

# Antibacterial Action Of Melaleuca Alternifolia Essential Oil And Propolis Extract On The Bacteria Staphylococcus Aureus: In The Treatment Of Acnea

Ruiz-Silva, KR<sup>1</sup>, Mello-Pinto, MV<sup>3</sup>; Ruiz-Silva,C<sup>2</sup>

<sup>1</sup>(Department, College/ Faculdade CTA, pharmaceutical)

<sup>2</sup>(Department, College/ Faculdade CTA, PHd, Msc, PT.)

<sup>3</sup>(Instituto Celulare)

---

## Abstract:

Acne is one of the most inflammatory conditions or chronic diseases that affects the skin. The bacteria *S. aureus* is able to aggravate it because it is easy to multiply, and can be found in acneic secretion. Thus, it is necessary to seek appropriate antibacterial treatment for the clinical presentation. To combat it it is important to carry out local asphyxiation by selecting the best treatment based on the type of skin and the severity of the acne presented. Topical treatments are indicated in mild and moderate forms, or the adjuncts to systemic therapies in lesions plus serious. The attention to treatments that use the resources of therapeutic use to combat infectious diseases based on complementary or alternative therapies, especially in cases of bacterial resistance. In the present study, they were reviewed in the literature on the action of essential oils of *Melaleuca alternifolia* and the extract of Propolis treatment of acne and proof of them the antibacterials on *Staphylococcus aureus* in experimental tests, by means of antibiograms, measuring the inhibition halos that as two services institutes on a bacteria in plates of samples, that demonstrate results that prove like antibacterial actions in the microorganism in analysis.

The oil of *Melaleuca* and the extract of Propolis, presenting a potentiating action when in association. For a proper application on acneic skin, it is suggested an *in vivo* test performance.

**Materials and Methods:** The experiment was carried out in the Clinical Analysis Laboratory of Universidade Paulista on the Tatuapé campus. The research was carried out in order to seek experimental proof of the antibacterial action of *Melaleuca alternifolia* (Tea Tree) essential oil and Propolis extract separately and in combination on the Gram-positive bacteria *Staphylococcus aureus*.

**Results:** After 48 hours in the incubator, bacterial growth was observed on all plates. From then on, the inhibition halos and results were observed through precise measurements (Antibiogram). It can be seen that the two culture media produced with *Melaleuca alternifolia* essential oil, both experiments one and two, obtained the same result, that is, both inhibited an average of 0.26 cm of the *S. aureus* bacterial colony. In the culture medium with the Propolis extract, the inhibition halo was only 0.02 cm different when compared to that of *Melaleuca*, that is, the extract inhibited 0.24 cm of halo. The concentration of *Melaleuca* oil together with the propolis extract obtained a potentiating result, with a growth inhibition of 0.46 cm, proving the effectiveness of the combination of these two natural substances in the same antibiogram sample. The antibiotic gentamicin used for comparisons, on the other hand, presented an average of 0.81 cm of inhibition halo.

**Conclusion:** It was concluded that *Staphylococcus aureus* was suitable for the evaluation of natural substances, since it was successfully cultivated. The evaluation of the essential oil of *Melaleuca alternifolia* and the extract of Propolis indicated antibacterial action, however, both demonstrated potentiating effects on the colony when associated. *In vivo* studies of the association in anti-acne formulations are suggested to improve these natural substances in the appropriate application on the skin conditioned to the treatment, defining the correct dosage.

**Keyword:** pharmacy, dermatological diseases, Gram-positive bacteria, natural products (anti-acne)..

---

Date of Submission: 05-10-2024

Date of Acceptance: 15-10-2024

---

## I. Introduction

Acne is one of the chronic inflammatory conditions/diseases that most affects the skin (CORREA; SILVA; OLIVEIRA 2010; DEUSCHLE et al., 2015). It can be chronically inflammatory, affecting the polysebaceous follicle, and is of genetic, hormonal and/or multifactorial origin. Taking medications, factors such as stress and an inappropriate diet can aggravate the condition (DEUSCHLE et al., 2015). The signs commonly begin to appear in adolescence; it is believed to have an incidence of 85% among young people aged 12 to 24 (RODRIGUES NETO et al., 2015). It affects both sexes, being normally more severe in men during

adolescence (UDA; WANCZINSKI, 2008) but more persistent in women (FIGUEIREDO et al., 2011a). Staphylococci are responsible for causing or aggravating several diseases, including acne (TEDESCO et al., 2014). As resistance (superbacteria) of human and animal pathogens has increased, in recent years the demand for natural antibacterial substances has increased (SILVA; MEJIA, 2015). Since the skin is an attractive, accessible and penetrable route for administering substances, this route of administration shows benefits such as systemic effect and ease of vectorization exclusively to the affected area when topical use is desired (BACCOLI et al., 2015).

Melaleuca alternifolia oil is a topical alternative widely used in the treatment of acne. On the skin, it plays an important role in reducing the inflammatory process of an infected wound, acting as a natural antibiotic, being bactericidal and bacteriostatic, with antiseptic, analgesic and healing action, among others (TEDESCO et al., 2014).

Propolis is an efficient natural antibiotic, rich in vitamins and minerals (MACHADO et al., 2012). Having antioxidant action, it helps in the healing process of wounds due to the presence of flavonoids (MATSUCHITA; MATSUCHITA, 2014).

In this study, the efficacy of the pure essential oil (without dilution) of Melaleuca alternifolia (Tea Tree) and the Propolis extract as natural antibacterials were analyzed through antibiograms, with the antibiotic gentamicin as a comparison for the analysis, aiming at its actions on the Gram-positive bacterial pathogens Staphylococcus aureus.

## **II. Material And Methods**

The experiment was carried out in the Clinical Analysis laboratory of Universidade Paulista on the Tatuapé campus.

The research was carried out in order to seek experimental proof of the antibacterial action of Melaleuca alternifolia (Tea Tree) essential oil and Propolis extract separately and in combination on the Gram-positive bacteria Staphylococcus aureus

The following materials were used to perform the antibiogram:

- Melaleuca alternifolia essential oil (bottle with dropper) from the BioEssência® brand, batch number 161113 and Propolis extract (bottle with dropper) from the Smells® brand, batch number: SC 17222
- Antibiotic Gentamicin
- Staphylococcus aureus bacteria
- Two-hole punch
- Filter papers
- Test tubes
- Petri dishes
- Mueller Hinton agar
- Sterile swab
- Tweezers
- Beaker
- Tips
- Autoclave
- Laminar flow hood

The S. aureus bacteria was stored in the refrigerator at 8°C in the laboratory. The natural products were obtained through physical purchase and stored under the due care described on the labels. The extract was kept in a cool, humid place and the essential oil was not exposed to heat, light or humidity as recommended.

The essential oil of Melaleuca alternifolia was not diluted, but applied pure to the Mueller Hinton Agar culture medium, while the Propolis extract was diluted in cereal alcohol with a minimum dry extract of 11%. Both were used as found commercially, with their original manufacturing aspects and within the specified expiration date.

The antibiotic gentamicin, which was stored in the laboratory refrigerator, was chosen as a means of comparison between the natural substances for performing the antibiogram, in which the inhibition halo was verified and compared.

### **Procedure methodology**

#### **Antibiogram**

All materials used in the preparation of the antibiogram were autoclaved at 121°C for 40 minutes.

After autoclaving, in the laminar flow hood, 30 drops (1.5 ml) of Melaleuca alternifolia essential oil were dripped into a test tube, 30 drops (1.5 ml) of propolis extract into another tube, and 30 drops of Melaleuca

essential oil and propolis extract were dripped into the third. Using tweezers, the discs were transferred to the test tubes, impregnating at least 4 or more discs in each tube. Staphylococcus aureus bacteria were seeded in a zigzag pattern using a sterile swab on the 6 plates containing Mueller Hinton Agar culture medium. After inoculation, triplicates impregnated with Melaleuca alternifolia essential oil and 1 disc impregnated with gentamicin were added to the first and second plates, creating a reserve experiment. In the third plate, triplicates of propolis extract and 1 disk impregnated with gentamicin were placed. In the fourth culture medium, 1 disk of extract, 1 of essential oil, the combination of both and the impregnated gentamicin disk were inserted. In the fifth Petri dish, triplicates impregnated with the combination of Melaleuca alternifolia and propolis extract plus 1 disk of gentamicin were placed. The sixth plate, containing Mueller Hinton Agar inoculated with Gram-positive bacteria, was only seeded to observe bacterial growth on the culture medium, with no disks being added to its surface. The covered Petri dishes were taken to the incubator, remained at 37°C for 24 hours, were removed the following day for analysis and then returned to the incubator for another 24 hours. Immediately after 48 hours, they were removed to make the desired measurements.

### III. Result And Discussions

#### Antibiogram

After 48 hours in the incubator, bacterial growth was observed on all plates. From then on, inhibition zones and results were observed through precise measurements, which are described in Table 1

**Table 1 - Measurement, in centimeters, of results of the inhibition zone of the growth of the Staphylococcus aureus bacteria in antibiograms.**

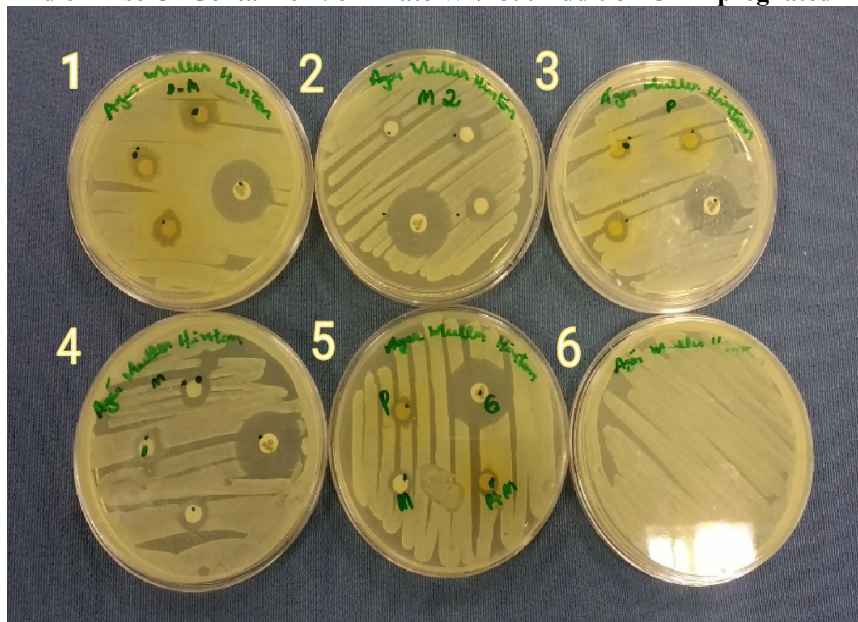
Diameter (end to end)	From the radius to the end	From the radius to the end
SUBSTANCES	LEFT	RIGHT
<b>1° - OE MELALEUCA 1</b>		
1,1 cm	0,3 cm	0,3 cm
1,0 cm	0,2 cm	0,2 cm
1,0 cm	0,3 cm	0,3 cm
Gentamicina		
2,0 cm	0,8 cm	0,7 cm
<b>2° - OE MELALEUCA 2</b>		
0,9 cm	0,1 cm	0,1 cm
1,1 cm	0,3 cm	0,4 cm
1,1 cm	0,3 cm	0,4 cm
Gentamicina		
2,3 cm	0,9 cm	0,9 cm
<b>3° - EXT. DE PRÓPOLIS</b>		
0,8 cm	0,1 cm	0,3 cm
1,1 cm	0,3 cm	0,3 cm
0,9 cm	0,1 cm	0,1 cm
Gentamicina		
2,0 cm	0,7 cm	0,8 cm
<b>4° - OE M + EXT P</b>		
1,6 cm	0,6 cm	0,5 cm
1,5 cm	0,7 cm	0,4cm
1,3 cm	0,4 cm	0,4 cm
Gentamicina		
2,0 cm	0,8 cm	0,8 cm
<b>5° - EXT. P OE M EXT. P + OE M</b>		
Própolis		
1,2 cm	0,4 cm	0,3 cm
Melaleuca		
0,9 cm	0,3 cm	0,2 cm
M+P		
1,0 cm	0,4 cm	0,3 cm
Gentamicina		
2,0 cm	0,9 cm	0,8 cm

EXT. = Extract  
EO = Essential oil  
M = Melaleuca  
P = Propolis

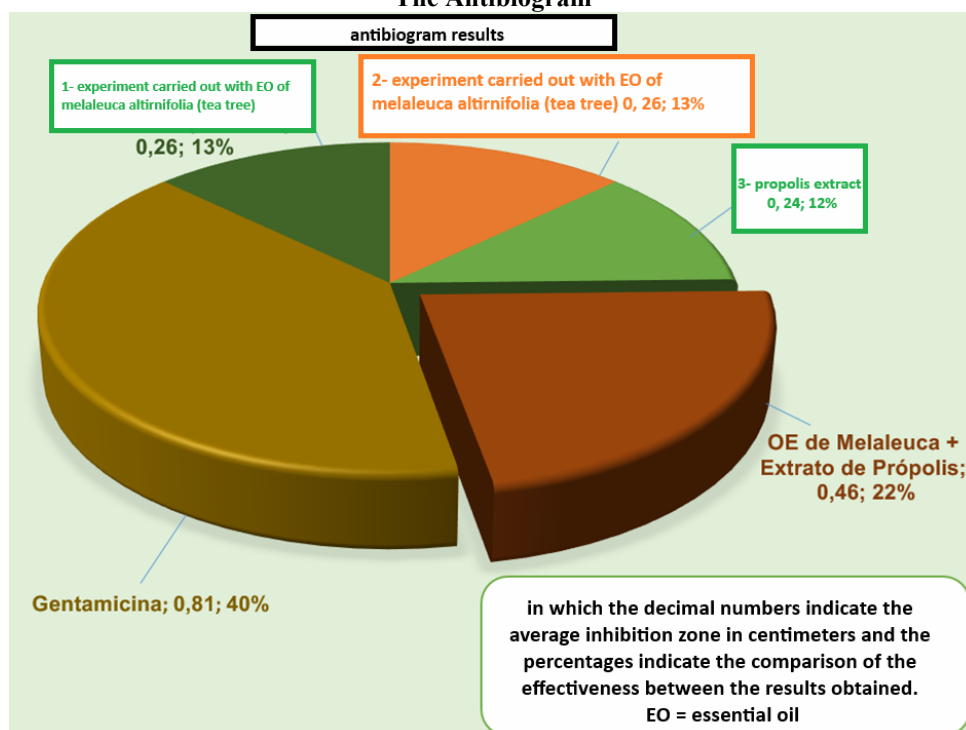
P + M = Propolis extract associated with Melaleuca oil

Source: author's own

**Figure 1-1- Triplicate Of Propolis Extract Associated With Melaleuca Alternifolia Oil And 01 Disc Impregnated With Gentamicin. 2- Triplicate Of Melaleuca Essential Oil And 01 Disc Of Impregnated Gentamicin. 3 – Triplicate Of Propolis Extract And 01 Disc Impregnated With Gentamicin. 4 – Triplicate Of Melaleuca Alternifolia Essential Oil And 01 Disc Of Impregnation With Gentamicin. 5 – In This Plate Were Added 01 Disc With The Impregnation Of Propolis Extract, 01 With Melaleuca Oil, Another Disc Containing The Impregnation Of Propolis Extract In Association With Melaleuca Alternifolia Essential Oil And 01 Disc Of Gentamicin. 6 – Plate Without Addition Of Impregnated Discs.**



**Figure 2: Graph Representing The Comparison, In Percentage, Of The Antibacterial Agents Obtained In The Antibiogram**



Source: Author Himself

It can be seen that the two culture media used with Melaleuca alternifolia essential oil, both experiments one and two, obtained the same result, that is, both inhibited an average of 0.26 cm of the S. aureus



bacterial colony. In the culture medium with the Propolis extract, the inhibition halo was only 0.02 cm different when compared to that of Melaleuca, that is, the extract inhibited 0.24 cm of halo. The concentration of Melaleuca oil together with the propolis extract obtained a potentiating result, with a growth inhibition of 0.46 cm, proving the effectiveness of the combination of these two natural substances in the same antibiogram sample. The antibiotic gentamicin used for comparisons presented an average of 0.81 cm of inhibition halo.

#### IV. Conclusion

It is concluded that *Staphylococcus aureus* proved to be suitable for the evaluation of natural substances, since it was successfully cultivated. The evaluation of the essential oil of *Melaleuca alternifolia* and the extract of Propolis indicated antibacterial action, however, both demonstrated potentiating effects on the colony when associated.

In vivo studies of the association in anti-acne formulations are suggested to improve these natural substances in the appropriate application on the skin conditioned to the treatment, defining the correct dosage.

#### References

- [1] Almeida, T. Ap. Et Al. Estudo Clínico De Formulações Contendo *Pereskia Aculeata* Mill. Para Tratamento Da Acne. In: Ix Epcc – Encontro Internacional De Produção Científica Unicesumar, Maringá/Paraná. Anais. Isbn – 978-85-8084-996-7, N. 9, P. 4-8, Novembro, 2015.
- [2] Alonso, M. C. F.; Fernández, A.T. Actualización Em Acné. An Pediatr Contin, España, V. 9, N. 3, P. 153-161, 2011.
- [3] Anvisa – Agência Nacional De Vigilância Sanitária. Descrição Dos Meios De Cultura Empregados Nos Exames Microbiológicos. Módulo Iv. 64 F. P. 61. Disponível Em: <Http://Www.Anvisa.Gov.Br/Servicosau/Manuais/Microbiologia.Asp>. Acesso Em: 04/08/2017.
- [4] Baccoli, B.C. Et Al. Os Benefícios Do Óleo De Melaleuca Na Acne Grau Ii E Iii: Uma Revisão De Literatura. Revista Da Universidade Vale Do Rio Verde, Três Corações/Mg, V. 13, N. 1, P. 536-547, 2015.
- [5] Barbosa, V. Et Al. Avaliação Da Atividade Antibacteriana Do Óleo Essencial De *Rosmarinus Officinalis* L. E Tintura De Própolis Frente À Bactéria Causadora Da Acne *Propionibacterium Acnes*. Revista Brasileira De Plantas Mediciniais, Campinas, V. 16, N. 2, P. 169-173, 2014.
- [6] Bastos, I. B. N. Própolis: Revisão Bibliográfica. 2010. 42 F. Monografia (Especialização) - Universidade Federal De Minas Gerais, Faculdade De Odontologia, Minas Gerais, 2010.
- [7] Bezerra, A. C. A.; Gonçalves, G. M. S. Desenvolvimento E Estudos De Estabilidade De Formulações Cosméticas Contendo Própolis Destinadas À Prevenção Da Acne. In: Anais Do Xiv Encontro De Iniciação Científica Da Puc-Campinas, Campinas/Sp. Anais. Issn 1982-0178. Setembro, 2009.
- [8] Buriol, L. Et Al. Composição Química E Atividade Biológica De Extrato Oleoso De Própolis: Uma Alternativa Ao Extrato Etanólico. Química Nova, V. 32, N. 2, 2009.
- [9] Correa, F. F. B.; Silva R. C.; Oliveira, A. C. Acne Inimiga Da Pele. In: X Congresso De Educação Do Norte Pioneiro, Jacarezinho/Pr, Uenp-Cche-Cica: Universidade Estadual Do Norte Do Paraná. Anais. Issn – 1808-3579, P. 172-180, 2010.
- [10] Costa, A.; Alchorne, M. M. A.; Goldschmidt, M. C. B. Fatores Etiopatogênicos Da Acne Vulgar. Anais Brasileiros De Dermatologia, Rio De Janeiro, 2008, V. 83, N. 5, P. 451-459, Setembro/Outubro, 2008.
- [11] Deuschle, V. C. K. N. Et Al. Caracterização Das Lesões E Tratamentos Utilizados Na Acne. Revint – Revista Interdisciplinar De Ensino, Pesquisa E Extensão, Rs/Brasil, Unicruz- Universidade De Cruz Alta. Issn – 2358-6036, Vol. 3, Nº1, P. 224-236, 2015.
- [12] Falcão, M. A.; Pereira, M. A. A.; Milão, D. Estudo Da Atividade Antimicrobiana Do Extrato De Própolis Da Abelha *Apis Mellífera* Produzido Na Região Da Grande Porto Alegre. In: X Salão De Iniciação Científica Pucrs, P. 595-597, 2009.
- [13] Figueiredo, A. Et Al. Avaliação E Tratamento Do Doente Com Acne – Parte I: Epidemiologia, Etiopatogenia, Clínica, Classificação, Impacto Psicossocial, Mitos E Realidades, Diagnóstico Diferencial E Estudos Complementares. Rev. Port. Clin. Geral, P. 59-65, 2011a.
- [14] Figueiredo, A. Et Al. Avaliação E Tratamento Do Doente Com Acne – Parte Ii: Tratamento Tópico, Sistêmico E Cirúrgico, Tratamento Da Acne Na Grávida, Algoritmo Terapêutico. Rev. Port. Clin. Geral, P. 66-76, 2011b.
- [15] Garcia, C. C. Et Al. Desenvolvimento E Avaliação Da Estabilidade Físico-Química De Formulações De Sabonete Líquido Íntimo Acrescidas De Óleo De Melaleuca. Rev. Bras. Fram. V. 90, N. 3, P. 236-240, 2009.
- [16] Garvil, M. P. Et Al. Ação Antimicrobiana Do Óleo De Melaleuca (*Melaleuca Alternifolia*). E-Rac - Revista Eletrônica Da Reunião Anual De Ciência, Unetri, Minas Gerais, V. 3, N. 1, P. 1-16, 2013.
- [17] Gonçalves, G.M.S.; Santos, N.P.; Srebernich, S.M. Antioxidant And Antimicrobial Activities Of Propolis And Açai (*Euterpe Oleracea* Mart) Extracts. Journal Of Basic And Applied Pharmaceutical Sciences, Issn 1808-4532, P. 349-356, 2011.
- [18] Lustosa, S. R. Et Al. Própolis: Atualizações Sobre A Química E A Farmacologia. Revista Brasileira De Farmacognosia, Issn 0102-695x, 18(3): 447-454, Julho/Setembro, 2008.
- [19] Machado, A. V. Et Al. Tratamentos Da Acne Com O Uso De Ácido Úsnico E Própolis. Fisioterapia Brasil – Ed. Atlântica, V. 13, N. 6, P. 414-418, Novembro, 2012.
- [20] Machado, B. F. M. T. Óleos Essenciais: Verificação Da Ação Antimicrobiana In Vitro, Na Água E Sobre A Microbiota Da Pele Humana. 2011. 17 F. Dissertação (Mestrado) - Universidade Estadual Paulista, Instituto De Biociências De Botucatu, 2011.
- [21] Machado, B. F. M. T. Et Al. Óleos Essenciais: Verificação Da Ação Antimicrobiana In Vitro, Na Água E Sobre A Microbiota Da Pele Humana. 2011. 111 F. Dissertação (Mestrado) - Universidade Estadual Paulista, Instituto De Biociências De Botucatu, 2011.
- [22] Maier, M. C.; Rene, M.; Lubi, N. Uso Da Argila No Tratamento Preventivo Da Acne Na Adolescência. 2013. 13 Folhas. Curso De Tecnologia Em Estética E Imagem Pessoal – Universidade Tuiuti Do Paraná, Curitiba/Pr, 2013.
- [23] Marques, L. C. Preparação De Extratos Vegetais. Jornal Brasileiro De Fitomedicina, V. 3, N. 2, P. 74-76, Abr./Maio/Junho, 2005.
- [24] Matsuchita, H. L.P.; Matsuchita, A. S. P. Uso Da Própolis Na Prevenção E Tratamento Da Acne Vulgar, Uniciências, Pr/Brasil, V. 18, N. 1, P. 19-23, Junho, 2014.
- [25] Meneses, C.; Bouzas, I. Acne Vulgar E Adolescência. Adolescência E Saúde. V. 6, N. 3, Setembro, 2009.

- [26] Montenegro, C. M.; Costa, S. C. C.; Branco, C. R. C. Avaliação De Formulações Tópicas Magistrais Para O Tratamento Da Acne. Revista De Ciências Farmacêuticas Básica E Aplicada, Issn 1808-4532, V. 34, N. 1, P. 87-94, 2013.
- [27] Oliveira, A. C.; Silva, R. S. Desafios Do Cuidar Em Saúde Frente À Resistência Bacteriana: Uma Revisão. Revista Eletrônica De Enfermagem, V. 10, N. 1, P.189-197, 2008.
- [28] Pereira, C. S. Et Al. Desenvolvimento De Uma Formulação Farmacêutica Utilizando Óleo Essencial De Melaleuca Alternifolia. Xiii Inic, Ix Epg E Iii Inic Jr. Univap, Outubro, 2009.
- [29] Pistore, M. Et Al. Avaliação Do Uso Tópico Do Óleo Essencial De Ruta Graveolens L. (Arruda) Na Cicatrização De Feridas Em Ratos. Perspectiva, Erechim/Rs, V. 38, N. 141, P. 57-68, Março, 2014.
- [30] Rodrigues Neto E. M. Et Al. Abordagem Terapêutica Da Acne Na Clínica Farmacêutica. Boletim Informativo Geum, Programa De Pós-Graduação Em Ciências Farmacêuticas: Universidade Federal Do Piauí, Issn – 2237-7387, V. 6, N. 3, P. 59–66, Julho/Setembro, 2015.
- [31] Santos, A. L. Et Al. Staphylococcus Aureus: Visitando Uma Cepa De Importância Hospitalar. Jornal Brasileiro De Patologia E Medicina Laboratorial, Rio De Janeiro, Issn 1678-4774, V. 43, N. 6, P. 413-423, Dezembro, 2007.
- [32] Silva, P. A. A.; Mejia, D. P. M. Ação Antimicrobiana Do Óleo Essencial De Melaleuca Alternifolia (Tea Tree) Para Uso Como Coadjuvante Em Antissépticos. Fisioterapia Ser, Issn – 1809-3469, V. 10, P. 34-39, 2015.
- [33] Tedesco, L. Et Al. Avaliação Antibacteriana Do Extrato De Melaleuca (Melaleuca Alternifolia) Frente À Cepa De Staphylococcus Aureus. Arq. Cienc. Saúde Unipar, Umuarama, V. 18, N. 2, P. 89-94, Maio/Agosto, 2014.
- [34] Uda, C.F.; Wanczinski, B. J. Principais Ativos Empregados Na Farmácia Magistral Para O Tratamento Tópico Da Acne. Informa – Informativo Profissional Do Conselho De Federal De Farmácia, Brasília, V.20, N.9/10, P.16-25, Setembro, 2008.
- [35] Vieira, A. P. Et Al. Ação Dos Flavonoides Na Cicatrização Por Segunda Intenção Em Feridas Limpas Induzidas Cirurgicamente Em Ratos Wistar. Semina: Ciências Biológicas E Da Saúde, Londrina, V. 29, N. 1, P. 65-74, Janeiro/Junho, 2008.
- [36] Williams, H. C.; Dellavalle, R. P.; Garner, S. Acne Vulgaris. The Lancet, V. 379, N. 9813, P. 361-372, 2012.