Antioxidant and antibacterial activities of the essential oils of *Synedrella nodiflora*, *Mikania cordata* and *Melanthera scandens* three plants of the Ivorian flora

ABSTRACT

Aims: The objective of this work is to contribute to the valorization of medicinal and aromatic plants of the Ivorian flora. We propose to evaluate the antioxidant and antibacterial activities of the essential oil of three species used in traditional medicine.

Study design: valorization of aromatic and medicinal plants and essential oil.

Methodology: The antioxidant potential of the extracts was evaluated using the Blois method.The antibacterial activity of the different oils at different concentrations was determined for each bacterial strain, by the technique of macro-dilution in solid medium (diffusion in wells). The minimum inhibitory concentration (MIC) and The Minimum Bactericidal Concentration (MBC) were determined. MBC / MIC ratios were calculated. When this ratio is less than 4, the extract is considered to be bactericidal. When it is 4, the extract is considered bacteriostatic.

Results: The antioxidant activity has pointed out the poor antioxidant power of the EO extracted. The EC_{50} values vary from 15 µg/mL 32 µg/mg. The antibacterial tests have shown that the samples exert an inhibitory effect on Gram (+) bacteria. The diameters of the inhibition zones vary between 14 and 25 mm for the extracts against 35 mm for the gentamycin. The MBC/MIC is 2 for the gentamycin and 4 for all the EO combined versus the resistant *S. aureus meticiline*. Therefore, the essential oil has shown a bacteriostatic effect on this strain. As far as *S. aureus* CIP 483 is concerned, the MBC/MIC has given 1 for the gentamycin, 2 for *M. scandens* . The EO extract of *M. scandens* has a bactericidal action against this bacteria strain.

Conclusion: All the essential oils have less antioxidant activity than that of vitamin C. The antibacterial activity of EO has given satisfactory results on all Gram (+) bacteria. *Melanthera scandens* Essential Oil shows antibacterial potential against Staphylococcus aureus CIP 483.

Keywords: M. cordata, *M. scandens*, *S. nodiflora*, antioxidant activity, antibacterial activity,

1. INTRODUCTION

From antiquity to the present day, medicinal and aromatic plants have played a crucial role in the prevention and / or treatment of various human diseases [1]. The use of plants for their medicinal properties is a very ancient practice. It has its origins in the oldest civilizations and has been well preserved over the centuries around the world. Over the past two decades, much attention has been paid to plants as new alternative therapeutic agents due to their natural bioactive compounds [2].Thus, the search for new molecules, taking into account criteria other than efficiency, has become essential. Biological control through the use of natural antioxidant and antibacterial substances that can be an alternative to chemicals. Among these natural substances are essential oils extracted from aromatic plants [3].

Our interest focused on the study of the antibacterial and antioxidant activities of the essential oils of three medicinal and aromatic plants often used in traditional medicine and as a food condiment by the local population in Côte d'Ivoire: *Melanthera scandens, Mikania cordata* and *Synedrella nodiflora*.

To our knowledge, no study of the antioxidant and antibacterial activities has been done on the essential oils of the three Ivorian species. However, studies have been done on the extracts thereof.

In the literature, previous studies have been done on the extraction and chemical composition of essential oils from the organs of all three plants [4,5].

Antioxidant activity studies were performed on methanolic extracts from the dried leaves of the Nigerian species of *Melanthera scandens*. They show that these have less important antioxidant properties than those of vitamin C. [6] Studies of antibacterial activity have also been carried out in Indonesia and Bangladesh on extracts from the leaves and aerial part of *Mikania cordata* by the diffusion method in a solid medium. In Indonesia, this was carried out against 3 Gram (+) and one Gram (+) bacteria. The results have shown that the ethyl acetate extract have given good activity against S. aureus with an inhibition diameter of 14 mm [7]. In Bangladesh, it was performed against 8 Gram (+) bacteria and 5 Gram (-) bacteria. It appears that the dichloromethane fraction exerted a strong activity against *Escherichia coli* with a zone of inhibition of 14 mm [8]. Antibacterial and antioxidant activity studies have also been done on extracts of *Synedrella nodiflora*. It shows that the methanolic extract of the leaves has an inhibition zone of 14 mm against *Bacillus cerus* [9]. The antioxidant capacities of the methanolic extract and the soluble fractions were tested using DPPH and BHT. The 50% inhibitory concentrations (IC50) in µg / mL of the fractions and extracts vary between 10.52 and 31.25 when that of ascorbic acid and BHT are respectively 5.8 ± 0.21 and 27.5 ± 0.54. The soluble fraction of dichloromethane has good antioxidant activity. Its IC50 is 10.52 µg / mL [10].This is why, in this work, in order to contribute to a valuation of the aromatic and medicinal plants of Ivory Coast, we propose to evaluate by spectrophotometry the antioxidant activity of essential oils vis-à-vis the DPPH. and their antibacterial activity.

2. MATERIAL AND METHODS

2.1 Equipment

The plant material consists of the EO extracted from the three plants.

Bacterial strains.

Eight (08 strains) of bacteria were used. For the most part, these are reference strains from the laboratory of the Swiss Center for Scientific Research (CSRS) with the names ATCC and SO. Other strains, on the other hand, are clinical strains from the Institut Pasteur with the name CIP. Thus, the antibacterial tests were carried out on the following strains:

GRAM (-): *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Salmonella typhimirium* SO66; *Proteus mirabilis* ATCC 14153 and *Proteus vulgaris* CIP 5860

GRAM (+): *Staphylococcus aureus* ATCC 25923; *Staphylococcus aureus* CIP 483 and *Staphylococcus aureus* meticilin resistant ATCC 43300.

2.2. Methods

2.2.1. Evaluation of the antioxidant activity of EOs.

The antioxidant potential of the extracts was evaluated using the Blois method.
The DPPH is dissolved in absolute ethanol to obtain a solution of 0.3 mM molar concentration. The solutions to be tested: are diluted in absolute ethanol in order to have the following concentrations in mg / mL: 0.002; 0.02; 0.125; 0.25; 1 and 2.
2.5 ml of test solution are introduced into dry and sterile hemolysis tubes and 1 ml of ethanolic solution of DPPH is added. After shaking, the tubes are placed in the dark for 30 min, protected from light.
For each solution to be tested, a blank is prepared consisting of 2.5 mL of pure absolute ethanol supplemented with 1 mL of ethanolic solution of DPPH.
For the negative control, a solution of DPPH is prepared by diluting 1 mL of the ethanolic solution of DPPH in 2.5 mL of ethanol. For the positive control, a solution of vitamin C (ascorbic acid) is used, the absorbance of which is measured under the same conditions. The measurement of the residual absorbance is carried out at 517 nm. It is translated into percentage inhibition by the following formula [11]:

$$\%I = \left(1 - \frac{\text{Abs test}}{\text{Abs DPPH}}\right) x100$$

% I: Percentage inhibition. Abs test: Absorbance of ethanolic solution of EO and DPPH.
AbsDPPH: absorbance of blank (ethanolic solution of DPPH).

Before each measurement, the absorbance of the blank is measured. For each extract exhibiting an antioxidant potential, the EC50 (effective concentration of the substrate which inhibits the oxidative potential of DPPH by 50%) is determined graphically [12].

2.2.2. Evaluation of the antibacterial activity of Eessential Oils.

2.2.2.1 Measurement of the diameters of the zones of inhibition.

The antibacterial activity of the different oils at different concentrations was determined for each bacterial strain, by the technique of macro-dilution in solid medium (diffusion in wells) [13] from a culture of 18 to 20 h (105- 106 CFU / mL). The inoculum of 1 ml is inoculated on the surface of Mueller Hinton (MH) medium previously poured into Petri dishes. After 15 min, 6 mm diameter wells were cut using Pasteur pipettes. The bottom of the wells is blocked by a drop of MH agar to limit the diffusion of oils under the agar. Then, 50 µL of the oil at different concentrations and 50 µL of a gentamycin reference are distributed in each well. After diffusion, the cultures are incubated in incubators at 37 ° C. for 24 h. The inhibition halos are measured by a caliper. The activity is considered zero for an inhibition diameter (i.d.) less than or equal to 8 mm; weak for d.i. between 8 and 14 mm, average for i.d. between 14 and 20 mm; strong for i.d.. greater than or equal to 20 mm [13].

2.2.2.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

For the determination of the minimum inhibitory concentration (MIC), a series of 10 sterile hemolysis tubes is used. Using a sterile graduated pipette, 4.6 mL of MH-tween 80 broth are introduced into the first tube and 2.5 mL of the same broth into the other tubes.
Four hundred (400) µL of the E O to be tested are taken and placed in the first sterile tube containing 4.6 mL of BM-H medium, supplemented with Tween 80 (0.01%, v / v). The tube is homogenized by vortexing. Then, a series of dilution in geometric progression is carried out in Mueller -Hinton (BMH) -Tween 80 (0.01%, v / v) broth medium, so as to obtain a range of concentrations of between 80 and 0.3 mg / mL. Finally, 13 µL of a bacterial inoculum, with a density equivalent to Mac Farland standard 0.5 (108 CFU. ML-1), are placed in each of the

tubes of the dilution range, which are then placed at 37 ° C under stirring for 24 h. A control of the bacterial growth for which 13 µL of the standardized inoculum were deposited in BMH-Tween 80 medium (0.01%, v / v), is also carried out. MIC is the smallest concentration of extract capable of inhibiting bacterial growth. The smaller is the most effective extract [14]. The Minimum Bactericidal Concentration (MBC) corresponds to the lowest concentration capable of killing 99.99% of the initial inoculum. The same range of concentrations is used. Samples are taken in the control tube and in each of the tubes devoid of bacterial pellet and then deposited in ≪stria≫ on Mueller Hinton agar (MHA) . The inoculated dishes are incubated for 24 hours at 37 ° C [14].

MBC / MIC ratios were calculated. When this ratio is less than 4, the extract is considered to be bactericidal. When it is 4, the extract is considered bacteriostatic [14].

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3. RESULTS AND DISCUSSION

3.1 Results of antioxidant activity

These results (Figure 1) show that the extracted EOs exhibit relatively low antioxidant activity compared to vitamin C; This is proven by the determination of the EC50 (figure 2). Indeed, the EC50 is defined as the concentration of the substrate which causes the loss of 50% of the activity of DPPH$^{\bullet}$ [12]. The lower this concentration, the more effective the extract.

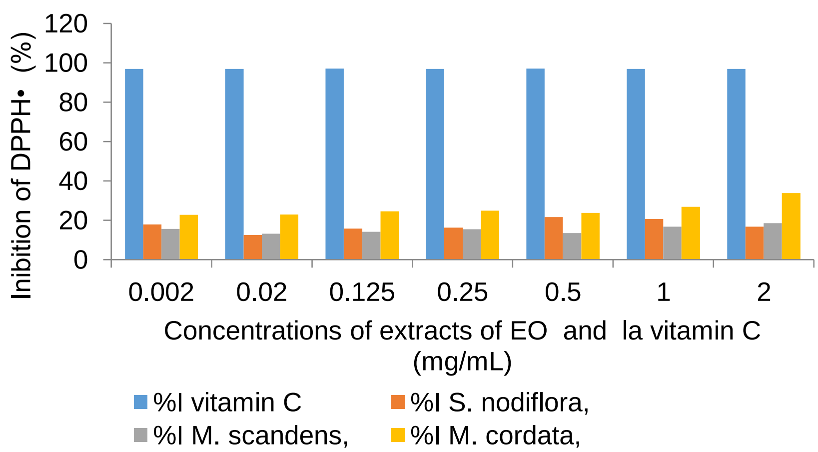
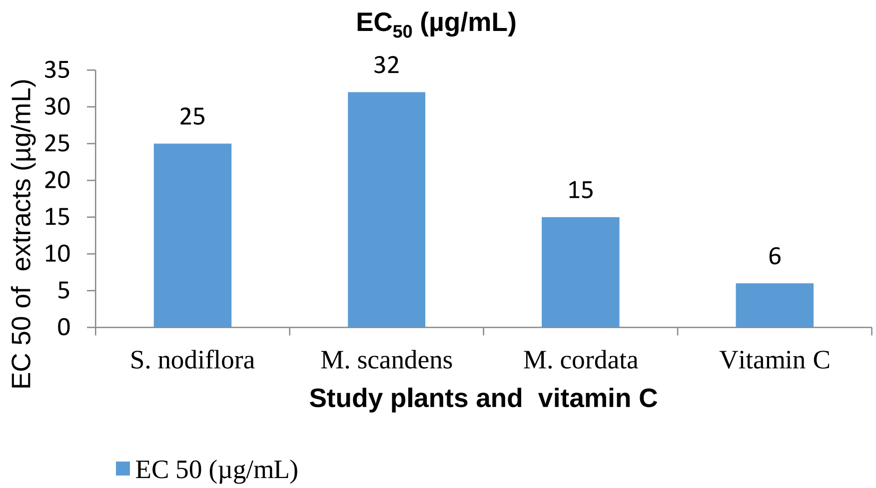


Figure 1: Inhibition of DPPH$^{\bullet}$ as a function of the concentration of EOs and vitamin C

The Essentiail Oils have a capacity of reduction of the free radical. The concentrations required for the neutralization of DPPH● vary between 32 µg / mL (Figure 2).



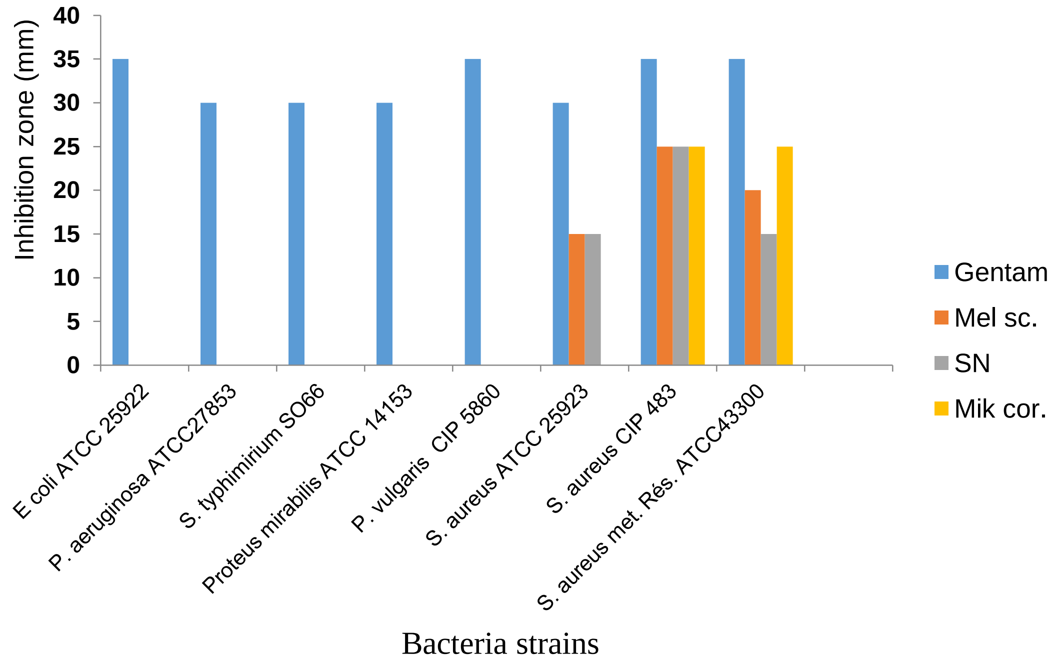
EC50: effective substrate concentration

Figure 2: EC50 of EO from study plants and vitamin C

The EC_{50} of *M. cordata* extract is twice that of *M. scandens*. Therefore, the antioxidant activity of EO from *M. cordata* is twice as effective as that of EO from *M. scandens*. The EC_{50} values show that the EO of *M. cordata* is more anti-free radical than that of *S. nodiflora* which is also more antioxidant than the EO extract of *M. scandens*. Furthermore, the EC50 of the extracted EOs was compared with that of vitamin C (6 µg / mL) determined by N'gaman [15] because the EC_{50} of vitamin C could not be determined for this study. It is two times smaller than that of *Mikania cordata*, four times smaller than that of *Synedrella nodiflora* and five times smaller than that of *M. scandens*. It follows that ascorbic acid (vitamin C) is twice as effective as extract of *M. cordata*, four times more anti-free radical than EO of *S. nodiflora* and six times more antioxidant than extract of *M. scandens*.
The relative antioxidant activity of EOs could justify the use of the plants from which they are derived in traditional therapy. This antioxidant activity is linked to the presence of terpenes because they are endowed with antioxidant power.

3-2-Results of antibacterial activity.

Antibacterial screening was performed. The diameters of the zones of inhibition are highlighted (Figure 3).
These results show that not all Gram (-) bacteria are sensitive to the EO extracts tested because there is no zone of inhibition of microorganisms. However, gentamycin has a strong inhibitory action on the growth of all the strains tested (d> 20 mm). All EO extracts are active against *S. aureus* CIP 483 and *S. aureus* methicilin resistant ATCC 43300, although it is resistant to methicilin.



Gentam: gentamicyn; Mel sc: Eo of Melanthera scandens; SN: EO of synedrella nodiflora; Mik cor: EO of Mikania cordata.

Figure 3: Zones of inhibition of bacteria by EOs and gentamycin at 1 mg / mL

The diameters of the zones of inhibition vary between 14 and 25 mm. It follows that EO extracts have remarkable activities on these bacteria. It should be noted that for the concentration of 1 mg / mL, all the EOs have a strong activity on *S. aureus* CIP 483 (d = 25 mm). Furthermore, *S. Aureus* ATCC 25923 is only sensitive to extracts of *Melanthera scandens* and *Synedrella nodiflora*. The diameter of the zones of inhibition is 15 mm compared to 30 mm for the reference antibiotic. So these EOs have an average activity against *S. aureus* ATCC 25923 (14 <d <20 mm). The EO of *Mikania cordata* is inactive on *S. aureus* ATCC 25923. The comparison of the chemical composition of the different extracts of EO reveals the absence of oxygenated monoterpenes in the EO of *M. cordata* when they are low. Amounts in extracts of *M. Scandens* (0.58%) and *S. nodiflora* (0.5%).The absence of EO activity of *Mikania cordata* on *S. aureus* strain ATCC 25923 is believed to be due to the absence of oxygenated monoterpenes. Moreover, concerning the majority compounds, in the extract of EO of *Mikania cordata*, the levels of α-caryophyllene (6.95%) and β-caryophyllene (8.45%) are low compared to the levels in the extracts. EO of *M. scandens* and *S. nodiflora.* Antibacterial activity could also depend on the percentage of major milk constituents.

Not all Gram (-) bacterial strains are sensitive to essential oil extracts. This would be due to the structure of their outer membrane. Indeed, the outer membrane of Gram (-) bacteria is rich in lipopolysaccharide. It makes the bacteria more hydrophilic, which prevents hydrophobic terpenes from adhering to it [16].

The results, of the MICs and MBCs determined and of the MBC / MIC ratios calculated of the extracted EOs and of gentamycin on the strains *S. aureus* CIP 483 and *S. aureus* methicilin resistant ATCC 43300, are given in Table1

Table 1: CMI, CMB and CMB / CMI ratios of extracts from EO and gentamycin

Souche	***S. aureus* methicilin resistant**			***S. aureus* CIP 483**		
	CMI mg/mL	CMB mg/mL	CMB/CMI	CMI mg/mL	CMB mg/mL	CMB/MC
Mel sc	**2.5**	**10**	**4**	**10**	**20**	**2**
Mik cor	**5**	**20**	**4**	**5**	**40**	**8**
SN	**5**	**20**	**4**	**10**	**80**	**8**
Gentamicine	**0,005**	**0,01**	**2**	**0,0025**	**0,0025**	**1**

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; Mel sc: EO extract from M. scandens ; SN: EO extract from S. nodiflora; Mikcor: EO extract from M. cordata

All EOs extracts have a bacteriostatic effect on the methicilin resistant *Staphylococcus aureus* strain (CMB / CMI = 4) while the *Melanthera scandens* extract has a bactericidal effect (CMB / CMI = 2) on the *S. aureus* strain CIP 483. This activity is similar to that of gentamycin on the resistant strain. These results would explain the use of *M. scandens* leaves in traditional medicine to treat certain pathologies such as malaria [17], diarrhea, dysentery, other gastrointestinal diseases [18] and diseases caused by fungi [7]. Although there are no studies in the literature on the antimicrobial activity of the essential oil of *Melanthera scandens*, the profile of the volatile compounds it contains, according to various studies, confirms the activity that **it** can exercise.

Indeed, according to Ultée et al., Mustafa et al, the antibacterial activity of EOs is mainly linked to the nature of their major compounds [19, 20]. But according to Delaquis et al. [21], the antimicrobial activity of certain EOs could be due to the presence of minority components. These compounds could exhibit an antibacterial activity by phenomena of synergy between the various constituents, much more pronounced than that foreseeable of the majority constituents. Thus, the antibacterial effects of EOs could be explained mainly by the presence of terpenes (monoterpenes and sesquiterpenes) and phenolic compounds. This is because the hydroxyls of the phenolic compounds are able to bind to the active sites of the target enzymes by hydrogen bonds. The presence of sesquiterpene alcohols in the EO of *M. scandens* could also explain this activity. Terpene alcohols are known for their antimicrobial power due to their solubility in water; which gives them an ability to penetrate bacterial cells [22]. The potential antibacterial activity of the essential oil of *Melanthera scandens*, obviously, seems to be linked to the presence of secondary metabolites [¨23-25].

Staphylococci are known for their involvement in food contamination and as agents causing many pathologies. Their antibiotic resistance is also known. It therefore appears imperative to find alternative antibiotics which could not only be used to eradicate certain pathologies but also as preservatives for certain foods such as juices and yoghurts [26]. EO from *M. scandens*' organs could also be used as an alternative to reduce these risks of food contamination.

4. CONCLUSION

The study of antioxidant activity by the DPPH•test has shown that the EOs analyzed**,** all have less antioxidant activity than that of vitamin C, taken as a reference antioxidant.
The evaluation of the antibacterial activity of EO has given satisfactory results on all Gram (+) bacteria. The diameters of the zones of inhibition vary between 15 and 25 mm. In

contrast, not all extracts have activity against all Gram (-) bacteria. *Melanthera scandens* EO shows antibacterial potential against *Staphylococcus aureus* CIP 483.

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COMPETING INTERESTS

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.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author KNS managed the bibliographical searches, wrote the protocol and the first edition of the manuscript. Author KKV help to evaluated the antioxidant activity of essential oils.. Author KBA provided the material and technical assistance for the study. Authors MBJA is the scientific supervisor. BY-A is the scientific director. All the authors read and approved the final manuscript.

NOTE:

THE STUDY HIGHLIGHTS THE EFFICACY OF " TRADITIONAL MEDICINE " WHICH IS AN ANCIENT TRADITION, USED IN SOME PARTS OF INDIA. THIS ANCIENT CONCEPT SHOULD BE CAREFULLY EVALUATED IN THE LIGHT OF MODERN MEDICAL SCIENCE AND CAN BE UTILIZED PARTIALLY IF FOUND SUITABLE.

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