The Study of the Antibacterial Activity of Asam Gelugor (*Garcinia Atroviridis*) against *Methecillin-resistant Staphylococcus Aureus* (MRSA), *Streptococcus Pneumoniae* and *Klebseilla Pneumoniae*

May Florence Dela Cruz Bacayo[1]*, Nurul Shahira Sajali[1], Wong Charng Choon[1], Santosh Fattepur[1], Kiran Chanabasappa Nilugal[1], Jiyauddin Khan[1], Fadli Asmani[1], Eddy Yusuf[1]
[1]School of Pharmacy, Management and Science University, 40100 Shah Alam Malaysia

Keywords: Antibacterial Activity, *Garcinia Atroviridis* Extracts, Zone of Inhibition.

Abstract: The curative ability of the endemic plants for different disorders has been described by traditional medicine practitioners. Some herbs have been reported to have antibacterial activity such as *Garcinia Atroviridis*. The antibacterial activity of *Garcinia Atroviridis* (Asam Gelugor) extracts was evaluated against Methicillin-resistant *Staphylococus aureus*, *Streptococcus pneumoniae* and *Klebseilla pneumoniae*. Asam Gelugor fruits (600 grams) were collected. The powder form were successively extracted with with 99.8 % of methanol. The extract was filtered and dried using the rotatory evaporator at a temperature not exceeding 50ºC. The antibacterial activity was determined by disc diffusion method for the zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The zone of inhibition were compared with that of ampicillin, vancomycin, and gentamicin antibiotic disc. The test by using disc diffusion method shows highest inhibition against the *Klebsiella pneumoniae* (15.33 ± 1.53) in 100% extract. Based on the result for MIC, inhibition is at 50mg/ml until 0.05 mg/ml against MRSA, while the MIC is positive in all concentration of extract against *Klebsiella pneumoniae* but the MIC result is negative in all concentration of the extract against *Streptococcus pneumoniae.* MBC result showed that there is bacterial growth in 500mg/ml of extract against MRSA while no bacterial growth in extract against *Klebsiella pneumonia*, bacterial growth is positive in the extract against the *Streptococcus pneumoniae*. The results shows that Asam Gelugor *(Garcinia Atroviridis)* may serve to the development of a new antibacterial agent against these type of bacteria.

1 INTRODUCTION

Disease can be cure not only by a processed medicine or medicine that can we can get at the market but home remedies can also be used to treat the disease furthermore, it is cheaper than the marketed drug. Example of home remedies are ginger, turmeric, garlic, black pepper, tamarind that can treat illness such as infection or to reduce body temperature. Back to ancient era, they use endemic plants to treat several illness. Thus, plants play a major role in the treatment of diseases. A very common example was morphine from the opium poppy and most of the sources of the drugs were from plant, animal and microorganism. (Evans, W.C. Trease and Evans Pharmacognosy., 2009)

Curative ability of many endemic plants has been described by the practitioners of traditional medicine for centuries. The increasing antibacterial activity of various medicinal plants were reported from the different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. (World Health Organization, WHO).

Herbs are widely exploited in the traditional medicine and their curative potentials are well documented. About 61 % of new drugs that were developed between 1981 and 2002 were of natural products focusing on infectious diseases and cancer therapy. Unfortunately, the discovery rate of active novel

Bacayo, M., Sajali, N., Choon, W., Fattepur, S., Nilugal, K., Khan, J., Asmani, F. and Yusuf, E.
The Study of the Antibacterial Activity of Asam Gelugor (Garcinia atroviridis) Against Methecillin-resistant Staphylococcus Aureus (MRSA), Streptococcus Pneumoniae and Klebseilla Pneumoniae.
DOI: 10.5220/0008358401180123
In *Proceedings of BROMO Conference (BROMO 2018)*, pages 118-123
ISBN: 978-989-758-347-6

chemical entities is declining. Thus, natural products from plants may give a new source of antibacterial activity with possibly novel mechanism of action. As the effects of plant extracts have been studied by many researchers. (Nayan et al, 2011)

Antibacterial agents are the well-known weapons to fight bacterial infections and helps to improve the quality of human life since its introduction. But, due to the emergence of drug-resistant bacteria, these antibacterial agents have become less effective. It is very essential to investigate newer drugs derived from natural sources for the prevention and treatment of bactrial infections as well as to combat antibiotic resistance.

A large rainforest of Peninsular Malaysia is the haven of *Garcinia Artroviridis*, known as Asam Gelugur, Asam Gelugo or Asam Keping in Malay. This species grows throughout in the rainforest and widely cultivated especially in the northern states. Asam Gelugor is commonly used for its weight reduction properties (Ensiklopedia Pengubatan Herba, 2013). The tree grows for about 20 meters high and has extended trunk, greyish smooth bark and hanging branches. The floret are darkish red with a yellowish to orange fruits borne singly on the ends of the twigs. The plant contains fruit acids such as citric acid, tartaric acid and ascorbic acid, that have antioxidant properties. Phytochemical investigations have isolated garcinia acid and its γ-lactone, atroviridin, atrovirisidone, atrovirinone, and some organic acids. (Amran et al, 2009). Hence, this study aims to investigate the antibacterial activity of *Garcinia Atroviridis* (Asam Gelugor) against MRSA, *Streptococcus pneumoniae* and *Klebsiella pneumoniae*.

2 METHODS

2.1 Extraction Process

Asam Gelugor fruits were collected and cut into small pieces (600g). The oven dried fruits were grind until its powder form which were extracted with 99.8% methanol using a Soxhlet extractor. The solution was filtered and dried using the rotatory evaporator at a temperature not exceeding 50ºC.

2.2 Disc Diffusion Method for Determination of Zone of Inhibition

The petriplates were inoculated with sterile swab dipped into the inoculums. The excess inoculum from the swab was removed by gently pressing and rotating the swab firmly against the side of the tube in order to remove excess fluid in the swab.
The sterile swab was streaked all over the surface of the petriplate three times for a lawn of growth, rotating the petriplate through an angle of 60° after each application. The sterile swab was passed round the edge of the agar surface. After the streaking, allow the petriplates to dry in a room temperatures for a 5 minutes, with the petriplates lid closed. A narrow hole was the bore in the petriplates and the extract were added in the hole. The petriplates were placed in an incubator at 37°C within 30 minutes
After 24 hours of incubation, use a metric ruler to measure the diameter of the zone (including the diameter disc) without opening the lid and record the diameter in mm.

2.3 Minimum Inhibitory Concentration (MIC)

Agar well diffusion method and the micro-broth dilution technique were employed to determine the minimum inhibitory concentration (MIC) for each extract and test organism. A reconstituted extract of 500 mg/mL concentration was serially diluted in two-fold up to 0.05 mg/mL. A 100 µL volume of each dilution was introduced into duplicate wells in the Mueller Hinton Agar plates that is pre‑inoculated with test bacterial strain; and incubated at 37 °C for 24 h. The minimum inhibitory concentration was taken and recorded as the lowest concentration of the extract showing measurable inhibition zone. For the micro‑broth dilution technique, a 100 µL volume of each dilution of the extract was introduced into duplicate tubes of 2.0 mL Mueller Hinton broth (MHB) seeded with 100 µL of the standardized suspension of the test bacterial strain. Incubation was at 37 °C for 24 hours and MIC was taken as the lowest concentration of the extract that made the culture show no visible growth.

2.4 Minimum Bactericidal Concentration (MBC)

A modified agar well diffusion technique was employed in the determination of the minimum bactericidal concentration (MBC). A 2 mm diameter agar disc cut out from the inhibition zone of the last three consecutive wells in each dilution showing inhibition was inoculated into a fresh sterile nutrient broth medium. The broth cultures were incubated at 37 °C for 24 hours after which 100 µL was spread over a fresh sterile MHA. The MHA culture was in turn incubated at 37 °C for 24 hours and the least concentration of the extract showing no growth was taken as the MBC. An MBC which coincided with or was next to the MIC value was considered bactericidal while those that differed markedly were considered bacteriostatic.

3 RESULT AND DISCUSSIONS

The antibacterial activity of *Garcinia Atroviridis* has been measured by the different test employed on it. The disc diffusion method was used to measure the zone of inhibition of the extract shows highest inhibition against *Klebsiella pneumoniae* (15.33 ± 1.53) in 100% concentration of the extract followed by inhibition against MRSA (10.76 ± 4.13) then *Streptococcus pneumoniae* (4.88 ± 0.44). The negative result is shown the inhibition of 25% concentration of the extract against *Streptococcus pneumoniae*.

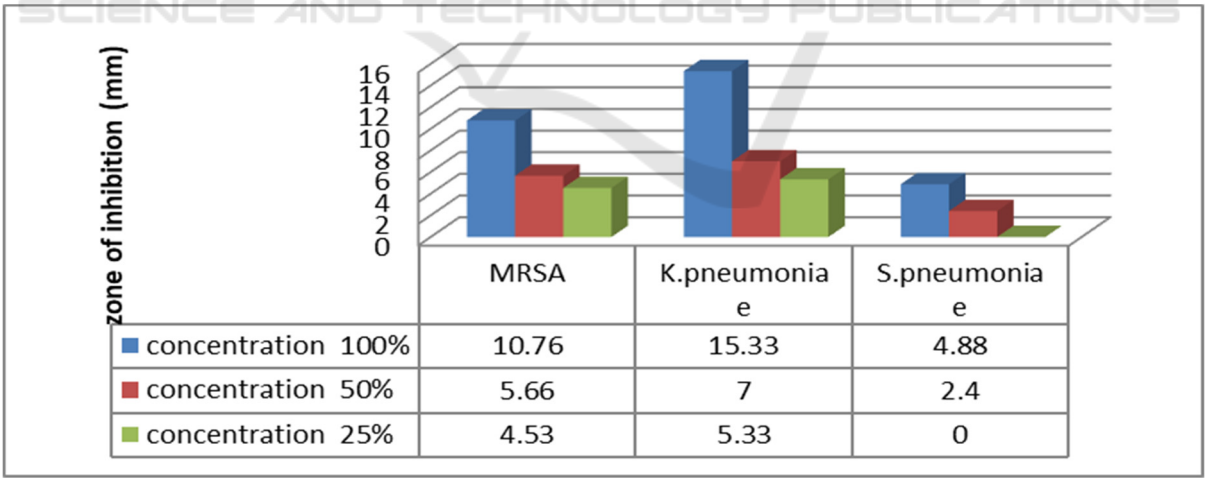
The statistical tool one-way ANOVA showed that the antibacterial activity of the fruit extracts is significant when compared with positive control with the P value ($p < 0.05$) against MRSA, *S. pneumoniae* and *K. pneumoniae*.

MIC results showed at 50mg/ml until 0.05 mg/ml against MRSA, while the MIC is positive in all concentration for inhibition against *Klebsiella pneumoniae* but the MIC result is negative in all concentration against *Streptococcus pneumoniae*.

The MBC result showed that there is bacterial growth in 500mg/ml of extract against MRSA while no bacterial growth in the lowest concentration of extract against *Klebsiella pneumoniae*. The bacterial growth is positive in the lowest concentration of extract against the *Streptococcus pneumoniae*.

The antibacterial activity of Asam Gelugor fruit extract which was shown in this study is attributed to the presence of the different active constituents of the plant. (Zakaria, 2011).

Table 1: Zone of inhibition vs bacterial species with different concentration of *G. Atroviridis* plant extract



	MRSA	K.pneumoniae	S.pneumoniae
concentration 100%	10.76	15.33	4.88
concentration 50%	5.66	7	2.4
concentration 25%	4.53	5.33	0

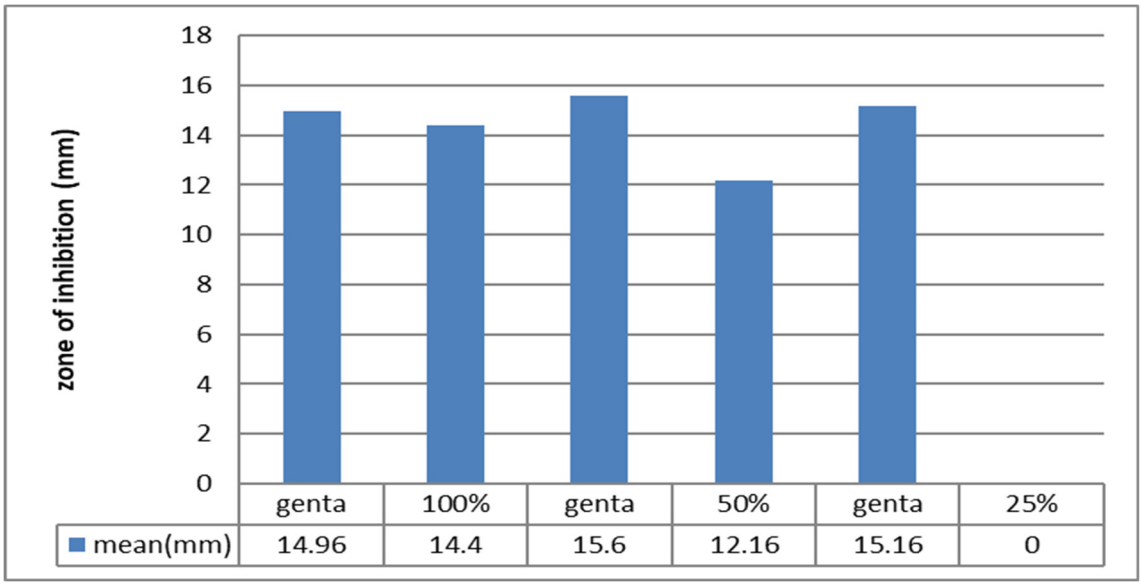


Figure 1: Comparison of zone of inhibition of plant extract vs gentamicin in *Klebsiella pneumoniae*

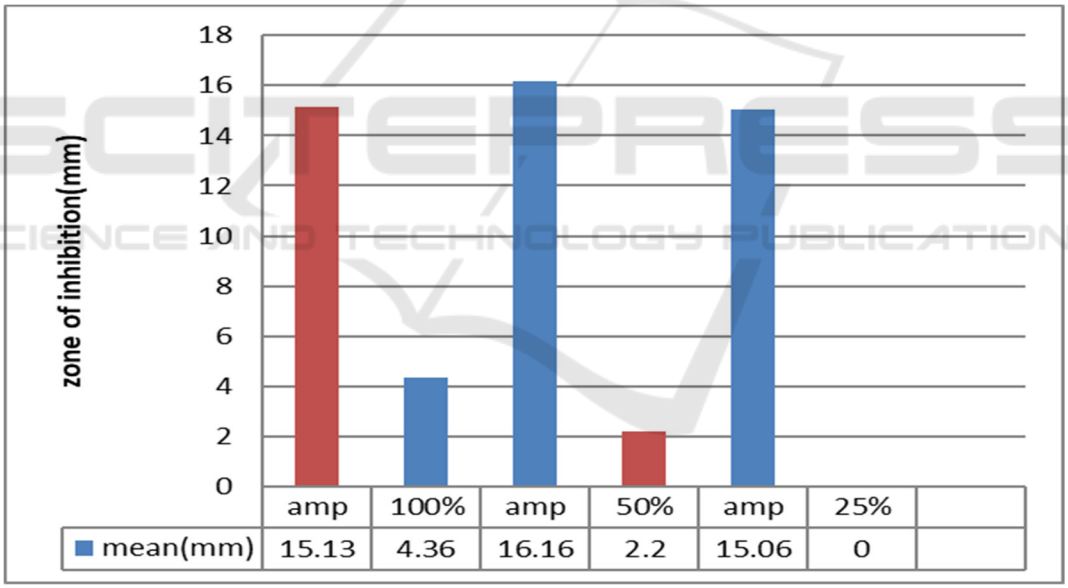


Figure 2: Comparison of zone of inhibition of plant extract vs ampicillin in *Streptococcus pneumoniae*

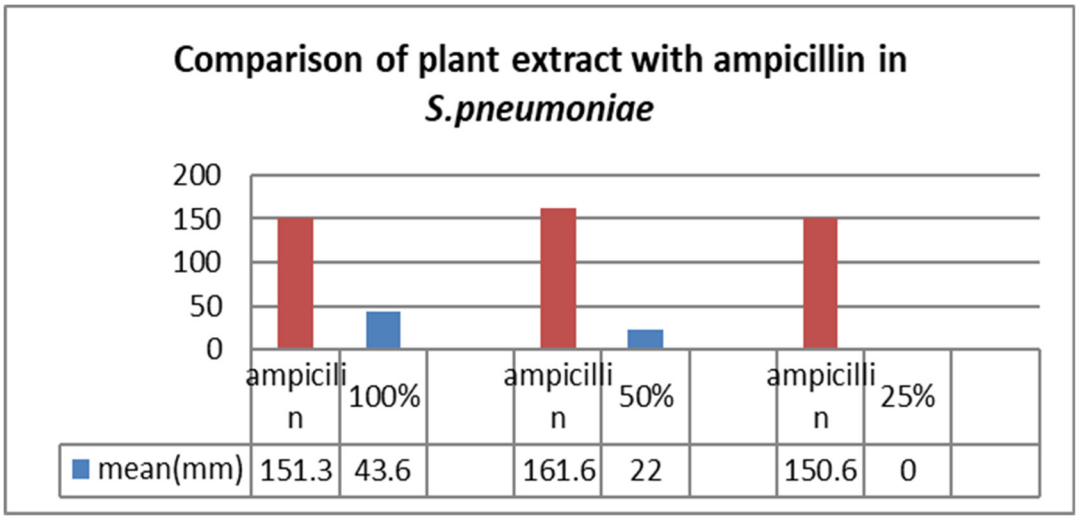


Figure 3: Comparison of zone of inhibition of plant extract vs ampicillin in *Streptococcus pneumoniae*

Table 2: Summary of *G. Atrovidis* plant extract zone of inhibition against the 3 strains of bacteria

BACTERIA	MRSA	K.PNEUMONIAE	S.PNEUMONIAE	SIG
CONCENTRATION	MEAN (mm) ± SD			
100%	10.76 ± 4.1	15.33 ± 1.53	4.8 ± 0.44	0.05
50%	5.66 ± 1.57	7.00 ± 1.00	2.43 ± 0.40	0
25%	4.53 ± 0.50	5.33 ± 0.58	0	0

4 CONCLUSION

The in-vitro antibacterial study of *Garcinia Atrovidis* against the selected gram-negative and gram-positive organisms conclude that *Garcinia Atrovidis* extract strongly inhibit the growth of *Klebsiella pneumoniae* as well as the standard antibiotic (Gentamicin). The plant extract has the potential to treat infection caused by *Klebsiella pneumoniae.*

ACKNOWLEDGMENT

The author is very grateful and thankful to the research committee and lecturers of Management and Science University, Malaysia for providing all the needed equipment and materials as well as the continuous guidance and support until the completion of this research project.

REFERENCES

Amran A A, Zaiton Z, Faizah O, Morat P, (2009), Effects of Garcinia atroviridis on serum profiles and atherosclerotic lesions in the aorta of guinea pigs fed a high cholesterol diet, 2009; 50 (3) : 295

Awatif Al-Judaibi, Ashwag Al-Zahrani, Khadijah A. Altammar, Salmah Binti Ismail and Nadia T. Darweesh,(2014), Comparative Study Of Antibacterial Activity Of Plant Extracts From Several Regions Of Asia, 139-147, 2014

Sawsan T. Abu Zied and Somaia A. L. Eissa ,(2000) Comparative Study On Antibacterial Activities Of Two Natural Plants Versus Three different intracanal Medications.

Gislene G. F. Nascimento; Juliana Locatelli, Paulo C. Freitas,Giuliana L. Silva,(2000), Antibacterial Activity Of Plant Extracts And Phytochemicals On Antibiotic Resistant Bacteria, 1678-4405

Nayan R. Bhalodia and V. J. Shukla,(201), Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant, 104–109.

Fuursted, Kurt(1997), Comparative Study of Bactericidal Activities, Postantibiotic Effects, and Effects on Bacterial Virulence of Penicillin G and Six Macrolides against Streptococcus pneumoniae, p. 781–784, 0066-4804/97

Liñares, J., Ardanuy, C., Pallares, R. and Fenoll, A. (2010), Changes in antimicrobial resistance, serotypes and genotypes in Streptococcus pneumoniae over a 30year period. Clinical Microbiology and Infection, 16: 402–410.doi 10.1111/j.14690691.2010.03182.x

Z. A. Zakaria,(2011), Antimicrobial activity of the aqueous extract of selected Malaysian herbs, pp. 5379-5383, DOI: 10.5897/AJMR11.874.

Todar's online textbook of bacteriology.

William Charles Evans, Daphne Evans, George Edward Trease (2009), Trease and Evans Pharmacognosy, Saunders/Elsevier Ltd.

Sana Mukhtar and Ifra Ghori,(2012), Antibacterial Activity Of Aqueous And Ethanolic Extracts Of Garlic,Cinnamon And Turmeric Against Escherichia Coli ATCC 25922 And Bacillus Subtilis DSM 3256, 0976-4550

Gislene G. F. Nascimento,Juliana Locatelli,Paulo C. Freitas, Giuliana L. Silva, (2000).

Kumar A , Tantry BA, Rahiman S, Gupta U,(2011), Comparative study of antimicrobial activity and phytochemical analysis of methanolic and aqueous extracts of the fruit of Emblica officinalis against pathogenic bacteria, Sep;31(3):24650.

Marcos J. Salvador; Paulo S. Pereira; Suzelei C. França; Regina C. Candido3\; Izabel Y. Ito; Diones A. Dias,(2003), Comparative study of antibacterial and antifugal activity of callus culture and adult plants extracts from Alternanthera Maritima (Amaranthaceae) 34:131-136.

Satyanshu Kumar, Shelly Sharma, Sunil Kumar Chattopadhyay,(2013), The potential health benefit of polyisoprenylated benzophenones from Garcinia and related genera: Ethnobotanical and therapeutic importance, Volume 89, September 2013, Pages 86–125

Ibrahim Jantan,(2004), Medicinal Plant Research in Malaysia: Scientific Interests and Advances, Jurnal Sains Kesihatan Malaysia 2(2) 2004: 27-46.

HABIBAH ZAINAL ABIDIN, June 2005, The Evaluation, Production, And Derivatisation Of Garcinia Acid In Garcinia Atroviridis.

Permana, Dharma; Lajisa, Nordin Hj.; Shaaria, Khozirah; Alib, Abdul M.; Mackeenb,

Mukram M.; Kitajimac, Mariko; Takayamac, Hiromitsu and Aimic, Norio.: A new prenylated hydroquinone from the roots of Garcinia atroviridis Griff ex T. Anders (Guttiferae). Z. Naturforsch. 58bb, 332D335 (2003).

Tisdale, Eric J.; Kochman, David A. and Theodorakis, Emmanuel A.: Total synthesis of atroviridin. Tetrahedron Letters 44 (2003) 3281–3284 Pergamon.

Achmadi, S.S. Hayati (Indonesia). (2001), Institut Pertanian Bogor (Indonesia). Fakultas Matematika dan Ilmu Pengetahuan Alam. The potency of potassium hydroxycitrate derived from gelugur fruit (Garcinia atroviridis) in reducing body weight and cholesterol levels in rats, v. 8(1) p. 23-26

Jena, B. S.; Jayaprakasha, G. K.; Singh, R. P.; Sakariah, K. K.: Chemistry and biochemistry of (-)-hydroxycitric acid from Garcinia. Journal of Agricultural and Food Chemistry 2002, 50, 1, p.10-22.

Shahab Qureshi, MD; Chief Editor: Michael Stuart Bronze,(2015), Klebsiella Infections Treatment & Management http://emedicine.medscape.com/article/219907treatment

Shahab Qureshi, MD; Chief Editor: Michael Stuart Bronze,(2015), Klebsiella Infections, http://emedicine.medscape.com/article/219907overview.

R. Podschun and U. Ullmann,(1998), Klebsiella spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors, Clin Microbiol Rev. 1998 Oct; 11(4): 589–603.

Mohammed Rahmatullah, Md. Nur Kabidul Azam, Md. Mizanur Rahman, Syeda Seraj, Mostafi Jumrut Mahal, Sadia Moin Mou, Dilruba Nasrin, Zubaida Khatun, Farhana Islam, Majeedul H. Chowdhury,(2011) A Survey of Medicinal Plants Used by Garo and Non-Garo Traditional Medicinal Practitioners in Two Villages of Tangail District, Bangladesh, American-Eurasian Journal of Sustainable Agriculture, 5(3): 350-357, 2011 ISSN 1995-0748.

Jan van de Kassteelea, Marga G. van Santen-Verheuvelb, Femke D. H. Koedijkc, Alje P. van Damd,e, Marianne A. B. van der Sandec,f and Albert J. de Neeling, (2011), New Statistical Technique for Analyzing MIC Based Susceptibility Data, Antimicrob. Agents Chemother. March 2012 vol. 56 no. 3 1557-1563.

Centers for Disease Control,The American Academy of Family Physicians. apriotti, T. Dermatology Nursing, Jan. 26, 2004.

Johnson, L. Infections in Medicine, 2005.

WebMD Feature: "Drug-Resistant Staph Spreads Across U.S.

Nahida Tabassum and Mariya Hamdani, Plants used to treat skin diseases, Pharmacogn Rev. 2014 JanJun; 8(15): 52–60. doi: 10.4103/09737847.125531.

Dr. Khalid Gadd, (2013), Ensiklopedia Pengubatan Herba, Terapi Alternatif Kedoktoran Islam, Al Hidayah House of Publishers Sdn.Bhd.