International Journal of Allied Practice, Research and Review
Website: www.ijaprr.com (ISSN 2350-1294)

Antibacterial Activity of *Cananga odorata* Flower Extracts against *Streptococcus pneumoniae* and *Klebsiella pneumoniae*

Kogila Vijayan[1], Anita Gnana Kumari [1]and Anbu Jeba Sunilson [2]
[1]School of Pharmacy, KPJ Healthcare University College, Nilai , Malaysia
[2] Department of Siddha Medicine,Faculty of Science,Tamil University,Thanjavur, India

Abstract - Microbial infections are continuously occurring in Malaysia where the risk of such infectious disease is observed to be high. Many generations of antibiotic classes such as Aminoglycosides, Cephalosporins, Carbapenem and Penicillin were established to fight against microbial infections yet they have become less effective due to emergence of drug resistance bacteria such as *Klebsiella pneumoniae* and *Streptococcus pneumoniae.* Recently, medicinal plant such as *Cananga odorata* or known as Ylang-ylang has been reported as an alternative approach to treat bacterial infections. It may due to some phytochemicals present in the plant such as amino acids, flavonoids, triterpenoids, tannins, phenols, volatile oils and resins and others. The aim of this current study was to evaluate the antibacterial activity of *Cananga odorata* flower extracts against pneumonia causing pathogens such as *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. Methanol and aqueous flower extracts of *Cananga odorata* were used for its anti-bacterial activity against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* by using disk diffusion method in which Ceftriaxone (30µg) was used as standard. The result which were analysed by ANOVA showed that methanol flower extract possessed maximum anti-bacterial activity against *Streptococcus pneumoniae* (30±0.440) while aqueous flower extract displayed maximum activity against *Klebsiella pneumoniae* (27±0.371). On the other hand, qualitative phytochemical screening of different flower extracts of *Cananga odorata* revealed the presence alkaloids, amino acids, carbohydrates, flavones, terpenoids, tannins, polyphenols and phytosterols in aqueous extract and alkaloids, amino acids, carbohydrates, flavonoids, flavones, terpenoids, tannins, phytosterols and polyphenols in methanol extract. Hence, *Cananga odorata* flower exhibit a great potential as an antibacterial agent and can be subjected for the treatment of pneumonial infections and various medicinal purpose.

Keywords : Antibacterial activity, *Cananga odorata*, Drug resistance, Ceftriaxone, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

I. Introduction

Pneumonia is a type of Acute Respiratory Tract Infection (ARTI) involving alveoli or air sacs of the lung. In most part of the world, it is known to be the leading cause of mortality in children below five than other major diseases such as AIDS, measles or malaria. Pneumonia is also marked as a common and leading cause of hospitalization and death worldwide [1]. It may be possibly caused by bacteria, viruses or fungi, yet the high rate of pneumococcal illness marked by bacteria. *Streptococcus pneumoniae* and *Klebsiella*

pneumoniae are common pneumonia causing bacteria. *Streptococcus pneumoniae* is a Gram-positive, facultative anaerobic, belongs to genus *streptococcus*, a cell which is lancet in shape. It is a leading cause of invasive bacterial disease in children and the elderly [2]. *Klebsiella pneumoniae* is a Gram-negative, facultative anaerobic, belongs to genus *Klebsiella*, a rod-shaped cell. It is an opportunistic pathogen that can lead to a wide range of infections such as pneumonias, bacteremias, septicaemia, urinary tract infections, liver abscesses and soft tissue infections in weak individuals. It is a Carbapenemase-producing Enterobacteriaceae that has increasing resistance towards Carbapenem antibacterial agents [3].

The treatments for bacterial pneumonia are mainly depend on synthetic drugs such as antibiotics. Unfortunately, antibiotics can cause unwanted effect on host such as sensitivity problems, killing of good gut and mucosal microbiota, produce allergic response and weaken immune system by supressing it [4]. Microorganisms may develop resistance due to non-selective or random use of an anti-bacterial agents[5]. Hence, alternative treatment is needed to treat those infectious diseases with milder or no side effects.

Since ancient times, natural products have been the backbone of medicinal field of healing and have also been an important part of history and culture. It has been proved that natural products played important roles in modern drug development, principally for antibacterial and antitumor agents. *Cananga odorata* is one of the medicinal plant which is commonly known as ylang-ylang [6]. It is very popular for its essential oil which has been widely used in perfume industry, food and supplement industry, cosmetic industry, massage and aroma therapy [7]. This plant also has many ethno medicinal uses and has been used as traditional medicine by people around the world [8]. Many studies have reported *that Cananga odorata* plant possess variety of pharmacological activities and secondary metabolites which are biologically active compounds. *Cananga odorata* plant reviewed to have potential pharmacological activities such as anti-microbial, anti-fungal, anti-protozoal, antioxidant, anticancer, anti-inflammatory, anti-diabetic and others [9]. In view of these aspects, the present *in-vitro* study was undertaken to evaluate the antibacterial activity of *Cananga odorata* flower extracts against the pneumonia causing pathogens (*Streptococcus pneumonia* and *Klebsiella pneumoniae).*

II. Materials and Methods

Plant materials

The fresh whole flowers of *Cananga odorata* (1.5kg) were obtained from Cyberjaya in month of December 2017. The flowers were authenticated by Biodiversity Unit of Institute of Bioscience, UPM. The flowers were rinsed with clean water to remove impurities and dried under sunlight. The dried flowers were size reduced into coarse powder using blender and stored in airtight bottle.

Preparation of extracts

The size reduced *Cananga odorata* flowers (60 grams) was equally parted into two portions and transferred in a 500ml bottle separately. Cold maceration was used to carry out the extraction. 150ml methanol and aqueous solvent was added in each bottle. 10ml of chloroform was added into the bottle containing aqueous reagent to prevent microbial growth. The containers were properly closed and allowed to stand for at least 7 days with frequent shaking at room temperature. After 7 days, plant extracts were filtered using Whatmann sterile filter paper. The filtered methanol and aqueous extracts were concentrated using heating mantle at 50°C and 80°C respectively. The extracts were transferred into sterile bottles and stored in the refrigerator until further use [10].

Collection of microbial stain

The microbial strains, *Streptococcus pneumoniae* (Gram-positive) and *Klebsiella pneumoniae* (Gram-negative) were obtained from Pusat Perubatan Universiti Kebangsaan Malaysia (HUKM).

Preliminary Phytochemical Screening on *Cananga odorata* Flower Extracts

Phytochemical analysis was done to identify the presence of phytoconstituents using standard methods.

***In-vitro* antimicrobial screening for extracts against tested pathogens**

Culture media

Muller Hinton (MH) agar media was used for antibacterial activity [11].

Preparation of subculture

The stock culture of each stain was stored in blood agar and Macconkey agar at 4°C. To maintain the stock culture, every week bacterial culture was sub-cultured to blood agar. The bacterial strain was incubated for 24 hours [12].

Preparation of Inoculum

A loop full of organism was taken from the stock culture and streaked on nutrient agar slant. The agar slant containing organism was incubated at 30-35°C for 24-48 hours [13].

Preparation of Standard

Antibiotic discs of Ceftriaxone 30µg were used as standard (positive control) to compare the antibacterial activity of flower extracts. Ceftriaxone is an antibiotic which is effective against both *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. The antibiotic discs of Ceftriaxone 30µg were prepared manually using standard method [14].

Preparation of Filter Paper Discs

Antibiotic discs of 6mm diameter were prepared using Whatmann No.1 filter papers. The discs obtained with appropriate shape were autoclaved for 121°C for 15minutes.

Preparation of Antibiotic Stock Solution (30µg) and Test Extracts (1000µ)

To obtain the stock solution, a known amount of antibiotic powder was dissolved in sterile distilled water. The working solution was prepared from stock solution by dilution at the time of disc preparation. A 6mm diameter paper disc able to absorb 0.02ml or 20µl of solution. The concentrations were expressed in µg/µl. Same method as preparation of antibiotic discs was applied to produce discs of extracts. The stock solution for extracts was prepared in higher concentration than antibiotic strength [14].

Impregnation of Discs

Sterile discs were impregnated with Ceftriaxone antibiotic solution (30µg), test extracts (1000µg) and distilled water (negative control). A fixed volume of 20µl was loaded on each disc using a mechanical pipette.

Drying and Storage

Loaded discs were placed in an incubator and allowed to dry at 37°C for 4 hours. After drying, the discs were transferred into a sterile air-tight container with desiccant and stored in freezer at -20°C. The discs were removed from the freezer one or two hours before its usage [15].

Preparation of plates

Commercially available dehydrated Mueller Hinton agar (MHA) was used to make the media for pneumonia susceptibility testing. 38gm of agar were suspended in one litre of distilled water, heated with frequent agitation and boiled for 1 minute to fully dissolve the medium. The mixture was autoclaved at 121°C for 15 minutes and then cooled to 45°C to 50°C. After cooling, agar was poured into petri dishes uniformly and allowed to solidify and dry. The final pH was adjusted to 7.3 ± 0.1 at 25ºC. Prepared plates were kept in sterile plastic bags and stored at 4ºC until use [16].

Evaluation of *in-vitro* antimicrobial activity

Antibacterial Assay

Disk diffusion method was used for the antibacterial testing. Muller Hinton agar media plate was labelled with name of organisms and date. A loop full of organism was taken using sterile loop and inoculated into Muller Hinton Agar Media using aseptic technique. The discs of *Cananga odorata* flower extract (methanol 1000µg and aqueous 1000µg), standard antibiotic disc (Ceftriaxone 30µg) and negative control (distilled water) were placed on MH agar plate using sterile forceps. The plates were incubated for 30-35°C for 24-48 hours. The area of inhibition was observed and measured [17].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of Aqueous and Methanolic Extracts of *Cananga odorata* Flowers

Minimum inhibitory concentration was determined using broth dilution method. The extract was serially diluted with 1 ml of nutrient broth to give concentrations (1000, 500, 250, 125, 62.5, 31.25 µg/µl). Each test tube containing plant extract was inoculated with 1ml of bacterial strain. The tubes were incubated at 35-37°C for 18-20 hours and the results were observed. The minimum concentration that does not have any microbial growth was selected as MIC. To determine the MBC, from the chosen test tubes in the MIC determination, a loopful of broth was collected from those test tubes that did not show any growth and inoculated onto sterile MH agar plate by streaking. All the plates were incubated at 37°C at 24 hours. The concentration with no visible growth of pathogen was identified as minimum bactericidal concentration [18].

Statistical Analysis

The data was expressed as mean value ± standard error of the mean (SEM). The assessment for antibacterial assay and MIC studies were performed in triplicate. The data was subjected to one way analysis of variance (AVOVA) using Dunnett 'T' test and p values < 0.05 was considered as significant [19].

III. Results

Qualitative phytochemical screening shows that the aqueous and methanol flower extracts composed of bioactive compounds as shown in Table no. 1. Aqueous extracts indicated presence of alkaloids, amino acids, carbohydrates, flavones, terpenoids, tannins, polyphenols and phytosterols. Meanwhile,

methanol extract displayed alkaloids, amino acids, carbohydrates, flavonoids, flavones, terpenoids, tannins, phytosterols and polyphenols.

Table no. 1 : Phytoconstituents present in flower extracts of *Cananga odorata*

S.No	Phytoconstituents	Aqueous extract	Methanol extract
1	Alkaloids	+	+
2	Amino acids	+	+
3	Carbohydrates	+	+
4	Fats	+	+
5	Flavonoids	-	+
6	Flavones	+	+
7	Terpenoids	+	+
8	Tannins	+	+
9	Steroids	-	-
10	Phytosterols	+	+
11	Saponins	-	-
12	Poly phenols	+	+
13	Oil/Resin	+	+

+ indicates presence of phytoconstituents
- indicates absence of phytoconstituents

The anti-microbial activities of aqueous and methanol flower extracts of *Cananga odorata* were summarized in Table no. 2, Figure 1 (a), Figure 1 (b).The results indicated that aqueous extract exerted maximum activity against *Klebsiella pneumoniae* while methanol extract displayed maximum activity towards *Streptococcus pneumoniae* when compared to standard.

Table no. 2 : Anti-Microbial Activity of Plant Extracts against tested organisms

Test organism	Inhibition Zone (mm)		
	Standard, Ceftriaxone (30µg/µl)	Aqueous Extract (1000µg/µl)	Methanol extract (1000µg/µl)
Klebsiella pneumoniae	33±0.333***	27±0.371***	-
Streptococcus pneumoniae	33±0.289***	-	30±0.440***

Results presented here are the mean ± SEM value of n=3; ***p< 0.001= highly significant, **p< 0.01= significant and *p< 0.05= less significant

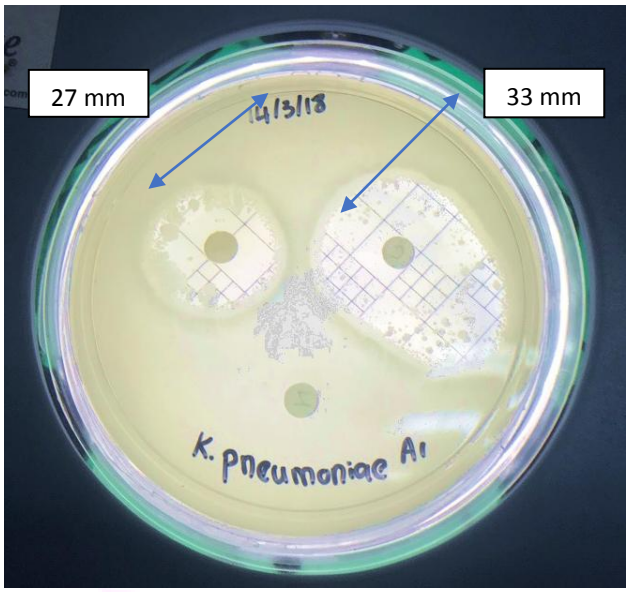

Figure 1 (a) : Zone of inhibition of Ceftriaxone and Aqueous extracts of *Cananga odorata* flower against *Klebsiella pneumoniae.*

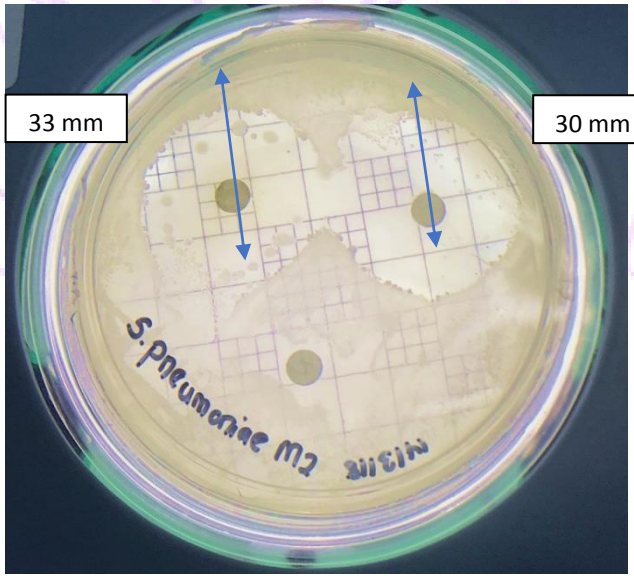

Figure 1 (b) : Zone of inhibition of Ceftriaxone and Methanol extracts of *Cananga odorata* flower against *Streptococcus pneumoniae.*

Minimum Inhibitory Concentration (MIC) of aqueous and methanol flower extracts of *Cananga odorata* were determined by dilution method. The MIC values were summarized in Table no. 3 and Table no.4. Both aqueous and methanol extracts showed inhibitory effect on tested organisms at low concentration of 125µg/µl – 1000µg/µl.

Table no.3 : Minimum Inhibitory Concentration (MIC) Activity of Aqueous Flower Extract of *Cananga odorata* on Test Organisms

Test organism	Concentration of Aqueous Extract (µg/µl)					
	1000	500	250	125	62.5	31.25
Klebsiella pneumoniae	-	-	-	-	+	+
Streptococcus pneumoniae	+	+	+	+	+	+

+ = visible growth ; - = no visible growth

Table no.4 : Minimum Inhibitory Concentration (MIC) Activity of Methanol Flower Extract of *Cananga odorata* on Test Organisms

Test organism	Concentration of Methanol Extract (µg/µl)					
	1000	500	250	125	62.5	31.25
Klebsiella pneumoniae	+	+	+	+	+	+
Streptococcus pneumoniae	-	-	-	-	+	+

Minimum Bactericidal Concentration (MBC) of aqueous and methanol flower extracts of *Cananga odorata* were determined by plate method. The bactericidal effect of aqueous extract on *Klebsiella pneumoniae* and *Streptococcus pneumoniae* was at low concentrations of 250µg/µl and 250µg/µl, respectively. The activity was summarized in Table no. 5 and Table no.6.

Table no.5 : Minimum Bactericidal Concentration (MBC) Activity of Aqueous Flower Extract of *Cananga odorata* on Test Organisms

Test organism	Concentration of Aqueous Extract (µg/µl)			
	1000	500	250	125
Klebsiella pneumoniae	-	-	-	+

+ = visible growth ; - = no visible growth

Table no.6 : Minimum Bactericidal Concentration (MBC) Activity of Methanol Flower Extract of *Cananga odorata* on Test Organisms

Test organism	Concentration of Methanol Extract (µg/µl)			
	1000	500	250	125
Streptococcus pneumoniae	-	-	-	+

IV. Discussion

Pneumonia is marked as a common and leading cause of hospitalization and death worldwide. The causative agents *Klebsiella pneumoniae* and *Streptococcus pneumoniae* are also act as the source of antibiotic resistance that have lead the need for alternative treatments. The present study was conducted to determine anti-bacterial activity of aqueous and methanol flower extracts of *Cananga odorata* on pneumonia causing organisms. Although this medicinal plant has been utilized traditionally as herbal treatments, there has been very few studies regarding its effect on pneumonia causing pathogens.

Based on the antibacterial assay, aqueous and methanol flower extracts of *C.odorata* exhibited significant anti-bacterial activity against the test pathogens. Aqueous flower extract (1000µg/µl) showed good activity on *Klebsiella pneumoniae* and methanol flower extract (1000µg/µl) displayed good activity on *Streptococcus pneumoniae* compared to the standard antibiotic, Ceftriaxone (30µg/µl). Gram-negative organism was more susceptible towards aqueous extract; while, Gram-positive organism was more susceptible towards methanol flower extract of *C.odorata*. The higher susceptibility of a particular group of bacteria to a particular solvent can be due to the difference in their cell wall structure and composition.
Gram-negative bacteria consist of a complex outer membrane rich in lipopolysaccharide which limiting the entry of hydrophobic compounds through it. Whereas, lipopolysaccharide is absent in Gram-positive bacteria which instead are composed of a thick peptidoglycan wall that not strong enough to resist small antimicrobial molecules into the cell membrane. In additional, due to the lipophilic ends of lipoteichoic acid present in cell membrane, diffusion of hydrophobic compounds will be easy in Gram-positive bacteria. Presence of secondary metabolites such as alkaloids, amino acids, flavonoids, triterpenoids, tannins, phytosterols, resins and others, in both aqueous and methanol extracts might be bound for anti-bacterial activity of the plant [20][21].

Although, the precise active principles for the high sensitivity observed need to be disclosed through further studies, it could be opinionated that, the antibacterial compounds in aqueous extract of *Cananga* flower may be of hydrophilic in nature which pass through the liposaccharide layer of Gram-negative cell wall leading to entry of the inhibitory molecules. While, the antimicrobial compounds in methanol extract may be of hydrophobic in nature made the Gram-positive attributed more susceptibility towards organic solvent. Even though methanol is more towards polar, it has the ability to extract hydrophobic compounds in low concentrations. Natural products, either as a pure compound or as a standardized plant extracts give diverse chances for new drug development due to the unrivalled availability of chemical diversity.

V. Conclusion

The findings concluded the antibacterial activity of *Cananga odorata* flower against the tested pneumonia causing pathogens and suggest that it can be subjected to study for various medicinal purposes. However, further study is required to isolate the antibacterial phytoconstituent(s) and predict the exact

mechanism of action of antibacterial activity of *Cananga odorata* flower as well as the safety and toxicity profile of *Cananga odorata* flower. It may lead the development of promising natural antibacterial agent for pneumonia from the selected plant, *Cananga odorata.*

VI. Acknowledgements

The authors would like to thank the Management, KPJ Healthcare University College, Kota Seriemas, Nilai, Malaysia, for their continuous encouragement and support.

VII. References

1) **Akhil et al. (2017). A Prospective Study on Prescribing Pattern of Antibiotics for Treating Pneumonia in Pediatrics in a Tertiary Care Hospital, Bangalore. World Journal of Pharmaceutical Research, 15 (6), 1227-1260.**

2) **Todar, K. (2012) Bacterial Resistance to Antibiotics. The Microbial World. Lectures in Microbiology, University of Wisconsin-Madison. www.textbookofbacteriology.net**

3) **Sanchez, G., Master, R., Clark, R., Fyyaz, M., Duvvuri, P., Ekta, G., & Bordon, J. (2013). Klebsiella pneumoniaeAntimicrobial Drug Resistance, United States, 1998–2010. Emerging Infectious Diseases, 19(1), 133-136.**

4) **Punopas, K., Eumkeb, G., Chitsomboon, B.Nakkiew, P.(2004). Study of antibacterial activity of some medicinal plants in Lamiaceae family. INTERNATIONAL INFORMATION SYSTEM FOR THE AGRICULTURAL SCIENCE AND TECHNOLOGY**

5) **Stephen T Odonkor, Kennedy K Addo (2011) Bacteria resistance to antibiotics: Recent trends and challenges. Int J Biol Med Res. 2(4); 1204-1210.**

6) **Parrotta, John A. 2009. Cananga odorata. In: Enzyklopädie der Holzgewächse [Encyclopaedia of Woody Plants]. Wiley-VCH, Germany.**

7) **Nazanin Saedi, BA; Glen H. Crawford, MD. (2005). Botanical Briefs: Ylang-Ylang Oil— Extracts From the Tree Cananga odorata: CLOSE ENCOUNTERS WITH THE ENVIRONMENT. (7). Page 149-150.**

8) **Elevitch, C.R., and H.I. Manner. (2006). Artocarpus heterophyllus (jackfruit), ver. 1.1v. In: Elevitch, C.R. (ed.). Species Profiles for Pacific Island Agroforestry. Permanent Agriculture Resources (PAR), Hōlualoa, Hawai'i. <http://www.traditionaltree.org>**

9) **Tan, L., Lee, L., Yin, W., Chan, C., Abdul Kadir, H., Chan, K., & Goh, B. (2015). Traditional Uses, Phytochemistry, and Bioactivities ofCananga odorata(Ylang-Ylang). Evidence-Based Complementary and Alternative Medicine, 1-30.**

10) **Bhumika D, Bijal AF, Antibacterial Activity and Phytochemical Screening of Different Parts of *Moringa oleifera* against Selected Gram Positive and Gram Negative Bacteria, *Journal of Pharmaceutical, Chemical and Biological Sciences*, 2015; 3: 421-425.**

11) **Hewitt W, Vincent S. Theory and application of microbiological assay. Academic Press; London; 1989.**

12) **Mackie, McCartney. Practical medical microbiology. 14th Edition. Churchill Livingstone; New York; 1996.**

13) **Pelezar MJ, Chan ECS, Krieg NR. Microbiology. Tata McGraw-Hill Publishing Company Limited; New Delhi; 1993.**

14) **MK Lalitha. (2004). Manual on Antimicrobial Susceptibility Testing. Retrieved 20 March 2018, from http://www.ijmm.org/documents/Antimicrobial.doc.**

15) **Vineetha et al. (2015). Preparation, Standardization of Antibiotic Discs and Study of Resistance Pattern for First-Line Antibiotics in Isolates from Clinical Samples. International Journal of Applied Research, 1 (11), 624-631.**

16) **Ahmed I, Mehmood Z, Mohammad F. (1998). Screening of Some Indian Medicinal Plants for their Antimicrobial Properties, Journal of Ethnopharmacology, 62: 183-193.**

17) **Alagumaruthanayagam et al. (2009). Evaluation of solid (disc diffusion)- and liquid (turbidity)-phase antibiogram methods for clinical isolates of diarrheagenic E. coli and correlation with efflux. The Journal of Antibiotics, 62(7), 377-384.**

18) **Acharya, T. (2017). Minimum Inhibitory concentration (MIC): Broth dilution method-procedure and interpretation - microbeonline. microbeonline. Retrieved 18 May 2017, from http://microbeonline.com/minimum-inhibitory-concentration-mic-broth-dilution-method-procedure-interpretation/**

19) **Rashid et al. (2017). Characterization of phytoconstituents and evaluation of antimicrobial activity of silverextract nanoparticles synthesized from Momordica charantia fruit extract. BMC Complementary and Alternative Medicine, 17:336. DOI 10.1186/s12906-017-1843-8.**

20) **K.L. Compean and R.A. Ynalvez. (2014). Antimicrobial Activity of Plant Secondary Metabolites: A Review. Research Journal of Medicinal Plants, 8: 204-213.**

21) **Murugan, T., Wins, J. A., & Murugan, M. (2013). Antimicrobial Activity and Phytochemical Constituents of Leaf Extracts of Cassia auriculata. Indian Journal of Pharmaceutical Sciences, 75(1), 122–125.**