INTERNATIONAL BACCALAUREATE BIOLOGY EXTENDED ESSAY

Research On Antibacterial Activity Of Rosmarinus Officinalis, Curcuma Longa, Hypercium Perforatum, Eucalyptus Globulus, And Melaleuca Alternifolia Of Medicinal Plants On Staphylococcus Aureus

Research Question: Do Rosmarinus Officinalis, Curcuma

Longa, Hypercium Perforatum, Eucalyptus Globulus, And Melaleuca Alternifolia Medical Plants Have An Antibactarial Affect On Staphylococcus Aureus?

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INTRODUCTION

I have always been interested in alternative medicine. I started to think whether it can be more effective than conventional medicine or provide benefits in a healthier way. I started to do research and read scientific literature and the more I read the more I realized that it is an effective method.

Alternative Medicine: In the most general definition, alternative medicine includes medical practices which are estimated to treat but cannot be proven for that with scientific methods. Plants that are used as active ingredients in medicines are mainly used in this method. Active ingredients in plants are mostly extracted by distilling. Medicines in which compounds from plants are used are produced with medicinal plants or with compounds of these plants extracted in laboratories.

The immune system fights against diseases and weakens due to several reasons such as poor eating habits, stress, viral infections, genetic diseases (e.g.: diabetes), anxiety, distress, severe infections, severe depressions. People with weak immune system cannot be protected against pathogens and their health is adversely affected. Alternative medicine therapies intend to support and reinforce the immune system to help people fight off diseases. There are many other substances/compounds in plants used in alternative medicine other than those used as active ingredients in medicines i.e. there can be a wide range of compounds in a plant including active ingredients and other compounds. Since pharmacological and pharmaceutical effects of these compounds are not well known, most physicians do not trust in alternative medicine products. Additionally, due to adverse effects caused by medicines, which can lead to health problems and due to pharmaceutical therapies that are inconvenient and last long, there is an increasing trend for using alternative medicine methods. Alternative medicine, together with natural foods boosts the immune system however it should be used under medical supervision and the dosage is very important. According to the World Healthcare Organisation data; 20,000 plants are used in medicine. Turkey has many medicinal plants thanks to its geographical location.

Some of the important medicinal plants that can be found in Turkey:

- * Linden Tea (*Tilia cordata*)
- * Laurel (*Laurus nobilis*)
- * Rosemary (*Rosmarinus officinalis*)
- * Eucalyptus (Eucalyptus globulus)
- * Spearmint (Mentha piperita)
- * Thyme (*Thymus vulgaris*)
- * Turmeric (*Curcuma longa*)
- * St John's Wort (Hypericum perforatum)

The group of compounds containing a benzene ring to which one or more hydroxyl groups are attached are called phenolic compounds or polyphenols. All compounds that contain at least one aromatic ring and a large amount of hydroxyl substituents in this ring are called phenolic compounds.

Based on what I have learned from the scientific literature I have read, I asked this question: is it possible to have healthier and more affordable products when compounds of medicinal plants are used in oral care products we use every day such as toothpaste and mouth wash? And I decided to conduct an experiment to find answers to this question. I started to do research on medicinal plants and I specifically chose those that have antibacterial and antiviral properties. Based on this research, I decided to use St John's Wort, rosemary and eucalyptus.

Oral flora has a rich diversity of microorganisms. This rich flora is boosted with food residue on teeth, aerobic and anaerobic parts of oral cavity, heat and decreased pH levels, which makes it hard to fight off diseases. Additionally, ineffective brushing, not brushing everywhere in the mouth which results in lack of effect of antibacterial properties of the toothpaste in all parts of the mouth are factors that have a negative effect on oral health. Therefore, chemical substances used in toothpastes, although they are individually successful, may be insufficient to protect oral flora since they stay in the mouth and have contact with teeth for a limited time. That's why I wanted to use oral care products in the experiment I planned to do.

I wanted to use turmeric (*Curcuma longa*) (picture 1) because it is a very popular plant thanks to its antibacterial and antiviral properties. When I did a research on this plant, I found out that it has been used for a very long time in medicine. Turmeric is native to Southeast Asia and it is cultivated in some regions in the world. In-vitro and in-vivo studies have found that curcumin, which is the active ingredient of turmeric is known for its antibacterial and antiviral properties.^{1 2 3} Curcumin is known to have therapeutic effects on diabetic wounds, rheumatic pains and liver diseases. It has also beneficial effects on cuts, burns and bruises. Additionally curcumin has also been shown to help wounds heal faster and prevent infection. However, since it has a high level of volatile oil content, it should be used in diluted form.

¹ Negi, P. S., Jayaprakasha, G. K., Jagan Mohan Rao, L., & Sakariah, K. K. (1999). Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. Journal of agricultural and food chemistry, 47(10), 4297-4300.

² Jayaprakasha, G. K., Rao, L. J. M., & Sakariah, K. K. (2005). Chemistry and biological activities of C. longa. Trends in Food Science & Technology, 16(12), 533-548.

³ Jurenka, J. S. (2009). Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. Alternative medicine review, 14(2).

Turmeric is generally considered safe in clinical studies however dosage is important and it is not recommended to be used concomitantly with some medicines for chronic diseases. Although it has no known toxicity, some people can be allergic to it.⁴



Picture 1: Turmeric (Curcuma longa)

The second plant I wanted to use was rosemary (*Rosmarinus officinalis*) (picture 2). It has become increasingly popular in our country because of both its use in the kitchen and in medicine. Rosemary is scientifically an important aromatic and medicinal plant. Studies in the literature have shown that rosemary has positive effects on the immune system.

Rosemary is known to have antioxidant and antimicrobial properties and even have anticarcinogenic effects.^{5 6} Rosemary essential oil and compounds can differ depending on the regions harvest season and part of the plant used. Rosemary is one of the plants that are rich in phenolic compounds and therefore there are ongoing studies to use it in foods together with other medicinal plants that are rich in phenolic compounds (e.g.: thyme, sage etc.). Main active ingredients of rosemary extract are carnasol and carnosic acid.⁷ The most powerful of these active ingredients is carnosic acid.

⁴ Labban, L. (2014). Medicinal and pharmacological properties of Turmeric (Curcuma longa): A review. Int J Pharm Biomed Sci, 5(1), 17-23.

⁵ Oluwatuyi, M., Kaatz, G. W., & Gibbons, S. (2004). Antibacterial and resistance modifying activity of Rosmarinus officinalis. Phytochemistry, 65(24), 3249-3254.

⁶ Malayoğlu, H. B. (2010). Biberiyenin (Rosmarinus officinalis L.) antioksidan etkisi. Hayvansal Üretim, 51(2).

⁷ Ayanoğlu, F., Başkaya, Ş., & Bahadırlı, N. P. (2016). Biberiye (Rosmarinus officinalis L.) bitkisinin uçucu yağ oranı, uçucu yağ bileşenleri ve antioksidan içeriğinde morfogenetik ve ontogenetik varyabilite. Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi, 21(1).

It is known to be used for digestive system disorders and rheumatism however its dosage, just like with any other medicinal plant is very important. It can lead to adverse reactions when used concomitantly with some medicines.⁸



Picture 2: Rosemary (Rosmarinus officinalis)

Another medicinal plant I wanted to use in my experiment is St John's Wort (Hypericum perforatum) (picture 3)⁹ which is used by people to help wounds heal.¹⁰ This plant is used widely in the world as a household remedy. The main ingredient of this plant extract is the red coloured hypericin. This ingredient makes it possible to be used pharmacologically. St John's Wort extract shows several pharmacological effects. I specifically investigated its antibacterial properties in my study.

⁸ Begum, A., Sandhya, S., Vinod, K. R., Reddy, S., & Banji, D. (2013). An in-depth review on the medicinal flora Rosmarinus officinalis (Lamiaceae). Acta scientiarum polonorum Technologia alimentaria, 12(1), 61-74.

⁹ Çırak, C., & Kurt, D. (2014). Önemli tıbbi bitkiler olarak hypericum türleri ve kullanım alanları. Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi, 24(1), 38-52.

¹⁰ Ernst, E. (Ed.). (2003). Hypericum: the genus Hypericum. CRC Press.

There are studies which show that its properties help to prevent hepatitis, intestinal tumours, infections caused by many viruses and growth of HIV virus.^{11 12}

Additionally St John's Wort is known to heal wounds and reduce inflammation. Above ground parts of the plant show good results when used to treat wounds, bacterial infections and headache. Antibacterial properties of St John's Wort are the result of two active ingredients: hyperforin and hypericin.¹³ Additionally studies have shown that it is highly effective against gram positive bacteria. That is why I believe it is a good choice for my study.



Picture 3: St. John's Wort (Hypercium perforetum)

The other medicinal plant I chose was Eucalyptus which we hear more often recently with the start of the pandemic due to its antibacterial properties. Eucalyptus (picture 4) has strong antiviral and antibacterial properties.¹⁴ Its pharmacologically active ingredient is eucalyptol. Eucalyptus has been used in a wide range of areas and for a wide range of illnesses.

¹¹ Barnes, J., Anderson, L. A., & Phillipson, J. D. (2001). St John's wort (Hypericum perforatum L.): a review of its chemistry, pharmacology and clinical properties. Journal of pharmacy and pharmacology, 53(5), 583-600.

¹² Shrivastava, M., & Dwivedi, L. (2015). Therapeutic potential of Hypericum perforatum: a review. Int. J. Pharm. Sci. Res, 6(12), 4982-4988.

¹³ Saddiqe, Z., Naeem, I., & Maimoona, A. (2010). A review of the antibacterial activity of Hypericum perforatum L. Journal of ethnopharmacology, 131(3), 511-521.

¹⁴ Mulyaningsih, S., Sporer, F., Reichling, J., & Wink, M. (2011). Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens. Pharmaceutical biology, 49(9), 893-899.

It is known especially to help in the treatment of respiratory diseases, asthma, bronchitis and even common cold. It is proven to have an alleviating effect on muscle and joint pains.¹⁵ Its smell is known to affect the nervous system and provide relief.¹⁶

In addition to the above mentioned effects, its antimicrobial properties are known to help alleviate headaches, reduce local bleedings, reduce fever and work against malaria, wounds and inflammation. Additionally, eucalyptus leaves are used for therapeutic purposes. Of course dosage and method of application should be controlled. The main reason why I chose eucalyptus is that it is used effectively for oral diseases and periodontal diseases.¹⁷



Picture 4: Eucalyptus (Eucalyptus globulus.)

The last medicinal plant I chose is the tea tree (*Melaleuca alternifolia*) (picture 5). The main reason why I chose this plant is that it is already used in many oral care products.^{19 20}

- ¹⁷ Tanaka, M., Toe, M., Nagata, H., Ojima, M., Kuboniwa, M., Shimizu, K., ... & Shizukuishi, S. (2010). Effect of eucalyptusextract chewing gum on oral malodor: a double-masked, randomized trial. Journal of periodontology, 81(11), 1564-1571.
- ¹⁸ Ragul, P., Dhanraj, M., & Jain, A. R. (2018). Efficacy of eucalyptus oil over chlorhexidine mouthwash in dental practice. Drug invention today, 10(5), 638-641.
- ¹⁹ Soukoulis, S., & Hirsch, R. (2004). The effects of a tea tree oil-containing gel on plaque and chronic gingivitis. Australian dental journal, 49(2), 78-83.
- ²⁰ Kamath, N. P., Tandon, S., Nayak, R., Naidu, S., Anand, P. S., & Kamath, Y. S. (2020). The effect of aloe vera and tea tree oil mouthwashes on the oral health of school children. European Archives of Paediatric Dentistry, 21(1), 61-66.

¹⁵ Vecchio, M. G., Loganes, C., & Minto, C. (2016). Beneficial and healthy properties of Eucalyptus plants: A great potential use. The Open Agriculture Journal, 10(1).

¹⁶ Kurt, N. C., & Çankaya, İ. İ. T. (2021). Aromatherapy Applications And Essential Oils. Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi.

Then I started to make some research about this plant. I found that it is endemic to Australia and grown on river banks and it is an oil which is produced by steaming tea tree leaves. Tea tree is believed to have antibacterial properties and additionally tea tree oil is used to treat acne, lice and bug bites.²¹ Studies investigating tea tree oil have shown that when tea tree oil is added in shampoos, it is effective in treating dandruff if used for a certain a period of time. Therefore it is not only used in oral care products but also in many skin care products. Tea tree oil is mostly used in skin disorders caused by bacteria.²² As with all medicinal plants, the dosage, application method and amount of tea tree oil is important. Generally it is a safe plant to use however studies have shown that it should not be ingested since ingestion can cause a toxic effect. In addition to that, people suffering from eczema are also recommended not to use it.



Picture 5: Tea tree *(Melaleuca alternifolia)* **Staphylococcus aureus** is a type of bacteria of the Staphylococcaceae family. It is a gram positive bacteria and there are approximately 20 types. It is a commensal bacteria which is found in the skin flora however can cause diseases in certain host and environmental conditions. The reason why I chose this bacteria is the fact that it can naturally be found in the nasal and oral areas.²³

²¹ Carson, C. F., Hammer, K. A., & Riley, T. V. (2006). Melaleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties. Clinical microbiology reviews, 19(1), 50-62.

²² Pazyar, N., Yaghoobi, R., Bagherani, N., & Kazerouni, A. (2013). A review of applications of tea tree oil in dermatology. International journal of dermatology, 52(7), 784-790.

²³ Stewart, G. C. (2005). Staphylococcus aureus. Foodborne pathogens: microbiology and molecular biology, 273-284.

Staphylococcus aureus is not considered a member of normal oral microbiota. There is limited information on the incidence of *S.aereus* in the oral cavity of health children.²⁴ It is also found in infected wounds and acne. Based on the information I gathered in my research, I wanted to use it to see whether it has any effect on the wounds found inside the oral cavity.

RESEARCH QUESTION

Do Rosmarinus officinalis, Curcuma longa, Hypercium perforatum, Eucalyptus globulus, and Melaleuca alternifolia medical plants have an antibactarial affect on *Staphylococcus aureus*?

HYPOTHESIS

Each of the plants selected show different antibacterial properties. Effects of the plants used can vary. In this study, the effect of active ingredients on the growth of bacteria selected when essential oils of these plants are included in a variety of oral care products will be evaluated.

Do Rosmarinus officinalis, Curcuma longa, Hypercium perforatum, Eucalyptus globulus, and Melaleuca alternifolia medical plants have an antibactarial affect on *Staphylococcus aureus*?

(H₀ Hypothesis):

Essential oils of medicinal plants have no effect on *Staphylococcus aureus* which can be found in the oral flora.

(H₁ Hypothesis):

Essential oils obtained from medicinal lice have a significant effect on Staphylococcus aureus, which can develop in the oral flora.

²⁴ Güven, Y., Topcuoğlu, N., Üstün, N., Aksakal, D., Doymaz, M. Z., Aktoren, O., & Külekçi, G. (2019). Pedodonti kliniği hastalarının ağız boşluğunda Staphylococcus aureus ve metisiline dirençli Staphylococcus aureus (MRSA) varlığı. 7tepe Klinik Dergisi, 15(3), 334-338.

VARIABLES

Independent Variables : Turmeric (Curcuma longa), Tea tree (Melaleuca alternifolia), St. John's Worth (Hypericum perforatum), Eucalyptus (Eucalyptus globulus.), Rosemary (Rosmarinus officinalis).

Dependent variable: Diameter of inhibition zonesControlled variable: Use of Gentamycin on bacteria (Staphylococcus aureus) as an
antibiotic instead of essential oils

LIST OF MATERIALS

| Materials | Quantity | Unit |
|--|----------|--------|
| Rosemary essential oil (Rosmarinus officinalis) | 1 | 10ml |
| Turmeric essential oli (Curcuma longa) | 1 | 10ml |
| St. John's Wort essential oil (Hypercium perforatum) | 1 | 10ml |
| Eucalyptus essential oil (Eucalyptus globulus) | 1 | 10ml |
| Tea tree essential oil (Melaleuca alternifolia) | 1 | 10ml |
| Staphylococcus aureus ATCC 29213 | | |
| Mueller-Hinton Agar | | |
| Mueller-Hinton Broth | | |
| Distilled water | | 1000ml |
| Precision scale | 1 | |
| Water bath | 1 | |
| Petri dishes | 35 | |
| Burette | 5 | |
| Incubator | | |
| Bunsen burner | | |
| Micropipette | | |
| Micropipette tips | | |
| Autoclave | | |
| Refrigerator | | |
| Magnetic stirrer | | |
| Pliers | | |
| Beads | | |
| Swap | | |
| Laboratory coat | | |
| Pen | | |
| Ruler | | |
| Data sheet | | |
| Waste bottle | | |

METHOD DEVELOPMENT AND PLANNING

MATERIAL AND METHOD

Microorganisms: The types of bacteria used in my study were chosen from the "American Type Culture Collection (ATCC)" quality control strains which are recommended by the "Clinical and Laboratory Standards Institute (CLSI)" and known for their high sensitivity. Accordingly, *Staphylococcus aureus* ATCC 29213, a Gram (+) bacteria was used in my study.

EXPERIMENT SUMMARY

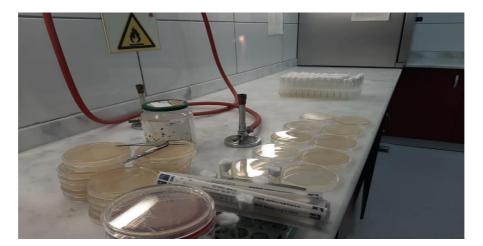
- 1. Commercially available essential oils of 5 medical plants were used on *Staphylococcus aureus* to understand their antibacterial activity.
- 2. With gas chromatography of the commercially available essential oils, phenolic compounds were determined. (Apendix)
- 3. Bacteria were cultured in the media using swaps in the solution that contain standard amount bacteria to determine the antibacterial activity.
- 4. Wells were bored using sterile beads in the media for the agar well diffusion method to evaluate antibacterial activity.
- 5. Essential oils were given numbers and then added in these wells and tested on the bacteria *Staphylococcus aureus*.
- 6. Media which also contain plant essential oils were kept at room temperature for 1 hour and then incubated at 37 C for 24 hours.
- 7. Gentamycin (antibiotic) was used as a positive control group.
- 8. The diameters of effects of essential oils post-incubation were measured.

Media: Mueller-Hinton Broth and Mueller-Hinton Agar broth were used.

METHOD

1. Preparation of the bacterial culture media

Bacterial culture media was prepared using commercially available MHA (Mueller Hinton Agar) and MHB (Mueller Hinton Broth). Powder media was weighed on a precision scale and dissolved in distilled water according to the ratios given in the package insert. Agar medium was placed in a boiling water bath and stirred occasionally until it was dissolved completely. Broth medium was dissolved using a magnetic stirrer. Media were sterilized at 1 atmospheric pressure at 121°C for 15 minutes. To prevent misting and moisture caused by the hot poured media, they were kept at 30°C. Sterilized media were poured on to the Petri dish with a thickness of 4mm (picture 6). Media were used to have subcultures of bacteria strains and grow bacteria. Culture media were kept in the fridge until used.



Picture 6: Preparation of the bacterial culture media

2. Preparation of the bacteria suspension

In my study, standard strains kept in glycerol stocks at -20° were used (selected from the "American Type Culture Collection (ATCC)" quality control strains which are recommended by the "Clinical and Laboratory Standards Institute (CLSI)" and known for their high sensitivity). Then they were taken out to thaw at +4°C and after being thawed MHB (Mueller Hinton Broth) broth was used to reactivate. Mueller Hinton Agar (MHA) was used for agar well diffusion method.

Then bacteria suspension was prepared in 3ml Mueller Hinton Broth to have all bacteria in 0.5 McFarland (1x 108 cell/ml) standard density (picture 7).



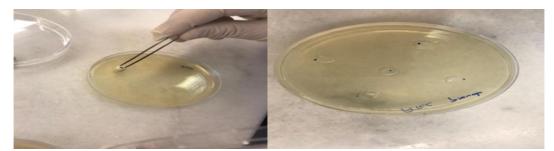
Picture 7: Preparation of the bacteria suspension

Staphylococcus aureus ATTC 29213 suspension was homogeneously cultured in Mueller Hinton Agar (MHA) using swaps. (picture 8).



Picture 8: suspension was homogeneously cultured in Mueller Hinton Agar

After the culture, media were kept to dry at room temperature for 5-10 minutes. Agar beads were used to punch holes in the inoculation agar and to create wells for the Agar Well Diffusion Method and the beads were sterilized by immersing in alcohol and then exposed to fire. Sterilized beads were held with pliers, five wells were created with sterile beads with 4 mm diameter, which were at the same distance to each other and to Petri dish walls in the media (picture 9). Each well was assigned a number from 1 to 5 and essential oils used were also given numbers. (Since there was a medium with a standard 4 mm thickness in the disk diffusion test, the well diameter which creates inhibition zones that comply with the disk diffusion result with this media thickness was calculated to be 8 mm. 4 mm agar thickness and 9 mm well diameter is required for standard measurement)



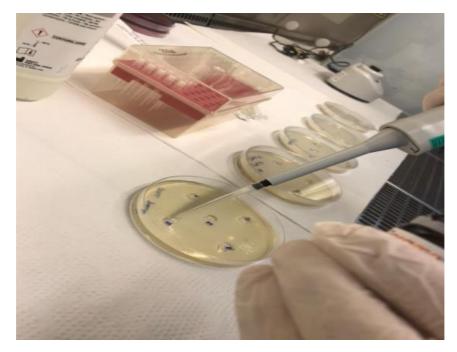
Picture 9: Sterilized beads were held with pliers, five wells were created with sterile beads Essential oils to be tested in wells were in numerical order;

- 1. turmeric in wells,
- 2. tea Tree in wells,
- 3. St. John's Wort in wells,
- 4. eucalyptus in wells,
- 5. Rosemary in wells

20µl from each suspension (picture 10).

After being kept at room temperature approximately for 1hour media were incubated at 37°C for 24 hours.

At the end of the incubation, antimicrobial activity diameters (inhibition zone) of the essential oils were measured.



Picture 10: 20µl from each suspension

RESULTS AND ANALYSIS

At the end of the incubation period, if the substance used was effective, inhibition zones where there was no apparent growth were formed around the wells.

Raw data table (table 1), Processed Data Table (table 2) and Anova (Table 3) These tables were made for analysis of variance at more than one value.

The inhibition values measured as a result of the experiment are shown in the table. (table 1)

Raw Data Table

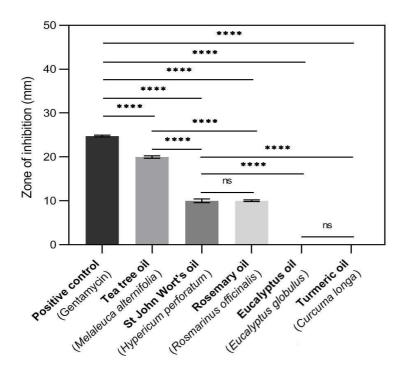
| Well No | Trails | The diemeter of inbision zone $(mm) \pm 0,01$ |
|---|--------|---|
| | 1 | 24,78 |
| | 2 | 24,55 |
| Positive control | 3 | 24,85 |
| | 4 | 25,05 |
| | 5 | 24,07 |
| | 1 | 0,00 |
| | 2 | 0,00 |
| 1. Turmeric (Curcuma longa) | 3 | 0,00 |
| - | 4 | 0,00 |
| - | 5 | 0,00 |
| | 1 | 20,00 |
| 2. Tea Tree (Melaleuca alternifolia) | 2 | 20,04 |
| | 3 | 19,70 |
| | 4 | 20,01 |
| | 5 | 19,85 |
| | 1 | 10,00 |
| | 2 | 10,25 |
| 3. St. John's Wort (Hypercium perforatum) | 3 | 10,6 |
| | 4 | 9,55 |
| | 5 | 9,70 |
| | 1 | 0,00 |
| | 2 | 0,00 |
| 4. Eucalyptus (Eucalyptus globulus) | 3 | 0,00 |
| | 4 | 0,00 |
| | 5 | 0,00 |
| | 1 | 10,00 |
| | 2 | 10,30 |
| 5. Rosemary (Rosmarinus officinalis) | 3 | 9,92 |
| | 4 | 9,80 |
| | 5 | 10,10 |

Table 1: Raw data of the inhabition zone of Turmeric oil, St. John's Wort oil, Eucalyptus oil, tea tree oil and rosemary oil of inhibition zones

Processed Data Table

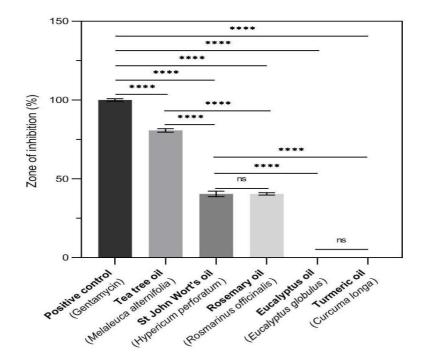
| Well No | Average diameter of inhibition zone | Standart deviation | Standart error |
|---|-------------------------------------|--------------------|----------------|
| Positive control (Gentamycin) | 24.79 | 0.19 | 0.08 |
| 1. Turmeric (<i>Curcuma longa</i>) | 0,00 | 0,00 | 0,00 |
| 2. Tea Tree (Melaleuca alternifolia) | 20.01 | 0.27 | 0.12 |
| 3. St. John's Wort (Hypercium perforatum) | 10.02 | 0.42 | 0.19 |
| 4. Eucalyptus (Eucalyptus globulus) | 0,00 | 0,00 | 0,00 |
| 5. Rosemary (Rosmarinus officinalis) | 10.02 | 0.19 | 0.08 |

Table 2: The average diameter of the inhibition zone, standart deviation and standart error in turmeric oil, tea tree oil, St. John's Wort oil, eucalyptus oil and rosemary oils



Graph 1: Average Inhibition zone graph of the groups in the study.(Error bars represent standart deviation)

(n:5, ****p<0,0001, ns p>0,05)



Graph 2: Inhibition zone graph of the groups in the study (%)

| | ANOVA | | | | | | | | | | |
|---------------------|-------------|----|-------------|-------------|-------------|-------------|--|--|--|--|--|
| Sourse of variation | SS | df | MS | F | P-value | F crit | | | | | |
| Between Groups | 2574.613027 | 5 | 514.9226053 | 9689.317042 | 7.87032E-39 | 2.620654148 | | | | | |
| Within Groups | 1.27544 | 24 | 0.053143333 | | | | | | | | |
| Total | 2575.888467 | 29 | | | | | | | | | |

Table 3: ANOVA

Anova was used because measurement was analysis of ariance based on more than one variable. (Table 3)

REPEAT #1

For *Staphylococcus aureus* ATTC 29213; inhibition zone with a 20 mm zone diameter in the well no 2 (tea tree) and inhibition zone with a 10 mm zone diameter in the well no 3 (St. John's Wort) and 5 (Rosemary) were measured. Other essential oils did not show any effect on the growth of *Staphylococcus aureus*.

REPEAT #2

For *Staphylococcus aureus* ATTC 29213; inhibition zone with a 20.4 mm zone diameter in the well no 2 (Tea tree) and inhibition zone with a 10.25 mm zone diameter in the well no 3 (St. John's Wort) and inhibition zone with a 10.3 mm zone diameter in well no 5 (Rosemary) were measured. Other essential oils did not show any effect on the growth of *Staphylococcus aureus*.

REPEAT #3

For *Staphylococcus aureus* ATTC 29213; inhibition zone with a 19.7 mm zone diameter in the well no 2 (Tea tree) and inhibition zone with a 10.6 mm zone diameter in the well no 3 (St. John's Wort) and inhibition zone with a 9.92 mm zone diameter in well no 5 (Rosemary) were measured. Other essential oils did not show any effect on the growth of *Staphylococcus aureus*.

REPEAT #4

For *Staphylococcus aureus* ATTC 29213; inhibition zone with a 20.1 mm zone diameter in the well no 2 (Tea tree) and inhibition zone with a 9.55 mm zone diameter in the well no 3 (St.

John's Wort) and inhibition zone with a 9.8 mm zone diameter in well no 5 (Rosemary) were measured. Other essential oils did not show any effect on the growth of *Staphylococcus aureus*.

REPEAT #5

For *Staphylococcus aureus* ATTC 29213; inhibition zone with a 19.85 mm zone diameter in the well no 2 (Tea tree) and inhibition zone with a 9.7 mm zone diameter in the well no 3 (St. John's Wort) and inhibition zone with a 10.1 mm zone diameter in well no 5 (Rosemary) were measured. Other essential oils did not show any effect on the growth of *Staphylococcus aureus*. Control Group

Positive control group Gentamycin (antibiotic) was used on *Staphylococcus aureus* ATTC 29213. Diameters of inhibition zones after repeated 5 tests were 24.78 mm, 24.55 mm, 24.85mm, 25.05 mm and 24.7 mm respectively.

Diameters of the inhibition zones measured in my study are shown in Table 1. No inhibition zone was formed in the turmeric oil and eucalyptus oil groups. Data was evaluated and the following mean values were found: Gentamycin 24.786 mm; Tee tree 20.01 mm, St John's Wort 10.02 mm, Rosemary 10.024 mm. According to the data obtained, the biggest inhibition zone was observed with Gentamycin used as the positive control group. A significant difference was found between the inhibition zones observed with essential oils and the inhibition zones of the control group, however among the groups, the biggest inhibition zone was in the tea tree group and the smallest inhibition zone was in the St. John's Wort group (Graph 1).

Using the inhibition zone data of each group, percentage values for antibacterial activity of each group on *staphylococcus aereus* were calculated (Graph 2). When results were reviewed, gentamycin used as the positive control group was found to be 100% effective. When group values were reviewed, tea tree had an antibacterial activity of 80.73%, St John's Wort had 40.42 %, and rosemary had 40.44% antibacterial activity. The best antibacterial activity was found in tea tree based on the inhibition percentages.

CONCLUSION

Based on the results of the experiments medicinal plants that have an effect on *Staphylococcus aureus are Hypericum perforatum* (St John's Wont), *Eucalyptus globulus* (Eucalyptus), *Melaleuca alternifolia* (tea tree) and *Rosmarinus officinalis* (rosemary). The effects of other essential oils were insignificant. As observed in other studies, phenolic compounds from these 3 medicinal plants have an effect on *Staphylococcus aureus*.

Based on the findings of this experiment, these medicinal plants can be used to develop the intended oral care products (toothpaste and mouth wash). Products developed with these medicinal products have an effect on *Staphylococcus aureus* which can be found in the oral flora. Thus using products that are developed with these medicinal plants can prevent potential infections. At the same time, oral care products that contain these plants will be more natural which can make the products popular. Thus it can be possible to produce products from more natural ingredients that both cost less and attract people's attention more. With the increasing interest in natural/organic products in the market, these products can be met with a higher demand than other products.

In the experiment performed on s.aureus bacteria, it was observed that essential oils taken from medicinal plants had antibacterial effects. H₀ hypothesis was rejected.

Tea tree essential oil, which was found to be effective in the evaluations, has a significant effect, as it was found in the Linda L Halcon's *Staphylococcus aureus* and wounds: A review St. John's Wort oil, another essential oil, is again Goran S Nikolić's Antimicrobial activity of imanin from St. John's wort from two localities against *Staphylococcus aureus* has a significant antimicrobial effect.

As seen in the Chemical composition and antimicrobial activity of the essential oil of Rosemary study by Yang Jiang et al., its antimicrobial effect gave significant values. In the study, essential oils of turmeric and eucalyptus plants did not have a significant effect on *S. aureus*. In the experiment there is the H_1 hypothesis for a significant effect of tea tree, S. John's Wort and rosemary essential oils on *S. aureus*.

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T.C. MERSİN ÜNİVERSİTESİ REKTÖRLÜĞÜ Tıp Fakültesi Dekanlığı

Temel Tıp Bilimleri Bölüm Başkanlığı Tıbbi Mikrobiyoloji Anabilim Dalı Başkanlığı

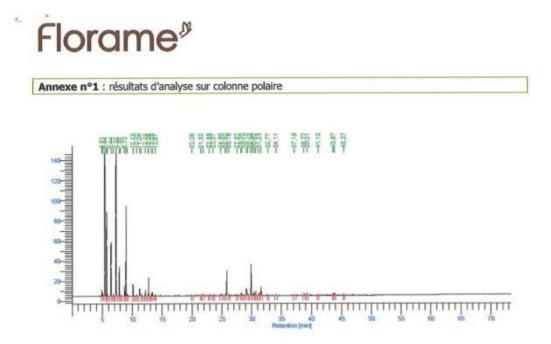
has done his IB diploma programme extended essay experiment in Mersin University Hospital Microbiology Laboratory by himself with the supervision of our staff.

Prof. Dr. Gönul ASLAN Mersin Ü fil. Prof. Dr. Gönül ASLAN

Chief of Microbiology Laboratory

APPENDIX: Gas Chromatography Results of Commercially Available Medicinal Plant Extracts From the Company Florame

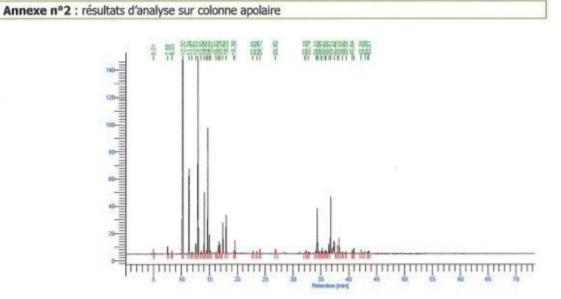
Hypericum perforatum



| Pics | Molécules Noms | Retention (min) | Aires [uV'sec] | Aires [%] | Pics ordre | Molécules Noms | Rétention [min] | Aires [uV*sec] | Aires [%] |
|------|--|--------------------|-------------------|--------------|----------------------|-----------------------|--------------------|-------------------|--------------|
| 1 | Contraction of the second seco | 4,911 | 9577.27 | 0.21 | 14 | MYRCENE | 10,134 | 33484.61 | 0,72 |
| 2 | | 5.074 | 5455.85 | 0,12 | 15 | A-TERPINENE | 10,697 | 3803,30 | 0.08 |
| 3 | METHYL-2-OCTANE | 5,442 | 2301104.06 | 49,37 | 16 | LIMONENE | 11,241 | 23055,11 | 0.49 |
| 4 | NONANE | 5.717 | 149125,15 | 3,20 | 17 | B-PHELLANDRENE | 11,551 | 6728,58 | 0.14 |
| 5 | Very states on the second states | 5,818 | 5536,35 | 0,12 | 18 | CINEOLE | 12,191 | 16604,36 | 0.36 |
| 6 | 3-METHYLNONANE | 6,441 | 120300.92 | 2,58 | 19 | | 12,646 | 8833.34 | 0,19 |
| 7 | TRICYCLENE | 6,860 | 4791,84 930436,04 | 0,10 | 20 | G-TERPINENE | 12,762 | 58914,83 | 1,26 |
| 8 | A-PINENE | 7,325 | 70865.42 | 1,52 | 21 | Se l'activ si factor | 13,249 | 8835,72 | 0,19 |
| 10 | 2-METHYLDECANE | 8,068 | 4966.50 | 0,11 | 22 | P-CYMENE | 13,408 | 13461,96 | 0,29 |
| 11 | | 8,734 | 23751,12 | 0.51 | 23 | TERPINOLENE | 13,866 | 6245,96 | 0.13 |
| 12 | B-PINENE | 8,979 | 291900.48 | 6.26 | 24 | Terri internation | 20,056 | 4628,09 | 0,10 |
| 13 | a r nearra | 9,195 | 6726.59 | 0.14 | 26 | A-COPAENE | 21,863 | 6528,07 | 0,14 |
| | | | | | 27 | CAMPHRE | 22,879 | 3613,52 | 0.08 |
| | | | | | 28 | LINALOL | 23,570 | 3565,20 | 0.08 |
| | | | | | 29 | LITTIC COL | 24,864 | 12261,74 | 0,26 |
| | | | | | 30 | | 25,604 | 8464,11 | 0,18 |
| | | | | | 31 | B-CARYOPHYLLENE | 25,844 | 119145,37 | 2,56 |
| | | | | | | DONNIOPTITELENE | 26,193 | 6119,27 | 0,13 |
| | | | | | 32 33 34 35 | | 27,573 | 4087,58 | 0,09 |
| | | | | | 34 | | 28,260 | 9962.68 | 0,21 |
| | | | | | 35 | | 28,424 | 8786,01 | 0,19 |
| | | | | | 36 | D CADINENE | 29,116 | 41267,94 | 0,89 |
| | | | | | 37 | D. CARDINGTON | 29,370 | 6547,77 | 0.14 |
| | | | | | 38 | D GERMACRENE | 29,939 | 154374,41 | 3,31 |
| | | | | | 39 | D GETTING GETE | 30,079 | 7117,80 | 0,15 |
| | | | | | 40 | | 30,421 | 25144.37 | 0.54 |
| | | | | | 41 | | 30,724 | 19564,55 | 0.42 |
| | | | | | 42 | | 31,235 | 7968,52 | 0,17 |
| | | | | | 43 | BICYCLOGERMACRENE | 31,548 | 52290,65 | 1,12 |
| | | | | | 44 | DIGTOLOGENINGHERE | 32,769 | 4134,88 | 0.09 |
| | | | | | 45 | | 34,107 | 4840.67 | 0,10 |
| | | | | | 46 | | 37,183 | 4044.42 | 0.09 |
| | | | | | 47 | | 38,771 | 7248,28 | 0,16 |
| | | | | | 48 | | 39,307 | 9941,46 | 0,21 |
| | | | | | 49 | | 41,122 | 4060,92 | 0.09 |
| | | | | | 50 | | 43.669 | 7722,62 | 0,17 |
| | | | | | 51 | | 43,879 | 7395,78 | 0,16 |
| | | | | | 52 | | 45.370 | 5553.66 | 0.12 |

1085,900 4660885,69 100,00

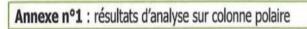
Florame

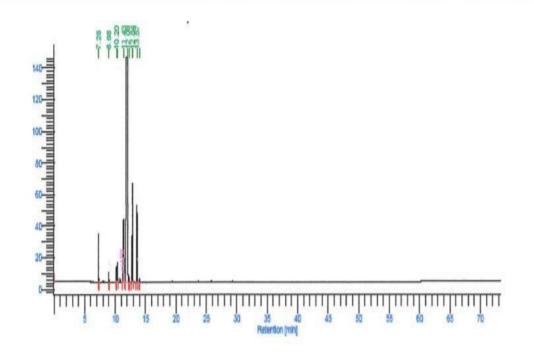


| Pics | Molécules Nome | Rétention (min) | Aires [uV/sec] | Ares [%] | |
|------|-----------------------------------|--------------------|----------------------|-------------|--|
| 1 | | 5,010 | 6101.57 | 0,12 | |
| 2 | | 7,558 | 10605,02 | 0,21 | |
| 3 | | 8,330 | 5069.70 | 0.10 | |
| 4 | METHYL-2-OCTANE | 10,298 | 2434464,61 | 48.23 | |
| 5 | NONANE | 11,244 | 103040,82 | 3.24 | |
| 0 | A TRADUCAL | 11,607 | 3274,84 | 0.06 | |
| 78 | A-THUYENE | 12,533 | 21369,35 | 0.43 | |
| 0 | A-PINENE CAMPHENE | 12,968 | 973651,71 7172,48 | 19.20 | |
| 10 | 3-METHYLNONANE | 14,050 | 132958,88 | 2.0 | |
| 11 | a ma contractoria | 14.428 | 7587.77 | 0.1 | |
| 12 | B-PINENE | 14,698 | 320940,23 | 0.3 | |
| 13 | MYRCENE | 14,931 | 38603.35 | 0.77 | |
| 14 | | 15,198 | 5773.07 | 0.1 | |
| 15 | A-TERPINENE | 10,198 | 4326,36 | 0.0 | |
| 16 | P-CYMENE | 16,539 | 16586,90 | 0.3 | |
| 17 | LIMONENE+B-PHEL+CINEOLE | 10,737 | 33435.50 | 0.0 | |
| 18 | CIS-B-OCIMENE | 16,903 | 19808,55 | 0,3 | |
| 19 | TRANS-B-OCIMENE | 17,399 | 67057.72 | 1,3 | |
| 28 | 2-METHYLDECANE | 17,997 | 90243,72 | 1,7 | |
| 21 | TERPINOLENE | 19.388 | 8167,12 | 0,1 | |
| 22 | LINALOL | 19,577 | 27019,84 | 0,50 | |
| 23 | BORNEOL | 22.847 | 5181,76 | 0,1 | |
| 24 | TERPINEN-4-OL A-TERPINEOL | 23,538 24,102 | 6671,17 10843,41 | 0.1 | |
| 26 | ATENTINEDL | 26,001 | 11858.04 | 0.2 | |
| 27 | | 32,160 | 3701.28 | 0.0 | |
| 28 | A-COPAENE | 32,342 | 6001.09 | 0.1 | |
| 29 | Proof Partie | 32,788 | 3513.83 | 0.0 | |
| 30 | | 34 000 | 8888.91 | 0,1 | |
| 31 | B-CARYOPHYLLENE | 34,338 | 132467,65 | 2,6 | |
| 32 | E-B-FARNESENE | 34,040 | 8174,51 | 0,1 | |
| 33 | AROMADENDRENE | 35,090 | 6478,75 | 0,1 | |
| 34 | | 35,200 | 14091.31 | 0.2 | |
| 36 | A-HUMULENE | 35.697 | 8178,04 | 0, 14 | |
| 36 | | 35,892 | 8113,41 | 0,1 | |
| 37 | a management | 36,022 | 6445,74 | 0,1 | |
| 38 | B-CUBEBENE | 36,510 | 20907,04 | 0,8 | |
| 40 | D GERMACRENE BICYCLOGERMACRENE | 30,834 37,417 | 100574,15 | 3,70 | |
| 41 | BICTCLOGENMACHENE | 38.032 | 20823.23 | 0.4 | |
| 42 | D-CADINENE | 38,313 | 44395.91 | 0.8 | |
| 43 | P. C. Horner Her | 38,918 | 4919.78 | 0.10 | |
| 44 | | 39,549 | 4258.07 | 0.0 | |
| 45 | | 40.643 | 7537.93 | 0.10 | |
| 40 | | 40,928 | 12895.04 | 0.25 | |
| 47 | | 42,258 | 10370,10 | 0,21 | |
| 48 | | 42,981 | 11411,80 | 0.23 | |
| 49 | | 43,428 | 5256,40 | 0,10 | |
| 50 | | 43,009 | 7198,60 | 0,14 | |
| | | | | | |

Eucalyptus globulus



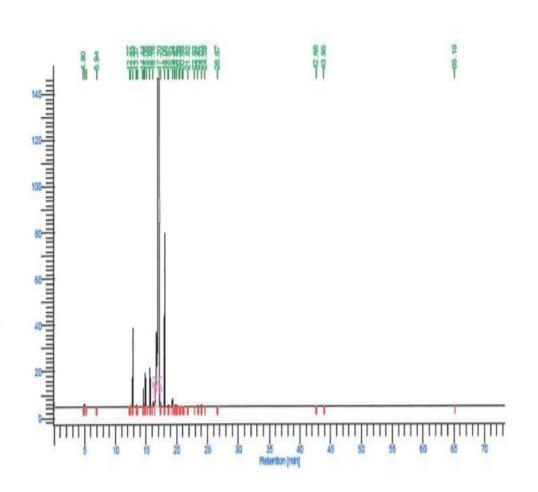




| Pics ordre | Molécules Noms | Rétention [min] | Aires [uV'sec] | Aires [%] |
|---------------|------------------------------|--------------------|-------------------|--------------|
| 1 | ALPHA PINENE | 7.280 | 83881.79 | 1.49 |
| 2 | BETA PINENE | 8.981 | 20587.90 | 0.37 |
| 3 | a phellandrene | 10.204 | 41001.98 | 0.73 |
| 4 | | 10.395 | 51983.97 | 0.93 |
| 23458 | LIMONENE + BETA PHELLANDRENE | 11.435 | 409158.21 | 7.28 |
| 8 | CINEOLE | 12.094 | 4586302.20 | 81.84 |
| 7 | | 12.338 | 9512.97 | 0.17 |
| 8 | GAMMA TERPINENE | 12.842 | 237885,47 | 4.23 |
| <u>ĝ</u> | PARA CYMENE | 13,570 | 109053.91 | 3.01 |
| 8 9 10 | | 13.987 | 8474.43 | 0.15 |
| | | 113.113 | 5617840.84 | 100.00 |

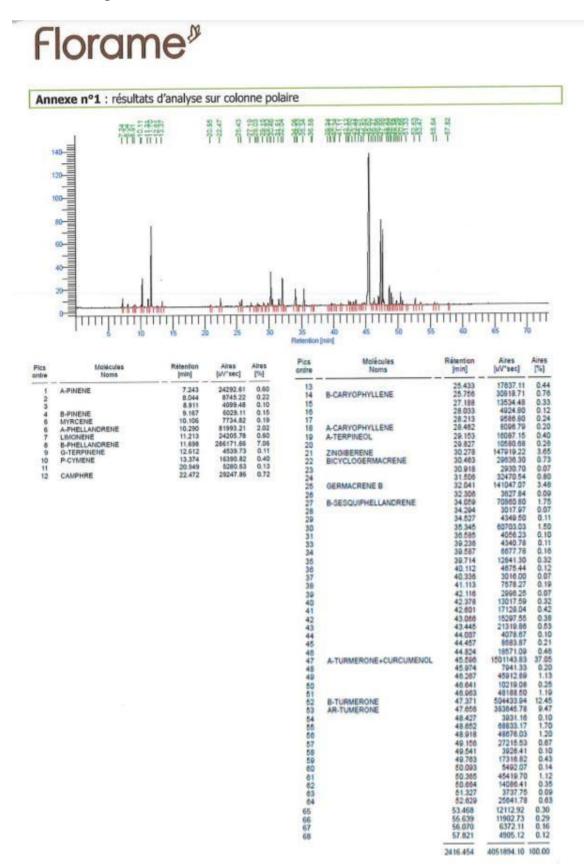
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Annexe n°2 : résultats d'analyse sur colonne apolaire

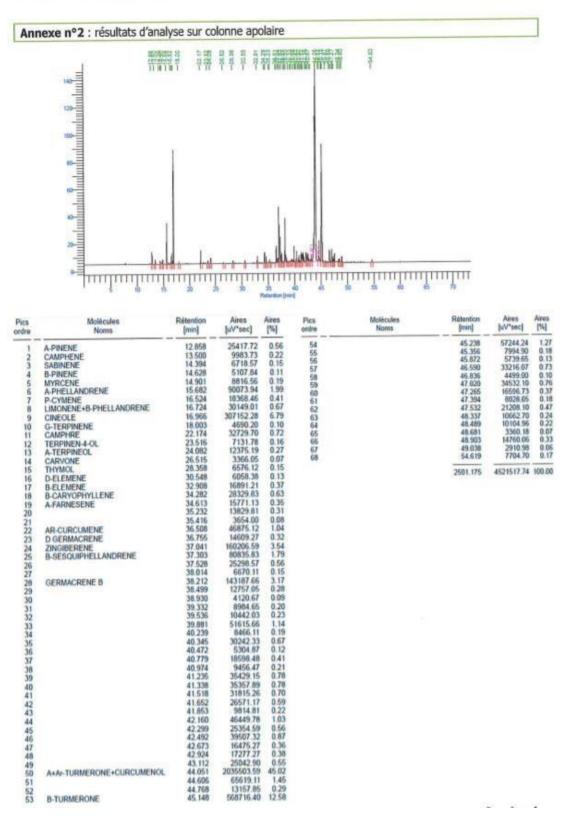


| Pics ordre | Molécules Noms | Rétention (min) | Aires [uV*sec] | Aires [%] |
|---------------|------------------------|--------------------|-------------------|--------------|
| 7 | ALPHA PINENE | 12.826 | 94716.08 | 1.54 |
| 12 | BETA PINENE | 14.591 | 23495.10 | 0.38 |
| 14 | MYRCENE | 14.874 | 47384.94 | 0.77 |
| | ALPHA PHELLANDRENE | 15,648 | 65205.96 | 1.08 |
| 18 17 | ALPHA TERPINENE | 16.163 | 12731.28 | 0.21 |
| 18 | B-PHEL+LIMO+1.8CINEOLE | 17.200 | 5827898.81 | 91.38 |
| | | 17.370 | 3744.96 | 0.08 |
| 19 20 | GAMMA TERPINENE | 18.050 | 271159.58 | 4.40 |
| 23 | | 19.339 | 12477.31 | 0.20 |
| | | 146.080 | 6159810.82 | 100.00 |

Curcuma longa



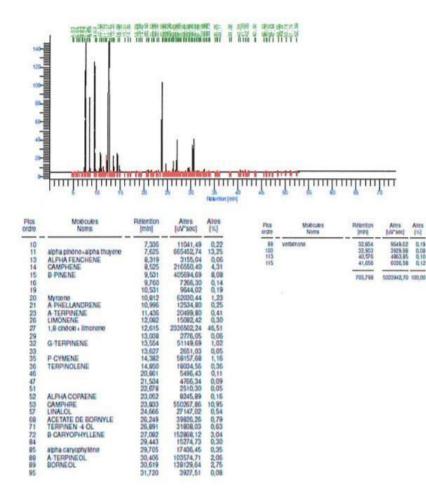
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Rosmarinus officinalis

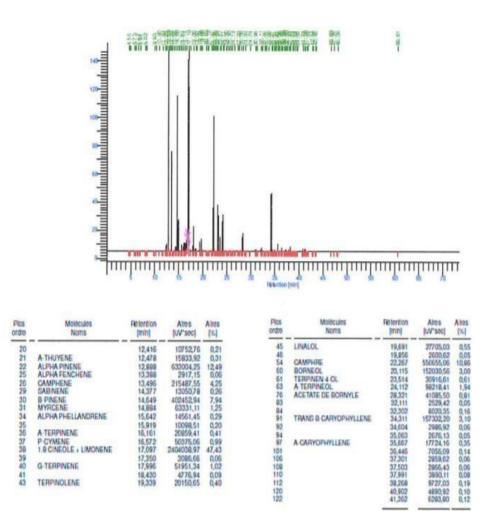
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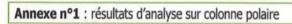
Annexe n°2 : résultats d'analyse sur colonne apolaire

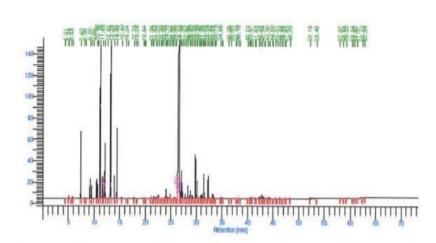


897,789 5058784,42 100,00

Melaleuca alternifolia

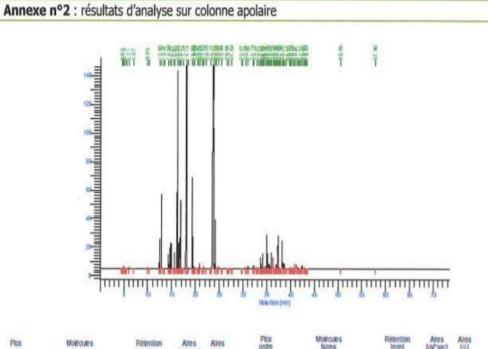
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| Pics ordro | Malecules Noms | Retention [min] | Aires [UV*sec] | Aires [%] | Pics ordro | Molecules Nome | Retention (min) | Aires [uV'soc] | Aires [%] |
|----------------|--------------------------------|--------------------|---------------------|--------------|----------------|-------------------|--------------------|-------------------|--------------|
| 3 | | 5.064 | 3553.99 | 0.06 | 64 | ALLOAROMADENDRENE | 28,342 | 40418.80 | 0.66 |
| 7 | ALPHA THUYENE+ALPHA PINENE | 7,399 | 222642.16 | 3,63 | 65 66 67 | | 28.661 | 3691.16 | 0,06 |
| 11 | BETA PINENE | 9,199 | 47584,15 | 0.77 | 66 | | 28,828 | 24969,11 | 0,41 |
| 12 | SABINENE | 9,466 | 31548.30 | 0,51 | 67 | | 29,167 | 9579,04 | 0,16 |
| 14 | MYRCENE | 10,477 | 56412,13 | 0,92 | 68 70 | ALPHA TERPINEOL | 29.385 | 3463,26 | 0.06 |
| 15 | ALPHA PHELLANDRENE | 10,656 | 38966.25 | 0.63 | 71 | ALFRATERFINEUL | 29,036 29,965 | 140670,62 7591,69 | 2,42 |
| 17 | ALPHA TERPINENE | 11,162 | 688694,50 | 11,22 | 72 | | 30,032 | 8762.09 | 0,12 |
| 18 | LIMONENE | 11,652 | 56300,79 | 0.92 | 72 73 | LEDENE | 30,163 | 67131,64 | 1.09 |
| 19 | 1.8 cineole + beta pheliandren | 12,072 | 233886.60 | 3.81 | 76 | | 30.635 | 6376.20 | 0.14 |
| 21 | GAMMA TERPINENE | 13,283 | 1340386.40 | 21,83 | 77 | | 30,998 | 7525.08 | |
| 21 | PARA CYMENE | 13,932 | 61041.76 | 0,99 | 78 | | 31,138 | 14603,06 | 0.12 |
| | TERPINOLENE | 14,459 | 252154.92 | 4.11 | 00 | BICYCLOGERMACRENE | 31,503 | 89268,17 | 1,45 |
| 35 | TENTINOLENE | 21,183 | 5008.26 | 0,08 | 61 | | 31,566 | 2606.30 | 0.04 |
| 23 35 37 | | 21,631 | 5164.32 | 0.08 | 84 | DELTA CADINENE | 32,334 | 68492,38 | 1,44 |
| 30 | | 22,251 | 5800.40 | 0.09 | 60 | | 33,201 | 12253,29 | 0,20 |
| 39 40 | ALPHA COPAENE | 22.557 | 11088.74 | 0,18 | 93 107 | | 34,896 40,933 | 3133,60 3645,60 | 0.05 |
| 45 | Acria coracia | 23,909 | 2648.13 | 0.04 | 110 | | 42,276 | 4952.25 | 0,08 |
| 40 | ALPHA GURGUJENE | 24,046 | | 0.47 | 111 | | 42.413 | 3810,12 | 0,06 |
| 45 46 47 | ALPHA GUHGUJENE | 24,145 | 28886.20 4554.65 | 0.47 | 112 | | 42,641 | 7139.25 | 0.12 |
| | | | | | 113 | GLOBULOL | 42,935 | 14008.97 | 0,23 |
| 49 50 56 | LINALOL | 24,534 | 3420.75 | 0.06 | 114 | VIRIDIFLOROL | 43.213 | 6004,48 | 0,10 |
| 50 | TERPINENE-4-OL | 24,839 26,587 | 13516,52 2254051,88 | 0.22 | 116 | | 43,901 | 2760,56 | 0,05 |
| | | | | 36,71 | 117 | | 44,187 | 5053,11 | 0,08 |
| 0/ | TRANS BETA CARYOPHYLLENE | 26,797 | 38628,98 | 0,63 | | | | - | |
| 57 58 59 | ADOLIADENDOCHE | 26,917 | 22192,27 | 0,36 | | | 1391,326 | 6140005,98 | 100,00 |
| DV CO | AROMADENDRENE | 27,085 | 88529,46 | 1,44 | | | | | |
| 60 61 62 | | 27,294 | 20008,89 | 0,33 | | | | | |
| 61 | | 27,543 | 2788,41 | 0.05 | | | | | |
| 62 | | 27,817 | 12428,15 | 0,20 | | | | | |

Florame[®]



| Norecures | Relention [min] | Altes [uV*soc] | Altes [14] | Pics | Noms | Rotention (min) | Altos [uV*sec] | Aros [%] |
|---|--|--|--|---|---|--|--|--|
| ALPHA THUYENE ALPHA PINENE | 5,124 12,478 12,839 | 3024,01 56725,65 146072,76 | 0,05 | 31 35 38 41 | LINALOL TERPINEN 4 OL | 19,674 20,907 21,746 23,811 | 3960,39 11732,72 8422,27 2083235,19 | 0,07 0,21 0,15 37,01 |
| BETA PINENE MYRCENE ALPHA PHELLANDRENE | 14,596 14,875 15,641 | 42582,48 50560,09 | 0,76 0,90 0,63 | 43 | ALPHA TERPINEOL ALPHA COPAENE | 24,174 24,348 24,824 31,049 | 131376,10 2551,28 4422,97 | 2,33 0,05 0,08 0,08 |
| PARA CYMENE LIMONENE BETA PHELLANDRENE | 16,505 16,705 16,783 | 55133,00 50766,03 64915,50 | 0,98 0,90 1,15 | 54 55 56 57 | | 32,155 32,295 32,345 32,972 | 4237,91 6656,83 2549,28 | 0,08 0,12 0,05 0,05 |
| 1.8 CINEOLE GAMMA TERPINENE TERPINOLENE | 16,922 18,132 19,388 | 148291,24 1240667,75 232690,66 | 2,63 22,04 4,13 | 58 60 64 | ALPHA GURJUNENE TRANS BETA CARYOPHYLLENE | 33,223 33,790 34,254 | 2826,81 25689,93 35229,78 | 0,05 0,46 0,63 |
| | | | | 69 | AROMADENDRENE | 34,903 35,075 35,190 | 4918,90 | 0,08 |
| | | | | 72 73 75 76 | ALLOAROMADENDRENE | 35,449 35,643 35,971 35,971 | 10817,70 8790,24 | 0,19 0,16 0,65 0,47 |
| | | | | 80 81 82 | LEDENE | 36,983 37,053 37,308 | 5097,61 14034,76 73677,69 | 0.09 0.25 1.31 |
| | | | | 84 | BICYCLOGERMACRENE DEL3A CADINENE | 37,656 38,275 | 2607,58 | 1,47 0,05 1,23 0,26 |
| | | | | 93 95 | | 38,673 39,992 40,245 | 11511,76 4599,97 2697,24 | 0,20 0,08 0,05 |
| | | | | 99 100 105 | GLOBULOL VIRIDIFLOROL | 40,869 41,199 42,273 42,421 | 11713,27 9739,92 4260,14 6141,99 | 0,21 0,17 0,08 0,11 |
| | ALPHA THUYENE ALPHA PINENE SABINENE BETA PINENE MYRCENE ALPHA PHELLANDRENE FARA CYMENE LIMONENE BETA PHELLANDRENE BETA PHELLANDRENE J.S CINEGLE GAMMA TERPINENE | 4.12HA THUYENE 5, 124 A.12HA THUYENE 12, 478 A.12HA PINENE 12, 339 GABRIENE 14, 302 BETA PINENE 14, 596 MYRCENE 14, 592 ALPHA PHELLANDRENE 15, 641 LIPHA THEPINENE 16, 233 RARA CYMENE 16, 050 BETA PHELLANDRENE 16, 705 BETA PHELLANDRENE 16, 705 GAMMA TERPINENE 16, 922 J.9 CINEQLE 16, 925 | ALPHA THUYENE 5,124 3024,01 ALPHA THUYENE 12,479 5,0725,65 ALPHA PINENE 12,809 146072,76 GABRIENE 14,002 27982,36 BETA PINENE 14,096 42582,48 MYRCENE 14,496 42582,48 ALPHA PIELIANDRENE 16,497 50560,96 ALPHA TERPINENE 16,505 637233,49 RARA CYMENE 16,505 55133,00 LIMONENE 16,705 50760,515,00 J.BOLNOLE 16,505 55133,00 J.BOLNOLE 16,202 14291,315,90 J.BOLNOLE 16,202 14291,21 J.BOLNOLE 16,222 1240067,75 | ALPHA THLYENE 5,124 3024,01 0,05 ALPHA THLYENE 12,478 56725,65 1,01 ALPHA PINENE 12,478 56725,65 1,01 ALPHA PINENE 12,039 140072,76 2,59 GABRIENE 14,362 27982,36 0,50 BITA PINENE 14,596 45482,48 0,76 MYRCENE 14,695 50560,09 0,90 ALPHA TERPINENE 15,641 35131,45 0,63 MYRCENE 16,055 507560,30 0,90 ALPHA TERPINENE 16,055 501560,09 0,90 MOYRCENE 16,055 501560,03 0,90 BETA PINELIANDRENE 16,055 501560,03 0,90 BETA PINELIANDRENE 16,760 64915,50 1,15 J.9 CINEOLE 16,922 148291,24 2,63 GAMMA TERPINENE 18,122 148297,24 2,63 | Noms (mm) ""><td>Noms [mm] [s/*soc] [x] ALPHA THUYENE 5,124 3024,01 0,05 31 LINALCK. ALPHA THUYENE 12,478 50725,55 1,01 30 31 LINALCK. ALPHA PINENE 12,478 50725,65 1,01 30 31 LINALCK. ALPHA PINENE 14,596 42582,48 0,50 42 ALPHA TERPINENCL. BETA PINENE 14,595 50506,09 0,76 43 ALPHA TERPINENCL. ALPHA PINENE 15,641 35313,46 0,63 53 54 BPRA CYMENE 16,505 55130,00 0,98 55 56 BETA PIRELLANDRENE 16,205 55130,00 0,98 55 15 1.9 CINSOLE 16,322 148291,24 2,63 59 22,04 60 ALPHA GURJUNENE TEHPINOLENE 19,398 232046;66 4,13 64 TRAND BETA CARYOPHYLLENE 69 GAMMA TERPINENE 19,398 232046;66 4,13 64</td><td>Noms (mm) <th< td=""><td>Noms [min] [M/16C] [14] ALPHA THUYENE 12,478 5024,01 0,05 35 LINALCL 20,907 11732,22 ALPHA THUYENE 12,478 56726,65 1,01 39 140,775 2,59 41 71746 6492,27 ALPHA PINENE 14,502 27998,35 0,55 41 71746 249,27 ALPHA PINENE 14,595 60560,09 0,64 42 ALPHA TERPINEOL 24,514 2551,38 ALPHA FHENIENE 15,641 3513,46 0,63 51 31 0,69 43 ALPHA FERPINENE 16,505 55130,00 0,98 55 32,215 429,24 429,27 JAPHA TERPINENE 16,505 55130,00 0,98 55 32,206 635,43 31,049 4312,13 LIMONENE 16,922 148291,34 2,63 32,220 286,47 32,220 286,47 J.9 CURSOLE 19,398 322966,65 4,13 64 TRAND BETA CARYOPHYLLENE</td></th<></td></th<> | Noms [mm] [s/*soc] [x] ALPHA THUYENE 5,124 3024,01 0,05 31 LINALCK. ALPHA THUYENE 12,478 50725,55 1,01 30 31 LINALCK. ALPHA PINENE 12,478 50725,65 1,01 30 31 LINALCK. ALPHA PINENE 14,596 42582,48 0,50 42 ALPHA TERPINENCL. BETA PINENE 14,595 50506,09 0,76 43 ALPHA TERPINENCL. ALPHA PINENE 15,641 35313,46 0,63 53 54 BPRA CYMENE 16,505 55130,00 0,98 55 56 BETA PIRELLANDRENE 16,205 55130,00 0,98 55 15 1.9 CINSOLE 16,322 148291,24 2,63 59 22,04 60 ALPHA GURJUNENE TEHPINOLENE 19,398 232046;66 4,13 64 TRAND BETA CARYOPHYLLENE 69 GAMMA TERPINENE 19,398 232046;66 4,13 64 | Noms (mm) ""><td>Noms [min] [M/16C] [14] ALPHA THUYENE 12,478 5024,01 0,05 35 LINALCL 20,907 11732,22 ALPHA THUYENE 12,478 56726,65 1,01 39 140,775 2,59 41 71746 6492,27 ALPHA PINENE 14,502 27998,35 0,55 41 71746 249,27 ALPHA PINENE 14,595 60560,09 0,64 42 ALPHA TERPINEOL 24,514 2551,38 ALPHA FHENIENE 15,641 3513,46 0,63 51 31 0,69 43 ALPHA FERPINENE 16,505 55130,00 0,98 55 32,215 429,24 429,27 JAPHA TERPINENE 16,505 55130,00 0,98 55 32,206 635,43 31,049 4312,13 LIMONENE 16,922 148291,34 2,63 32,220 286,47 32,220 286,47 J.9 CURSOLE 19,398 322966,65 4,13 64 TRAND BETA CARYOPHYLLENE</td></th<> | Noms [min] [M/16C] [14] ALPHA THUYENE 12,478 5024,01 0,05 35 LINALCL 20,907 11732,22 ALPHA THUYENE 12,478 56726,65 1,01 39 140,775 2,59 41 71746 6492,27 ALPHA PINENE 14,502 27998,35 0,55 41 71746 249,27 ALPHA PINENE 14,595 60560,09 0,64 42 ALPHA TERPINEOL 24,514 2551,38 ALPHA FHENIENE 15,641 3513,46 0,63 51 31 0,69 43 ALPHA FERPINENE 16,505 55130,00 0,98 55 32,215 429,24 429,27 JAPHA TERPINENE 16,505 55130,00 0,98 55 32,206 635,43 31,049 4312,13 LIMONENE 16,922 148291,34 2,63 32,220 286,47 32,220 286,47 J.9 CURSOLE 19,398 322966,65 4,13 64 TRAND BETA CARYOPHYLLENE |

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