

Antibacterial and Antifungal Activity of Essential Oil of *Eucalyptus camendulensis* on a Few Bacteria and Fungi

M. Mehani, N. Salhi, T. Valeria, S. Ladjel

Abstract—Red River Gum (*Eucalyptus camaldulensis*) is a tree of the genus *Eucalyptus* widely distributed in Algeria and in the world. The value of its aromatic secondary metabolites offers new perspectives in the pharmaceutical industry. This strategy can contribute to the sustainable development of our country. Preliminary tests performed on the essential oil of *Eucalyptus camendulensis* showed that this oil has antibacterial activity vis-à-vis the bacterial strains (*Enterococcus faecalis*, *Enterobacter cloacae*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) and antifungal (*Fusarium sporotrichioides* and *Fusarium graminearum*). The culture medium used was nutrient broth Muller Hinton. The interaction between the bacteria and the essential oil is expressed by a zone of inhibition with diameters of MIC indirectly expression of. And we used the PDA medium to determine the fungal activity. The extraction of the aromatic fraction (essentially oil-hydrolysis) of the fresh aërial part of the *Eucalyptus camendulensis* was performed by hydrodistillation. The average essential oil yield is 0.99%. The antimicrobial and fungal study of the essential oil and hydrosol showed a high inhibitory effect on the growth of pathogens.

Keywords—Essential oil, *Eucalyptus camendulensis*, bacteria and Fungi.

I. INTRODUCTION

ESSENTIAL oils have many therapeutic properties. In herbal medicine, they are used for their antiseptic properties against infectious diseases of fungal origin, against dermatophytes [4], those of bacterial origin.

Humans use plants for thousands of years to treat various ailments, in many developing countries; most of the population relies on traditional doctors and their collections of medicinal plants to cure them [1].

The MAP are plants that have grown or have picks in his natural environment for its medicinal and had an infinite variety of jobs, to report the therapeutic area, food, cosmetics, industrial, etc.. Herbs can play an important role in conserving biodiversity. These plants are actually very familiar to rural people who are very sensitive to their scarcity and their disappearance. Indeed, medicinal plants play an important role of health care population and represent a significant source of income for many families in the countryside and cities [2].

Throughout history, the plant kingdom has provided the essential human resources to its feeding, hygiene and health.

M. Mehani is with the Department of Natural Science and Life, Gardaia University, Algeria (e-mail: mounameh@gmail.com).

L. Segni is with the Department of Process Engineering Kasdi Mebah Ouargla University, Algeria.

Since ancient times, the fragrances of these same plants are associated with mystic rites, artistic and aesthetic.

It is known that some plants emit odors to attract insects to defend themselves. These smells come from small glands on the surface of leaves, stems or flowers that contain the essential oil. The essential oil is a volatile aromatic substance extracted from the plant. Little or no fat, it is called oil because it does not mix with water. Like gasoline, it ignites. Once extracted from plants, essential oils are used in perfumery, cosmetics, in food and other industries.

Essential oils have many therapeutic properties. In herbal medicine, they are used for their antiseptic properties against infectious diseases of fungal origin, against dermatophytes, those of bacterial origin.

In this work we try to study the antibacterial and antifungal activity of essential oils on the plant: *Eucalyptus camaldulensis*.

II. WORK METHODOLOGY

A. Hardware Plant

The aromatic plant harvested in the month of May 2010 is among the most abundant species in the region northwest of Algeria. The *Eucalyptus camaldulensis*: has been selected for testing antibacterial and fungal activity.

Eucalyptus camaldulensis is about 800 genders. It is a plantation species in many parts of the world but is native to Australia where it is widespread especially beside the continental water courses. Oddly, it is named for a private estate garden near the Camaldoli monastery near Naples (L'Hortus Camaldulensis di Napoli), from where the first specimen came to be described. Material from this tree was used by Frederick Dehnhardt, Chief Gardener at the Botanic Gardens in Naples, to describe this species in 1832 [3].

Leaves contain 0.1–0.4% essential oil, 77% of which is cineol. There is some cuminal, phellandrene, aromadendren (or aromadendral), and some valerylaldehyde, geraniol, cymene, and phellandral [4]. Leaves contain 5–11% tannin [5].

It is reported to be anesthetic, antiseptic, astringent, the redgum eucalyptus is a folk remedy for colds, colic, coughs, diarrhea, dysentery, hemorrhage, laryngalgia, laryngitis, pharyngitis, sore throat, spasm, trachalgia, and wounds [6].

B. Classification

Kingdom: Plantae

Under kingdom: Angiosperms

Class: Eudicots

Order: Myrtales

Family: Myrtaceae

Genus: *Eucalyptus*

Species: *Eucalyptus camaldulensis*

C. Extraction of Essential Oils by Hydrodistillation

The hydrodistillation of *Eucalyptus camaldulensis* (leaves dry) is performed using a Clevenger-type device (1928) [2]. The setup used is shown in Fig. 1.

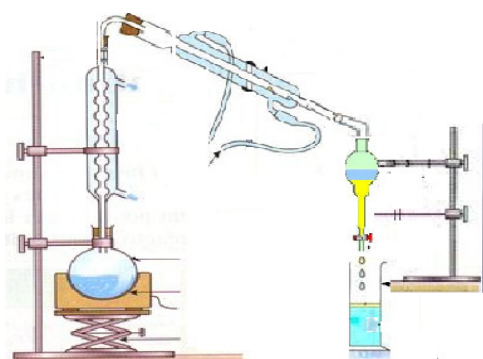


Fig. 1 Mounting a hydrodistillateur

D. Study of the Biological Activity of Essential Oil

Five bacteria (*Enterococcus faecalis*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus microsiliis* and *Pseudomonas aeruginosa*) and two yeasts (*Fusarium sporotrichioides* and *Fusarium graminearum*) were chosen for their high frequency in human infections.

E. Technique in Solid Medium: Method of Aromatogrammes

The aromatogram is based on a technique used in medical bacteriology, called antibiogram [7], [8]. It has the advantage of being very flexible in the choice of products to test and apply to many bacterial species [9], [10].

In this method, we use filter paper discs of 6 mm in diameter, impregnated in different concentrations of essential oil diluted in DMSO at 25%, 50% and 75%. These discs we deposit on the surface of an agar medium inoculated with the surface of a bacterial suspension. The incubation was carried out in an oven at 35°C for 24 h for bacteria and at 25°C for 5 days for yeasts.

1. Preparing Disks

The antibiogram is starting from the preparation of records, including Whatman paper was cut into 6 mm white disc. After sterilizing oven pastor for 20 min at 160°C, the discs were soaked in solutions with paravant prepares. Then, they are placed in petri dishes where they suffered a drying before being placed on the culture medium.

2. Preparation of Microbial Suspension

Preparation of microbial suspension is done by introducing two well isolated pure colonies of each species studied, in 10 ml of saline contained in a test tube.

3. Inoculation

The microbial suspension prepared was cast on the Muller Hinton agar. After soak up of all surface of the medium by the microbial suspension, the supernatant was discarded. Therefore, these plates were left to dry for 15 min at 37°C.

4. Application of Discs

The discs are prepared disposer to the surface of culture medium, pressing lightly with a sterile forceps, and then these dishes are incubated in an oven at T 37°C for 24 h.

5. Reading Results

We have methods to measure the diameter of the zones of inhibition in the case of microbial sensitivity around the discs of 6 mm in diameter.

D. Antifungal Activity

For the realization of the antifungal activity was adopted method of direct contact. To prepare the different concentrations were taken different concentrations of essential oil of *Eucalyptus* namely (50, 10, 5, 2.5, 1.25, μ l) and adjust to 20 ml PDA then stirred for 5 minutes to homogenize the medium PDA with essential oil.

III. RESULTS

A. Antimicrobienne Activity

The study of antibacterial extracts of *Eucalyptus camaldulensis* by the agar diffusion method or the method of absorbing disc to determine the antimicrobial activity of this plant *in vitro* including the diameter disk (6 mm) to measurement of the inhibition zones

The following Fig. 2 shows the results of the antimicrobial activity of the HE (classical diffusion method) of the *Eucalyptus* plant on bacterial strains *microsiliis Proteus*, *Enterococcus faecalis*, *cloacaei Enterobacter*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*.

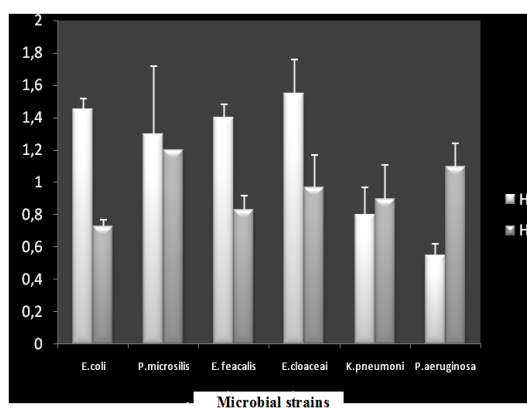


Fig. 2 Antimicrobial activity of HE *Eucalyptus camaldulensis*

The HE has *in vitro* inhibitory activity against the bacteria tested. By taking into consideration the inhibition diameters, HE is active on *E. coli*, *P. microsiliis*, *E. cloacaei* and *E. faecalis* respectively with a muting area 1.45, 1.3, 1.4 and 1.55 cm. On the contrary, it is less active vis-à-vis *P. aeruginosa*

and *K. pneumoni* (0.55 and 0.8 cm). In contrast to *P. aeruginosa* and *P. microsiliis* do not indicate great inhibitory action (insensitive) on essential oil of Eucalyptus.

Similar results were reported by [11]. They show that the essential oil of *Eucalyptus tereticornis* Sm.a, a broad inhibitor against microorganisms studied power; [12] also showed that the essential oils of *Clausena anisata*, *Eucalyptus camadulensis* and *Ocimum basilicum* have biological activity on microorganisms. Our results are consistent with those reported by [13], which showed that Eucalyptus is endowed with an efficient biological activity of microorganisms.

B. Antifungal Activity

The antifungal activity is revealed by the absence or the presence of mycelial growth. The results of the diameter of antifungal activity of essential oil of *Eucalyptus camendulensis* are presented in the graph 3. They vary between 13 and 55 mm (diameter including the disc) in the *Fusarium sporotrichioides* and *Fusarium graminearum*, the mycelial growth is varied between 11 and 55 mm.

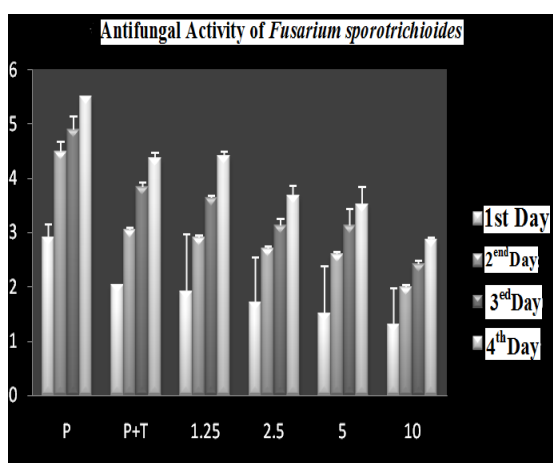


Fig. 3 Antifungal Activity of *Fusarium sporotrichioides*

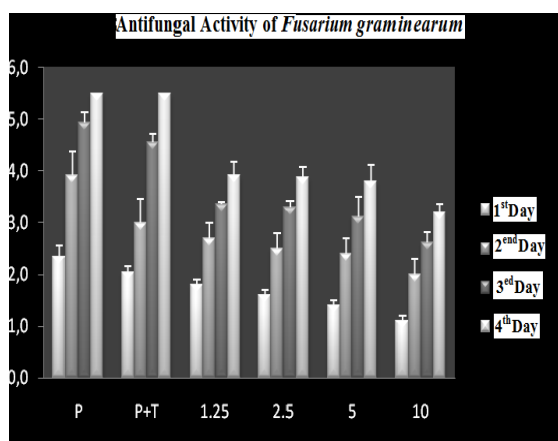


Fig. 4 Antifungal Activity of *Fusarium graminearum*

With different concentrations of essential oil extracted from Eucalyptus, it is observed that mycelial growth is remarkable

after 72 h for the control and different concentrations of essential oil of Eucalyptus namely 1.25, 2.5, 5 and 10 μl , by against at 25 μl no mycelial growth of *Fusarium sporotrichioides* is observed.

According the graph N° 4, which represents the activity antifonguigue of *Fusarium graminearum* depending on the incubation time and the concentration of essential oil of *Eucalyptus camendulensis* we note that there is an increase in mycelial growth over time with the exception of the incubation 50 μl concentration / 20ml of PDA that shows no mycelial growth.

IV. CONCLUSION

Many herbs contain chemical compounds having antimicrobial properties. Several research studies have been focused on the essential oils of these herbs.

The search for new therapeutic herbs character used mainly to show the validity of their use by traditional practitioners. It also showed that our country has to offer a rich and varied plant biomass. This is an immeasurable source for the development and the development of new active molecules for therapeutic purpose.

The use of volatile formulations based on medicinal and aromatic plants may have many advantages over existing products syntheses.

Better understanding of our study was to *Eucalyptus camendulensis* by studying of its aromatic fraction (HE-HA). We were able to evaluate and verify some of its biological properties and highlight its pharmacological potential.

In addition to its potential antimicrobial and antifungal verified on standardized gelose medium germs and vapor phase, the aromatic fraction has an undeniable anti-inflammatory action. Therefore, it may be proposed eventually as an asset of choice in the local treatment of inflammation.

It might be interesting to further phytochemical and biological investigations on these plants including purification of the extracts obtained to isolate the molecules responsible for the antimicrobial activities, which will larger armamentarium of herbal plants.

ACKNOWLEDGMENT

The author would like to acknowledge Dr. Ladjel Segni at University of Ouargla.

REFERENCES

- [1] A. F. Barrero, M.M. Herrador, P. Arteaga, J. Quitz, M. Aksira, F. Mellouki and S. Akkad "Chemical composition of essential oils of leaves and wood of *Tetraclinis articulata*" (Vahl) Masters; J. Essent. Oil Res., 2005, 17, pp. 166-167.
- [2] J. F. Clevenger, "Determination of volatile oil". J. Ann. Pharm. Assoc, 1928, 17(4), pp. 346-351.
- [3] "River Red Gum". *Eucalyptus camaldulensis* var. *obtusa*. Centre for Plant Biodiversity Research.
- [4] .S.I.R. (Council of Scientific and Industrial Research), "The wealth of India". 11 vols. New Delhi, 1948-1976.
- [5] J.M. Watt, M.G. Breyer-Brandwijk, "The medicinal and poisonous plants of southern and eastern Africa". 2nd ed. E.&S. Livingstone, Ltd., Edinburgh and London, 1962.

- [6] A. Duke, K. K. Wain, "Medicinal plants of the world". Computer index with more than 85,000 entries.. 3 vols, 1981
- [7] B. Benjlali, A. Tantaoui-elarki and M. Ismaili-alaoui, "Méthode d'étude des propriétés antiseptiques des huiles essentielles par contact direct en milieu gélosé". *Plant. Méd. Phytothér.* 1986, 20, pp. 155-167.
- [8] B. Satrani, H. Fougrach, B. Bourkhiss, D. Boustia, and M. Talbi., "Composition chimique et activité antimicrobienne de l'huile essentielle de *Cladanthus mixtus*". *Bull. Soc. Pharm. Bordeaux*, 2007, 146, pp. 85-96.
- [9] V.G. De Billerbeck, C. Roques, P. Vaniere and P. Marquier, "Activité antibactérienne et antifongique de produits à base d'huiles essentielles". *HYGIENES - 2002 - Vol X - n°3*, pp. 248-251.
- [10] M.C. Pibiri, "Assainissement microbiologique de l'air et des systèmes de ventilation au moyen d'huiles essentielles". Thèse de doctorat ès sciences, école polytechnique fédérale de Lausanne, 2005. pp. 19-55.
- [11] G. Alitonou, Guy, Felicien Avlessi., D. Valentin Wotto, A. Edwige, J. Dangou, and C.K. Dominique Sohounhloue, "Composition chimique, propriétés antimicrobiennes et activités Sur les tiques de l'huile essentielles d'*Eucalyptus tereticornis* Sm'' . 2004, C. R. Chimie 7.
- [12] D.K. Sohounhloue, A. Agbaka, E.B. Dockimo, L. Djossou, K. Fouabi, J. SOC.Ouest Afr.chim, 1999.8.87.
- [13] A. Kesbi, and S. Ladjel, "Étude des propriétés physico chimiques et évaluation de l'activité biologique des huiles essentielles d'*Eucalyptus globulus* dans la région d'Ouargla", 2011.

Mehani Mouna received his bachelor's degree in natural science life and Engineer in Biology Option: Ecology Saharan and steppic from Kasdi Merbeh Ouargla University, Algeria in 2006 and Magister's degree in Agronomy option: Protecting Ecosystems Saharan and Steppic from Kasdi Merbeh Ouargla University, Algeria (2009). She is presently working as Assistant Professor in Department of natural science and life, Gardaia University, Algeria and joined Institute in 2010. Her main research interests are extraction of essential oils from medicinal plants and their antimicrobial effect and salt stress.

Valeria Tersi Educated in the classic lyceum G.D. Romagnosi, Parma, graduated with maximum in Biological Science at the University of Parma. With a scholarship of Accademia dei Lincei she spent two years training in genetics and molecular biology in Cereal Research Institute at Fiorenzuola. Permanent position as scientist and then senior scientist at the same Institute. At present Research Director at CRA-GPG. At the beginning of her carrier she activated the first strategic research on heat and cold shock response in barley. Since then her work has been increased on basic and applied molecular research on cereal genetics and quality. She had the opportunity to participate to different breeding programs to develop barley, oats and durum wheat varieties. She had an important role in supporting the organization to build the new laboratories of the Centre. Invited Professor of Plant Genetics in Universities of Modena-Reggio Emilia, Parma and Napoli. Supervision of many undergraduated, graduated and PhD students and post-docs. She has published more than 200 papers in national and international journals. Responsible in the last decade of more than 40 national and international research projects. Member of small grain cereals working group of ECPGR. Member of Accademia dei Georgofili of Florence. Member of the scientific committee of PhD Schools at Universities of Parma and Reggio Emilia.

Segni Ladjel received his bachelor's degree in natural science life and Engineer in Petroleum Option: Production from I.A.P Boumerdes University, Algeria in 1989 and Magister's degree in Industrial Chemicals from Guelma University, Algeria 1994 and Doctor of chemical science (Ph.D.) from Constantine University Algeria 2003. He is presently working as Lecturer in Department of Process Engineering Ouargla University, Algeria and as a Director of Research: Laboratory of Process Engineering and joined Institute in 1994. His main research interests are extraction of essential oils from medicinal plants and their antimicrobial effect, toxins from scorpions, phytochemistry and Valuation of natural substances.