Evaluation of the Antimicrobial Activities of Hydroalcoholic Extracts of Four Aromatic Plants on the *in vitro* Growth of Germs Responsible for Cutaneous-Mucosal Infections

Bolou, GEK.[1]*, Koffi, D.[2], Ahon, GM.[3], N'Goran, AF.[4], N'Guessan, RCD.[5], Lidji JCSR.[5], Konan GKNA. [5], Acka, JAAB.[4], N'Guessan, JD.[5], Zirihi GN.[5], Djaman AJ.[5]
[1]National Center of Floristics, University Félix HOUPHOUËT-BOIGNY, Côte d'Ivoire
[2]Pasteur Institute of Cote d'Ivoire, Côte d'Ivoire
[3]National Pedagogical Institute of Technical and Professional Education, Côte d'Ivoire
[4]Ufr Agroforestry, University Jean Lorougnon GUEDE, Côte d'Ivoire
[5]Ufr Biosciences, University Félix HOUPHOUËT-BOIGNY, Côte d'Ivoire

Abstract. Several studies have shown that the essential oils of some aromatic plants have very good antimicrobial activities. But, knowing their low extraction yield and their sometimes toxic character, it was necessary to analyze the antimicrobial parameters of the crude extracts of four aromatic plants which are: *Ocimum gratissimum, Ocimum basilicum, Ocimum canum* and *Cymbopogon citratus.*

Thus, dilution tests were used to evaluate the antimicrobial activities of the hydroalcoholic extracts on *Staphylococcus aureus* and *Candida albicans*. The results obtained show that the four plants have a power of inhibition of the *in vitro* growth of *S. aureus* and *Ocimum gratissimum* proved to be the most active on the various tested germs. Its MIC was 6.25 mg/mL and its MBC was 12.5 mg/mL on the *Staphylococcus aureus* strain, while on the two *Candida albicans* strains, its MIC and MBC were equal to 50 mg/mL. *Cymbopogon citratus* gave the best extraction yield which was 6.95%. This study demonstrated that hydroalcoholic extracts of aromatic plants have antibacterial activity just like their essential oils, although the latter are more active.

Key words: aromatic plant, extraction, antimicrobial activity

Introduction

Mucocutaneous infections (MCI) are diseases of the skin and various mucous membranes. These infections damage the skin flora which is naturally normal. These infections are very often of bacterial or fungal origin. In Côte d'Ivoire, as in developing countries, people commonly use plant resources to satisfy their primary health needs (Salhi et al., 2010). Thus, medicinal plants remain an important source of medicine in environments where the modern health system is absent (Tabuti et al., 2003). The effectiveness of essential oils on MCI is no longer in question as scientific studies have proven it (Fontanay et al., 2015; Koba et al.,2004; Dalia et al., 2020). However, the availability of these essential oils sometimes remains problematic due to their low extraction yield (Lidji, 2020). Moreover, they sometimes contain toxic compounds for human health (Franchomme et al., 1990). It is therefore important to research the pharmacological properties of aromatic plants in extracts other than their essential oils. The present study is therefore in line with the development of the Ivorian pharmacopoeia. The objective is to analyze the antimicrobial powers of the total crude extracts of four local aromatic and medicinal plants in order to substitute them for their essential oils. It will be specifically to carry out the ethanolic extraction of these plants and to determine their antimicrobial parameters.

Materials and Methods

* Corresponding Author

Biological Material

The plant material consisted of fresh leaves of the four aromatic plants below. These plants were collected and identified at the National Center of Floristics of the University Félix HOOUPHOUET-BOIGNY, under the herbarium numbers written before their names.

Ocimum gratissimum (Lamiaceae): UCJ008879; *Ocimum basilicum* (Lamiaceae): UCJ008874

Ocimum canum (Lamiaceae): UCJ008877; *Cymbopogon citratus* (Poaceae): UCJ007004

The germs tested consisted of two strains of *Candida albicans* and one strain of *Staphylococcus aureus* with resistance profiles as follows:

Candida albicans 370 (Amphotericin B^R)

Candida albicans 479 ($Fluconazole^R$ $Itraconazole^R$ $Voriconazole^R$)

Staphylococcus aureus 1174 ($Amoxicillin^R$ $Ampicillin^R$ $Oxacillin^R$ $Ceftazidin^R$ $Fosfomicin^R$ $Vancomycin^R$ $Cefsulodin^R$)

Methods

Preparation of Plant Extracts

The fresh leaves of the aromatic plants were harvested and weighed on an electronic scale. They were then washed with clean water and cut. The cut leaves were used to prepare the hydroethanol extract according to the method described by Zirihi et al. (2003) with some modifications. Indeed, 100 g of the cut leaves were ground in 1L of 70% ethanol using a blender. The resulting crushed material is sieved and the residue is homogenized in 500 mL of 70% ethanol twice in a row. Another filtration is performed, once on percale cloth and twice on increasingly tightly packed hydrophilic cotton in a funnel. The resulting liquid hydroalcoholic extract is evaporated in an oven at 50°C to obtain a dry extract.

Calculation of the Extraction Yield

The extraction yield (Ey) is defined as the ratio between the mass of dry hydroalcoholic extract obtained (M') and the mass of fresh plant material (leaf) used (M). The yield is expressed as a percentage. It is calculated by the following formula:

$$Ey\ (\%) = \frac{M'}{M}\ X100$$

Ey: Extraction yield in %.
M': Mass of dry hydroalcoholic extract obtained in gram.
M: Mass of fresh plant material (leaf) used in gram.

Evaluation of Antibacterial Activities

Preparation of the bacterial inoculum

A few 18 hours old colonies were collected with a platinum loop and bubbled in 1mL of sterile distilled water (SDW). The turbidity of this suspension was adjusted to 0.5 Macfarland using an opacity control (Bio-Rad). This estimated 10^8 cfu/mL bacterial suspension was diluted 1:100 in sterile Mueller-Hinton (MH) broth prepared as a twofold concentration (0.1 mL of bacterial suspension in 9.9 mL of MH broth) to yield an estimated 10^6 cfu/mL bacterial inoculum (Bolou et al., 2011).

Preparation of plant extract concentration ranges and inoculation

Concentration ranges were prepared by the double dilution method in seven (07) hemolysis tubes. To perform the double dilution, the first tube T1 contained 2 mL of sterile extract stock solution concentrated to 100 mg/mL, the other six tubes (T2 to T7) each contained

1 mL of SDW. Thus, 1 mL of the first tube was taken and diluted into the second tube. After shaking, 1 mL of the second tube was taken and diluted into the third. This operation was repeated successively in the other tubes. Finally, 1mL of the last tube (T7) was discarded to adjust the volumes. The resulting concentration range was then spread as follows: 100; 50; 25; 12.5; 6.25; 3.12 and 1.56 mg /mL. Then, in each of these different tubes, 1mL of bacterial inoculum at 106 cfu/mL was added. The concentration range of plant extract tested was finally halved from 50 mg/mL to 0.78 mg/mL. A growth control (GC) tube containing 1 mL of sterile distilled water and 1 mL of inoculum and a sterility control (SC) containing 1 mL of sterile distilled water and 1 mL of sterile Mueller-Hinton broth, were also prepared. All tubes were incubated in the oven for 24 h at 37°C.

Preparation of the bactericidal control

From four sterile test tubes containing 9 mL of SDW each, successive one-tenth dilutions of the inoculum to 10^6 cfu/mL are made, giving dilutions 10^{-1}; 10^{-2}; 10^{-3} and 10^{-4}, respectively. The starting inoculum is dilution 10^0. Using a loop, 10 µL of each dilution was streaked onto an agar medium in a Petri dish, already prepared for this purpose. This dish was then incubated at 37°C for 24 hours and stored as a bactericidal control.

Determination of antibacterial parameters

After 24 h of incubation of the bacterial culture in the concentration range, the Minimum Inhibitory Concentration (MIC) is determined by observing the microbial growth in each tube. The MIC is the lowest concentration of extract showing no visible growth of the germ. For the determination of the Minimum Bactericidal Concentration (MBC), all tubes with no visible growth were subcultured on a 5 cm streak on Mueller-Hinton agar using a 10 µL loop. This subculture was also incubated at 37°C for 24 h. After 24 h of incubation, the streaks will be compared to the 10^{-4} streak of the bactericidal control. The MBC is then the smallest concentration whose streak shows a colony number less than or equal to that of the 10^{-4} streak.

Evaluation of the Antifungal Activity on *C. albicans*

The antifungal activity is evaluated by the dilution method in solid medium and the antifungal parameters that are the Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) are determined (Fézan et al., 2007).

Incorporation of plant extracts into Sabouraud agar

The incorporation of the plant extracts into the agar was done by the double dilution method in tilted tubes. Each series has 7 tubes containing the plant extract incorporated into the culture medium and 2 control tubes, one without plant extract for germ growth control (GC) and the other without plant extract or germ for sterility control (SC) of the culture medium (Ahon, 2014; Thès, 2001). The test tubes contain a range of decreasing extract concentration that varies from 50 mg/mL to 0.78 mg/mL describing a geometric sequence of reason 1/2. To perform the double dilution, 1 g of plant extract was homogenized in 20 mL of liquefied Sabouraud agar in tube T1, giving a concentration of 50 mg/mL. Half of the volume of this homogeneous mixture was transferred to the next tube (T2), containing 10 mL of Sabouraud agar and then this mixture was homogenized. This operation was repeated successively for the other tubes until tube 7 (T7), with the lowest concentration (0.78 mg/mL). For this last tube, half of the content was discarded to adjust the volumes. The tubes thus prepared were sterilized at 121°C in an autoclave for 15 min and tilted at laboratory temperature to allow cooling and solidification of the agar (Zirihi et al., 2003; Bagré et al., 2011).

Fungal inoculum preparation, plating and incubation

The fungal inoculum was prepared from 24 h old colonies. A perfectly isolated colony of *C. albicans* was suspended in 10 mL of sterile distilled water to give a microbial suspension estimated at 10^6 cfu/mL. A 1:10 dilution of this bacterial suspension was then performed to give our fungal inoculum estimated at 10^5 cfu/mL. Next, All tubes were inoculated by

exhaustion with 10 µL of the inoculum, except for the sterility control which received 10 µL of sterile distilled water. The inoculated tubes were incubated at 30°C for 48 h (Guédé-Guina et al., 1997; Kra, 2001).

Determination of antifungal parameters (MIC and MFC)

After 48 h of incubation, the growth of the germ is observed in each tube of each series of culture. The Minimum Inhibitory Concentration (MIC) is determined for each extract. This is the minimum concentration for which there is no visible growth of the germ with the naked eye. The Minimum Fungicidal Concentration (MFC) is also determined. This is the lowest concentration of extract that results in 99.99% inhibition of germ growth, compared to the growth control. In practice, the MFC is determined by a sterility test of tubes without visible growth from the MIC. Tubes with no visible growth are plated on a Petri dish containing new Sabouraud agar, containing neither plant extract or any antifungal substance. The platinum loop was passed over the entire surface of each tube without visible growth and then streaked on new agar. After 48 hours of incubation at 30°C, the CMF was identified as the smallest concentration that did not show visible germ growth when streaked.

Results

Extraction Yields

The different extraction yields are presented by figure 1 below. Indeed, the extraction of *Cymbopogon citratus* gave the highest yield (6.95%). After, come successively in a decreasing order, *O. gratissimum* (4.61%), *O. canum* (3.33%) and finally, *O. basilicum* with the lowest yield (2.27%).

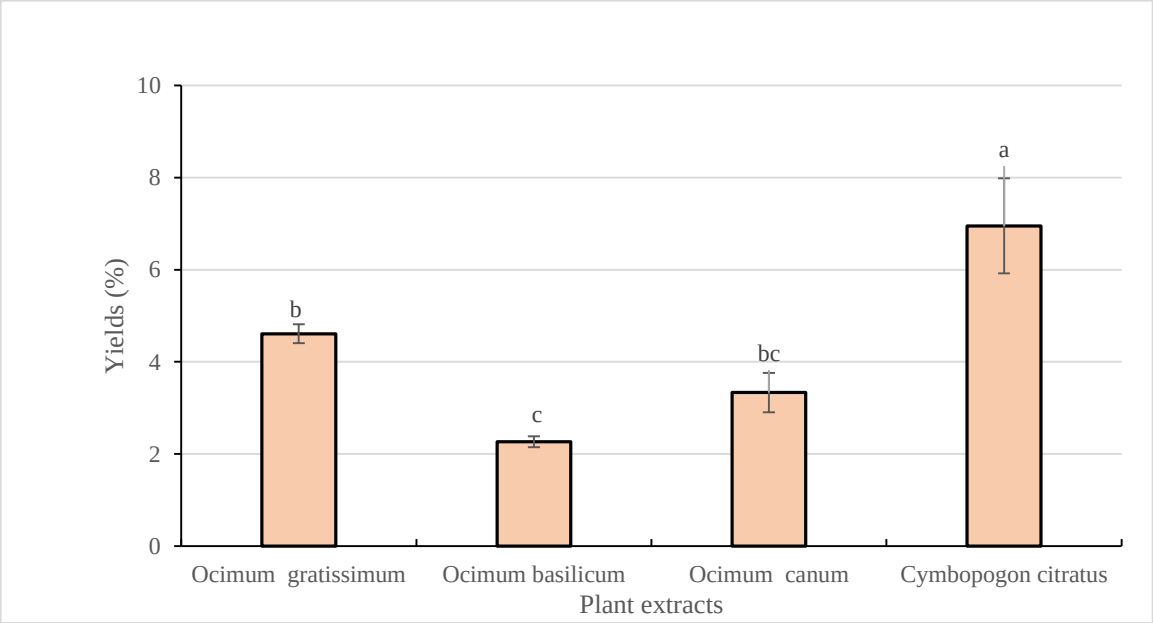

Figure 1. Yields of the different plant extractions

Antibacterial Activities of Plant Extracts on *Staphylococcus aureus*

The results reveal that the four aromatic plants have different activities (Table I). The MIC obtained are: 3.12 mg/mL for *Ocimum canum*, 6.25 mg/mL for *Ocimum gratissimum*, 12.5 mg/mL for *Ocimum basilicum* and 25 mg/mL for *Cymbopogon citratus*. The MBC of *Ocimum gratissimum* is 12.5 mg/mL while for the other three extracts, subculturing showed regrowth of bacteria for all tubes without visible growth. Therefore their MBC is higher than 50 mg/mL.

Table 1. Antibacterial parameters of plant extracts on a strain of *S. aureus*

Plant extracts	MIC (mg/mL)	MBC (mg/mL)
Ocimum gratissimum	6,25	12,50
Ocimum basilicum	12,50	>50
Ocimum canum	3,12	>50
Cymbopogon citratus	25	>50

Note: MIC: Minimum Inhibitory Concentration
MBC: Minimum Bactericidal Concentration

Antifungal Activities of Plant Extracts on *Candida albicans*

The hydroalcoholic extract of *O. gratissimum* was able to inhibit the growth of fungal germs at 50 mg/mL. Characterization of this inhibition also showed that subculturing on neutral agar resulted in no regrowth of germs at the same inhibitory concentration. The MIC and BMC of O. gratissimum are therefore identical. Their values are equal to 50 mg/mL on both *C. albicans* strains. However, for each of the other three plants, susceptibility testing showed no inhibition of germ growth at all concentrations used. The MIC and BMC of these three plant extracts are therefore greater than 50 mg/mL. These results are confined in Table 2 below.

Table 2. Antibacterial parameters of plant extracts on a strain of *C. albicans*

Plants	*Ca* 370		*Ca* 479	
	CMI (mg/mL)	CMF (mg/mL)	CMI (mg/mL)	CMF (mg/mL)
Ocimum gratissimum	50	50	50	50
Ocimum basilicum	>50	>50	>50	>50
Ocimum canum	>50	>50	>50	>50
Cymbopogon citratus	>50	>50	>50	>50

Note: Ca 370 and Ca 479 are *Candida albicans* strains
MIC: Minimum Inhibitory Concentration
MFC: Minimum Fungicidal Concentration

Discussion

This study consisted in determining the *in vitro* antimicrobial activity of hydroalcoholic extracts of four aromatic plants used in Côte d'Ivoire in folk medicine. The hydroalcoholic extractions showed that the extraction yields were all significantly different (at the 5% threshold). This indicates that the mass of extract obtained varies from one plant to another. Even the three species of the genus *Ocimum* did not give similar yields. This means that from a quantitative point of view, the four aromatic plants do not have the same phytochemical compositions. The extract of *Cymbopogon citratus* was obtained with the highest yield (6.95%). This reflects that this plant is richer in metabolites extractable by the ethanol-water mixture (70/30; v/v) than the other three plants. Next comes *Ocimum gratissimum* (4.61%). *Ocimum basilicum* has the lowest yield (2.37%). Indeed, the solvent used is very polar, it has the ability to extract the majority of fat and water soluble compounds of various polarity. It is therefore a very good solvent to analyze the totality of the extracts (Ambe et al., 2016). The extraction yield of *Ocimum gratissimum* is four times higher than that of its essential oil obtained in work done by Felicia et al. (2018) where the yield was 1.1%.

Ocimum gratissimum is the most active plant on *S.aureus.* This activity is corroborated by the work done by Adebolu et al, (2005) who showed that fresh leaves of *O. gratissimum* had antibacterial activity. Oussou et al, (2004) showed that the essential oil of *O. gratissimum is* rich in antibacterial and antifungal compounds. It is therefore not surprising that hydroalcoholic extract of the same plant, which certainly contains aromatic compounds, also

has antimicrobial powers. In this study, all aromatic plant extracts generally showed inhibitory power against the strain of S*taphylococcus* used. These results justify the use of these plants in the traditional treatment of skin diseases of staphylococcal origin. Indeed, in Côte d'Ivoire, fresh leaves of O. gratissimum are used extensively against impetigo in infants. The work of several authors has shown that this antibacterial activity can be generalized to several other bacteria (Aguidissou, 2015; Hossain et al., 2021). On the other hand, the study of antifungal potency on *C. albicans* revealed that only *O. gratissimum* had a notable anticandidosis potentiality. The other plants had no effect on the *Candida albicans* strains used. Comparison of the antimicrobial activities of the alcoholic extracts of these aromatic plants with the activities of their essential oils, studied by other authors, shows that the essential oils are generally more active on microorganisms than the alcoholic extracts (Hossain et al., 2021; Ohno et al., 2003; Bokobana et al., 2014). This demonstrates that the antimicrobial active ingredients could be the volatile compounds such as terpenes or monoterpenes that are abundant in the essential oils of these aromatic plants.

This study shows that essential oils remain essential components in the study of antimicrobial properties of aromatic plants. Although these oils are in very small quantities, they would concentrate most of the bioactive principles at the level of this category of plants, with regard to many pharmacological properties that are attributed to them (Rouibi et al., 2018; Soualeh et al., 2016). The extraction of all oils from fresh leaves of aromatic plants studied and particularly *Ocimum gratissimum*, could certainly give satisasfaction both in terms of yield and activities. This study justifies the frequent use of extracts and essential oils of this plant in ethnocosmetics and particularly against acne and various skin and mucous membrane problems.

Conclusion

The objective of the present study was to analyze the antimicrobial power of hydroalcoholic extracts of four aromatic plants widely used in the African pharmacopoeia. The extract of *O. gratissimum* was the most active on the microbial strains tested. The extracts of the other three plants also have an inhibitory power on S. aureus but are not active on the yeast strains. The level of antimicrobial activity of these hydroalcoholic extracts allows us to realize that despite their low availability, the essential oils of these aromatic plants remain more active than these crude extracts. This study is a contribution to the valorization of medicinal plants and the floristic heritage of Côte d'Ivoire.

References

Adebolu, T.T. & Oladimeji, S.A. (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. *African Journal of Biotechnology*, *4*, 682-684.

Aguidissou, O.N. (2015). *Evaluation in vitro de l'activité antibactérienne de quelques extraits de plantes médicinales sur des souches de salmonelles isolées chez les pintades*. Université d'abomey calavi. Memoire de Licence, 62 pages.

Ahon, G.M. (2014). Evaluation et essai d'optimisation de l'activité antifongique des extraits de *Terminalia superba* Engl. Et Diels (Combretaceaea), sur la croissance in vitro de *Aspergillus Fumigatus, Candida albicans* et *Cryptococcus neoformans*. Thèse de Doctorat d'université; Université Félix HOUPHOUET-BOIGNY, Abidjan, Cote d'Ivoive, 117 pages.

Bagré, I., Bahi, C., Ouattara, K., Zirihi, G.N., Djaman, A.J., Coulibaly, A., & N'Guessan, J.D. (2011). Étude botanique et exploration de l'activité antifongique de *Morinda morindoides*

(Baker) Milne-Redh. sur la croissance *in vitro de Cryptococcus neoformans*. *Phytothérapie, 9*, 136-141.

Bokobana, E.M., Koba, K., Poutouli, W.P., Akantetou, P.K., Nadio, N.A., Laba, A.B., Tozoou, P., Raynaud, C. & Sanda, K. (2014). Evaluation du potentiel insecticide et répulsif de l'huile essentielle de *Cymbopogon citratus* sur *Aphis gossypii*glover (*homoptera: aphididae*), ravageur du cotonnier au Togo. *Science de la vie, de la terre et agronomie, 2*(2), 48-55.

Bolou, G.E.K., Bagré, I., Ouattara, K. & Djaman, A.J. (2011). Evaluation of the antibacterial activity of 14 medicinal plants in Côte d'Ivoire. *Tropic Journal Pharmacology Ressources, 10*(3), 335-340.

Dalia, F. & Benkhemissa, M. (2020). L'aromathérapie: exploration du potentiel anti-infectieux des huiles essentielles. *Bulletin de Santé Hospitalo-Universitaire Constantine, 8*, 7-9.

Felicia, J., Kouamé, R.O., Kanko, C., Zanahi, F.T., Kouahou, F. & Yao, T. (2018). Bioefficacité des Huiles Essentielles de Trois Espèces Végétales (*Ocimum Gratissimum, Ocimum Canum et Hyptis Suaveolens*), de la Famille des Lamiacees dans la Lutte Contre *Sitophilus Zea mais*. *European Journal of Scientific Research, 150*(3), 273-284.

Fezan, H.T., N'guessan, F.K., Favel, A. & Fallague, K. (2007). Activité antifongique de quelques plantes de la flore ivoirienne. *Sciences et Nature, 4*(2), 117-122.

Fontanay, S., Mougenot, M.E., & Duval, R.E. (2015). Évaluation des activités antibactériennes des huiles essentielles et/ou de leurs composants majoritaires. *Hegel, 2*(2), 109-118.

Franchomme, P., Jollois, R. & Penoel, D. (2001). L'aromathérapie exactement : Encyclopédie de l'utilisation thérapeutique des huiles essentielles. Editions *Jollois,* Toulouse, France, 250p.

Guédé-Guina, F., Kra, A.K.M., Vangah Manda, M. & Bonga, G.M. (1997). Inhibition par MISCA-F2 de la croissance de Aspergillus fumigatus, Candida albicans et Cryptococcus neoformans trois germes opportunistes du SIDA. *J. Afrique Bioméd., 2*, 11-16.

Hossain, M.A., Kabir, M.J., Salehuddin, S.M., Rahman, S.M., Das, A.K., Singha, S.K., Alam, M.K., & Rahman, A (2010). Antibacterial properties of essential oils and methanol extracts of sweet basil Ocimum basilicum occurring in Bangladesh. *Pharm Biol., 48*(5), 504-511.

Koba, K., Sanda, K., Raynaud, C., Nenonene, Y.A., Millet, J., & Chaumont, J.P. (2004). Activités antimicrobiennes d'huiles essentielles de trois *Cymbopogon sp*. africains vis-àvis de germes pathogènes d'animaux de compagnie. *Ann. Méd. Vét., 148*, 202-206.

Kra, A. K. M. (2001). Evaluation et amélioration par séquençage chromatographique d'une action fongique de MISCA contre Aspergillus fimugatus, Thèse, Pharma., Bioch. Univ. Abidjan, 126p.

Lidji, J.C.S.R. (2020). Caractérisation physicochimique, activité antimicrobienne d'huiles végétales, d'huiles essentielles et des extraits végétaux sur quelques germes responsables d'infections cutanéo-muqueuses, Mémoire de Master en pharmacologie des substances naturelles, Ufr Biosciences, université Felix Houphouët-Boigny de Coccody, Abidjan Côte d'Ivoire, 55p.

Ohno, T., Kita, M., Yamaoka, Y, Imamura, S., Yamamoto, T., Mitsufuji, S., Kodama, T., Kashima, K. & Imanishi, J. (2003). Antimicrobial activity of essential oils against Helicobacter pylori. *Helicobacter, 8*(3), 207-215.

Oussou, K.R., Coffi, K., Guessend, N., Séri, Y., Dosso, M., Yao, T., N'Guessan, T., Gilles Figueredo, G., Chalchat, J.C. & Koukoua, G. (2004). Activités antibactériennes des huiles essentielles de trois plantes aromatiques de Côte-.d'Ivoire. *Comptes Rendus Chimie, 7*, 1081-1086.

Rouibi, A., Megateli, S., Saidi, F., Cherif, H.S., Fenagra, L., & Bouriach, M. (2018). Propriétés pharmacologiques de l'extrait aqueux et des huiles essentielles des graines de fenugrec (*trigonella foenum-graecum*. l.). *Revue Agrobiologia*, *8*(1), 913-919.

Salhi, S., Fadli, M., Zidane, L. & Douira, A. (2010). Etudes floristique et ethnobotanique des plantes médicinales de la ville de Kénitra (Maroc). *Lazaroa, 31*, 133-146.

Soualeh, N. & Soulimani, R. (2016). Huiles essentielles et composés organiques volatils, rôles et intérêts. *Phytothérapie, 14*(1), 44-57.

Tabuti, J.R.S., Lye, K.A., & Dhillion, S.S. (2003). Traditional herbal drugs of Bulamogi: plants, use and administration. (Uganda) *Journal of Ethnopharmacology, 88*, 19-44.

Thès PM (2001), Recherche du profil antimicrobien des huiles de G243 et MISCA sur quelques Agents de mycose de la peau, Mémoire de DEA de biotechnologies, option pharmacologie microbiologie. Université de Cocody, Abidjan, Côte-d'Ivoire 34p.

Zirihi, G.N. & Kra, A.K.M. (2003). Evaluation de l'activité antifongique de *Microglossa sapyrifolia* (LARMARCK) O. KUNTZE (Asteraceae)"PYMI" sur la croissance *in vitro* de *Candida albicans*. *Revue Médicale et Pharmacologique Africaine*, *17*, 11-19.