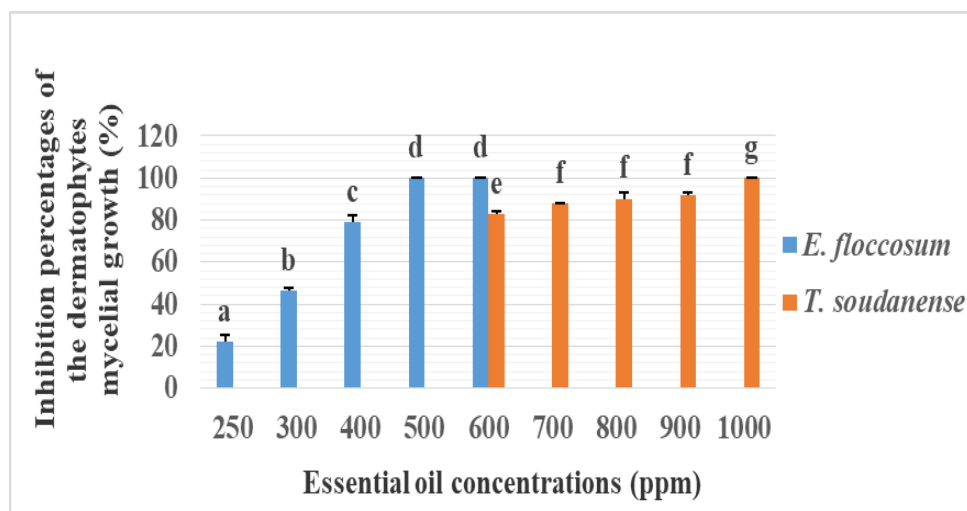


Figure 3 Inhibition Percentages of the two dermatophytes mycelial growth depending on the concentration of the essential oil of flower buds of *S. aromaticum* after 09 and 14 days (*E. floccosum* and *T. soudanense* respectively) of incubation

The analysis of figure 3 shows that the minimal inhibitory concentration of the essential oil was 500 ppm against *E. floccosum* and 1000 ppm against *T. soudanense*. This shows that the essential oil had a better activity against *E. floccosum* growth. These concentrations also represent the minimum fungistatic concentration for each germ.

3.4. Antidermatophytic activity of griseofulvin

It emerges from figure 4 that the inhibition of each dermatophyte mycelial growth was not total, as 100 % inhibition was not achieved at any dose level tested: 500 ppm, 1000 ppm, 2000 ppm and 4000 ppm. So, griseofulvin MIC was greater than 4000 ppm against both *E. floccosum* and *T. soudanense*.

The inhibition percentages of the two dermatophytes mycelial growth by griseofulvin is illustrated in figure 4 below.

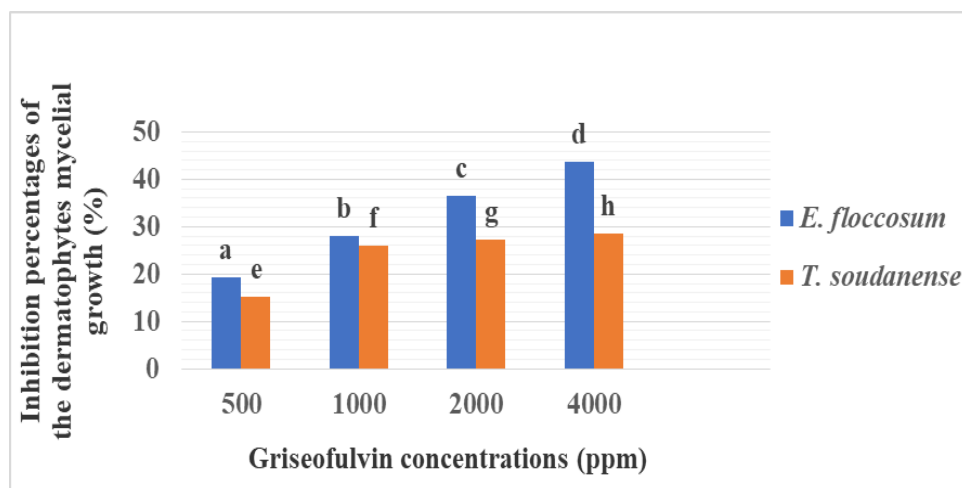


Figure 4 Inhibition Percentages of the two dermatophytes mycelial growth depending on the concentration of griseofulvin after 09 and 14 days (*E. floccosum* and *T. soudanense* respectively) of incubation

4. Discussion

4.1. Antioxidant and antiradical activities

Irrespective of methods used (FRAP and ABTS tests), the essential oil of the dry flower buds of *Syzygium aromaticum* has a significantly higher activity than that of BHT. Thus, in pathological conditions, the EO can use both scavenging capacity and reducing power to inhibit the proliferation of free radicals and oxidation reactions, which can lead to oxidative stress and involve skin damages and disease processes [23].

According to several authors the biological activity of an essential oil is related to its chemical composition [24, 25]. In the context of this work, the strong antioxidant and anti-free radical activity of the essential oil could be justified by the high level of eugenol (87.62 %) [26]. In fact, many authors reported a significant anti-radical activity of eugenol [27-29]. This strong activity can also be justified by the presence of some compounds such as eugenyl acetate, isoeugenol and β -caryophyllene, which could have a synergistic effect with the activity of Eugenol [30].

4.2. Antidermatophytic activity

The essential oil of *Syzygium aromaticum* was more active than griseofulvin, with MIC of 500 ppm against *E. floccosum* and 1000 ppm against *T. soudanense*. The activity of the essential oil can be justified by the presence of Eugenol, a major compound known for its antifungal properties [31]. This activity can also be explained by the complexity of the chemical composition of the essential oil [32] or by a possible synergistic effect between the major compounds and the minor compounds present in this essential oil [33]. Indeed, synergy is the result of the interaction between several compounds, so that the biological effects of a plant or a part of a plant are greater than the sum of the effects of its constituents studied separately [34].

These results are different from those of Pinto *et al.* [35], who evaluated the antifungal activity of the essential oil of *Eugenia caryophyllata* against *E. floccosum*. They have shown that EO induces a total inhibition of the growth of *E. floccosum* at a concentration of 0.16 mg/ml (160 ppm). The difference in activity observed between the results of these authors and those obtained in this work can be explained by the difference in sensitivity of the microorganisms or by the variation in the chemical composition of the essential oil.

5. Conclusion

The results obtained in this study show that the essential oil of the dry flower buds of *Syzygium aromaticum* can be used as an alternative to conventional antifungal drugs in the treatment of dermatophytosis and skin damages caused by *Epidermophyton floccosum* and *Trichophyton soudanense*. Further studies on the stability, toxicity and efficacy of this essential oil formulations *in vivo* are needed.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

References

- [1] Nabila EH. Mycoses: Study of a series listed in the parasitology-medical mycology department of the IBN SINA Hospital in Rabat over a period of 5 years (2007-2011). [Pharmacy thesis]. Rabat: University of Mohammed – Souissi. 2013.
- [2] Ndiaye M, Diongue K, Badiane A, Seck M, Ndiaye D. Epidemiological profile of superficial mycoses isolated in Dakar: Retrospective study 2011-2015. *J Mycol Med.* 2017; 27(3): 35.
- [3] Hassani L. Superficial yeast infection. *Dermatology course for students enrolled in 6th year of Medicine.* University of Constantine. 2020; 3: 2020.
- [4] AL-Khikani FH, Ayit AS. Major challenges in dermatophytosis treatment: current options and future vision. *Egypt J Dermatol Venereol.* 2021; 41(1): 1–9.
- [5] Lopes G, Pinto E, Salgueiro L. Natural products: An alternative to conventional therapy for dermatophytosis. *Mycopath.* 2017; 182(1-2): 143-67.
- [6] Brans A. Superficial mycoses: "Pharmacology of antifungals". [Thesis in Pharmacy]. Lille: University of Lille. 2015.
- [7] Minkoumou S, Fabrizi V, Papini M. Onychomycosis in Cameroon: a clinical and epidemiological study among dermatological patients. *Inter J Dermatol.* 2012; 51(12): 1474-7.
- [8] Cambier L. Contribution to the study of the immune response during infection with *Microsporum canis* and establishment of a mouse model of dermatophytosis. [Thesis in veterinary sciences]. Belgique: University of Liège. 2014.
- [9] Falah HOA, Aalae SA. Major challenges in dermatophytosis treatment: current options and future visions. *Egypt J Dermatol Venereol.* 2020; 41(1): 1–9.
- [10] Ghannoum M. Azole resistance in dermatophyte: prevalence and mechanism of action. *J Am Podiatr Med Assoc.* 2016; 106(1): 79–86.
- [11] Ozturk P, Arican O, Kurutas EB, Karakas T, Gungor M. Local oxidative stress in interdigital *Tinea pedis*. *J Dermatol.* 2013; 40(2): 114-7.
- [12] Zellagui A, Gherraf N, Rhouati S. Chemical composition and antibacterial activity of the essential oils *Ferula vesceritensis* leaves, endemic in Algeria. *Org med chem lett.* 2012; 2(1): 1-4.
- [13] Razafimamonjison G, Jahiel M, Duclos T, Ramanoelina P, Fawbush F, Danthu P. Bud, leaf and stem essential oil composition of *Syzygium aromaticum* from Madagascar, Indonesia and Zanzibar. *Int J Basic Appl Sci.* 2014; 3(3): 224-233.
- [14] Sameza ML, Mabou NLC, Tchameni NSS, Bedine BMA, Tchoumboungang F, Jazet DPM, Fekam BF. Evaluation of clove essential oil as a mycobiocide against *Rhizopus stolonifer* and *Fusarium solani*, Tuber rot causing fungi in yam (*Dioscorea rotundata* pair). *J phytopath.* 2016; 164(7-8): 433-440.
- [15] Boukhatem F. Antibacterial activity of two spices: *Syzygium aromaticum* and *Illicium verum*. [Master memory]. Mostaganem: University of Abdelhamid Ibn. 2017.
- [16] Clevenger J. Apparatus for the determination of volatile oil. *J Am Pharm Assoc.* 1928; 17(4): 346-49.
- [17] Boubrit S, Boussad N. *In vitro* determination of the antibacterial power of essential oils of eucalyptus, myrtle, cloves and savory, and their application to the preservation of fresh minced meat. [Thesis for the diploma of State

Engineer in Biology, quality control and analysis option]. Algeria: Mouloud Mammeri University of Tizi-ouzou. 2007.

- [18] Oyaizu M. Antioxidative activities of browning reaction prepared from glucosamine. *Jpn J Nutr.* 1986; 44(6): 307-315.
- [19] Kanoun K. Contribution to the phytochemical study and antioxidant activity of extracts of *Myrtus communis* L. (Rayhane) from the region of Tlemcen (Honaine). [Magister's thesis in Biology]. Tlemcen: aboubekr belkaid tlemcen university. 2011.
- [20] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved radical cation bleaching assay ABTS. *Free Radic Biol Med.* 1999; 26(9-10): 1231-1237.
- [21] Jazet D, Tchoumboungang F, Ndongson B, Agwanande W, Sandjon B, Amvam Z. Chemical characterization, antiradical, antioxidant and anti-inflammatory potential of the essential oils of *Canarium schweinfurthii* and *Aucoumea klaineana* (*Burseraceae*) growing in Cameroon. *Agric. Biol. J. N. Am.* 2010; 1(4): 606-611.
- [22] Lahlou M. Methods to study the phytochemistry and bioactivity of essential oils. *Phytother Res.* 2004; 18(6): 435-448.
- [23] Karapehlivan M, Uzlu E, Kaya N, Kankavi O, Ural K, Cital M. Investigation of Some Biochemical Parameters and the Antioxidant System in Calves with Dermatophytosis. *Turk. J. Vet. Anim. Sci.* 2007; 31(2): 85-89.
- [24] Jazet D, Tatsadjieu L, Ndongson B, Kuate J, Amvam Z, Menut C. Correlation between chemical composition and antifungal properties of essential oils of *Callistemon rigidus* and *Callistemon citrinus* of Cameroon against *Phaeoramularia angolensis*. *J Med Plants Res.* 2009; 3(1): 9-15.
- [25] Aboughe S, Aworet S, Eyele M. Some properties of essential oils from medicinal plants from Gabon. *Phytother.* 2015; 13(5): 283-287.
- [26] Mekemzeu FP, Nguikwie KS, Sameza ML, Tchoumboungang F, Tchabong R, Tatsadjieu NL, Jazet DPM. Antioxidant and antifungal of cocoa butter (*Theobroma cacao*), essential oil of *Syzygium aromaticum* and a combinaison of both extracts against three dermatophytes. *ASRJETS.* 2017; 37(1): 255-278.
- [27] Barbelet S. The clove tree: history, description and uses of the plant and its essential oil. [Thesis dissertation in Pharmacy]. France: University of Lorraine. 2015.
- [28] Dai J, Zhao X, Zeng J, Wan Q, Yang J, Li W. Drug screening for autophagy inhibitors based on the dissociation of Beclin1-Bcl2 complex using BiFC technique and mechanism of eugenol on anti-influenza a virus activity. *PLoS One.* 2013; 8(4): 1-9.
- [29] Nam H, Kim M. Eugenol with antioxidant activity inhibits MMP-9 related to metastasis in human fibrosarcoma cells. *FCT.* 2013; 55: 106-112.
- [30] Sohilaït HJ, Kainama H. Free Radical Scavenging Activity of Essential Oil of *Eugenia caryophyllata* from Amboina Island and Derivatives of Eugenol. *Open Chem.* 2019; 17(1): 422-428.
- [31] Hmiri S, Amrani N, Rahouti M. *In vitro* determination of the antifungal activity of the vapors of Eugenol and essential oils of *Mentha pulegium* L. and *Tanacetum annuum* L. against three molds responsible for rotting potatoes. *Acta bot gall.* 2011 ; 158(4) : 609-616.
- [32] Mohammedi Z. Etude Phytochimique et Activités Biologiques de quelques Plantes médicinales de la Région Nord et Sud-Ouest de l'Algérie. [Thèse de Doctorat en Biologie]. Algérie : Université Abou Bekr Belkair. 2013.
- [33] Baptista EB, Zimmermann-Franco DC, Lataliza AAB, Raposo NRB. Chemical composition and antifungal activity of essential oil from *Eucalyptus smithii* against dermatophytes. *Rev Soc Bras Med Trop.* 2015 ; 48(6) : 746-752.
- [34] Biabiany M. Recherche et développement d'extraits antifongiques issus de la flore Guadeloupéenne : Caractérisations phytochimiques, pharmacologiques et formulation. [Thèse de Doctorat]. Nord de France : Université de Lille. 2011.
- [35] Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species. *J Med Microbiol.* 2009; 58(11): 1454-1462.