

Antibacterial activities of plant extract *Cinnamomum zeylanicum* bark against multidrug-resistant bacteria

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ABSTRACT:

Background : Due to the increased occurrence of bacterial resistance to multiple antimicrobial drugs, there is a constant search for novel therapeutic strategies; medicinal plants represent an important source for obtaining such substances. The antimicrobial activity of extracts and essential oils of medicinal plants has been proven in several studies conducted in countries with diverse flora.

Aim : To evaluate the antibacterial activity of cinnamon against multidrug- resistant bacteria.

Material and Method: Bacterial susceptibility testing was conducted by the diffusion method on Mueller-Hinton agar using paper discs impregnated with test substances.

Results: All test strains were sensitive to the essential oil of cinnamon. We observed low synergistic potential between essential oils against the strain of *S. aureus*.

Conclusion: The essential oil of cinnamon has antibacterial activity against both Gram-positive and Gram-negative strains. Future research should be conducted to define the best concentration and better extraction solution for mastic and cinnamon peels.

Keywords: Antibacterial activity, *Cinnamomum zeylanicum*, Aqueous extract, Ethanolic extract.

المدخل : بسبب الزيادة لمقاومة البكتريا للعديد من الادوية المضادة للميكروبات، هناك استمرارية للبحث عن استراتيجيات علاجية جديدة . تعتبر النباتات الطبية مصدرا مهما للحصول على مثل هذه المواد. تم اثبات النشاط المضاد للميكروبات من المستخلصات والزيوت الاساسية للنباتات الطبية في العديد من الدراسات التي تجرى في البلدان الحافية على النباتات المتنوعة. **الهدف :** لتقييم الفعالية المضادة للبكتريا المقاومة للأدوية المتعددة. **المواد وطرائق العمل:** تم اجراء اختبار الحساسية البكتيرية بطريقة الانتشار على الاكار مولر-هينتون باستخدام اقراص مرقية مشبعة بمواد الاختبار. **النتائج :** جميع سلالات الاختبار كانت حساسة للزيت العطري للقرفة. لاحظنا انخفاض امكانية التأزر للزيوت الاساسية ضد *S. aureus* سلالة. **المخلص :** أن الزيت العطري للقرفة نشاط مضاد ضد السلالات الموجبة الكرام والسالبة الكرام . يجب اجراء بحث مستقبلي لتحديد افضل تركيز وافضل محلول استخلاص لقسور المستكي والقرفة. **الكلمات المفتاحية :** مضاد لفعالية البكتريا ، القرفة ، مستخلص مائي ، مستخلص الايثانول .

INTRODUCTION:

Bacterial resistance to antimicrobial multi-drugs is one of the most serious health problems in the world today, and it is becoming increasingly difficult to deal with, especially in the case of hospital infections caused by this microorganism, which can hardly be cured effectively [1].

Resistance to antimicrobial agents occurs due to a genetic phenomenon related to changing genes in microorganisms, which encodes various biochemical mechanisms that prevent the action of drugs. Secondary metabolic products accumulated by plants can stimulate the antibacterial activity of antibiotics whose effect is limited by multi-drug resistance mechanisms and thus improve the host's immune system response against infection [2,3].

On the other hand, over the past decades, there has been increasing evidence that some plants are rich sources of various classes of antimicrobial substances that act as defense systems, protecting them from both biotic and abiotic stresses. Some of these secondary metabolites, like alkaloids, polyphenols, polypeptides, and lectins, have been proven as effective antimicrobial agents. Also, these metabolites are also approved as safe materials for use in food products, with few side effects [4,5,6].

One of the most effective plants used in traditional medicine is cinnamon, due to its rich supply of natural, biologically active substances and its curative effects without side effects. According to traditional Chinese medicine, which dates back almost 4,000 years, cinnamon has been used not

only as an antioxidant and flavoring agent but also as a treatment for many health problems and diseases such as coughs and colds, diabetes, inflammation, gastrointestinal problems, and urinary tract infections. Cinnamon also has proven antifungal, antibacterial, and antiparasitic activity [7,8,9].

Recent studies have shown that cinnamon has antimicrobial activity against some microorganisms and that, when used with antibiotics, it has a favorable potential against multi-resistant bacteria [10].

In A study by Mariri and Safi (2014) who conducted an *in vitro* study using 28 plant extracts, oils, and some antibiotics with a concentration of 5% against Gram-negative bacteria, reported that among the substances that showed positive results was *Cinnamomum zeylanicum* [11].

Most of the antimicrobial activity seems to be associated with phenolic compounds. The antimicrobial effect is mainly related to changes in the permeability and integrity of the bacterial cell membrane [12].

The objective of the present study was to evaluate the antibacterial activity of cinnamon leaf and bark extracts on the multidrug-resistant bacteria: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus spp*, *Streptococcus agalactiae*, *Listeria monocytogenes*, and *Klebsiella spp*.

Material and Methods:**Clinical bacterial collection and isolation**

In the present study, seven clinical bacterial isolates were used. Three of them were Gram- positive: *Streptococcus agalactiae*,

Staphylococcus aureus, *Listeria monocytogenes*, and four Gram-negative bacteria: *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas*. These samples were collected from the Duhok Specialized Laboratory Center, Duhok city, Kurdistan Region of Iraq, and subjected to proper identification and confirmation. The study was conducted in the College of Health Sciences, Microbiology Department, University of Duhok, Iraq.

Collection and Preparation of Plant extract

The *C. zeylanicum* (cinnamon) barks were purchased from a local supermarket in Duhok province of Iraq. A total of 200 g of cinnamon bark was washed and dried in an oven dryer at 40 °C for 48 h. The dried barks were then grounded into powder, stored in dark glass bottles, and kept at -20°C until further analysis.

The extraction was done according to Parekh and Chanda [13], in which 2.5gm of *C. zeylanicum* barks powder was soaked into 100 ml of ethanol in conical flasks for two weeks at room temperature with regular shaking. Then, it was filtered using Whatman No.1 filter paper and evaporated. The final concentrated extract is kept in dark bottles at 4 °C until used. The same procedure was carried out for aqueous extraction using distilled water as a solvent.

Gram Staining

This was employed in the identification of Gram-positive and Gram-negative microorganisms. A smear of the bacterial isolate is made on a clean slide and stained with gram staining reagents. Their gram

reaction and morphology were observed under the microscope using a x100 objective lens.

Preparation of Stock and Standard Concentrations of Extract

In this study, the crude and undiluted extract of *C. zeylanicum* assumed 100% (v/v) was used as the stock concentration. From the stock, several concentrations of 10, 20, 30, and 40% (v/v) were prepared by diluting with distilled water [14]

Antimicrobial Susceptibility Test

The plant extracts were tested for antibacterial activity using the agar well diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) [15]. All clinical samples in this study were sub-cultured for 24 hours at 37° C in 5% sheep blood agar plate (BAP), Mannitol Salt Agar, Mueller-Hinton Agar (MHA), and MacConkey Agar, respectively for 24h at 37° C. The colonies were inoculated with distilled water. The turbidity was then adjusted to equal the turbidity of the 0.5 McFarland standard, giving a final inoculum of 1.5 x 10⁸ CFU/ml.

Using a sterile cotton swab, 145 µl of inoculum gram negative-bacteria and 280 µl of test organism gram-positive bacteria were spread on Mueller-Hinton Agar plate. Wells of 7 mm in diameter were punched into the inoculated plates using a sterile cork borer and filled with 100 µl of each concentration of plant ethanol extract and allowed to diffuse at room temperature for one hour. The plates were then incubated at 37 °C for 16 to 24 hours. Wells containing the same

volume of each concentration of plant distilled water extract served the purpose of comparison between both of them.

At the same time a comparison antibiotic control test was conducted using commercial disks (Imipenem, Kanamycin, Chloramphenicol, Bacitracin and Tetracycline) of the test isolates based on susceptibility patterns, and the zones of inhibition measured by mm, and comparing

the inhibition zone diameter with the measured zones standardized by the CLSI (Clinical and Laboratory Standard Institute) and reported as Susceptible (S), Intermediate (MR), and Resistant (R).

After 24 h of incubation, each plate was properly examined and antibacterial activity was evaluated by measuring the diameters of zones of inhibition in mm using a ruler against the test organism.

Results:

In this study, the good diffusion method for antimicrobial susceptibility test was initially performed to determine the antibacterial activities of various concentrations of ethanolic and aqueous extracts of the *C. zeylanicum* plant against seven multi-drug resistant bacteria: *Streptococcus agalactiae*, *Staphylococcus aureus*, *Listeria*

monocytogenes, *Escherichia coli*, *Klebsiella spp*, *Proteus spp* and *Pseudomonas spp*.

The results showed that most of these clinical bacteria isolates were susceptible to the ethanolic and aqueous extracts except *E. coli* and *Staphylococcus aureus* as seen in Table 1.

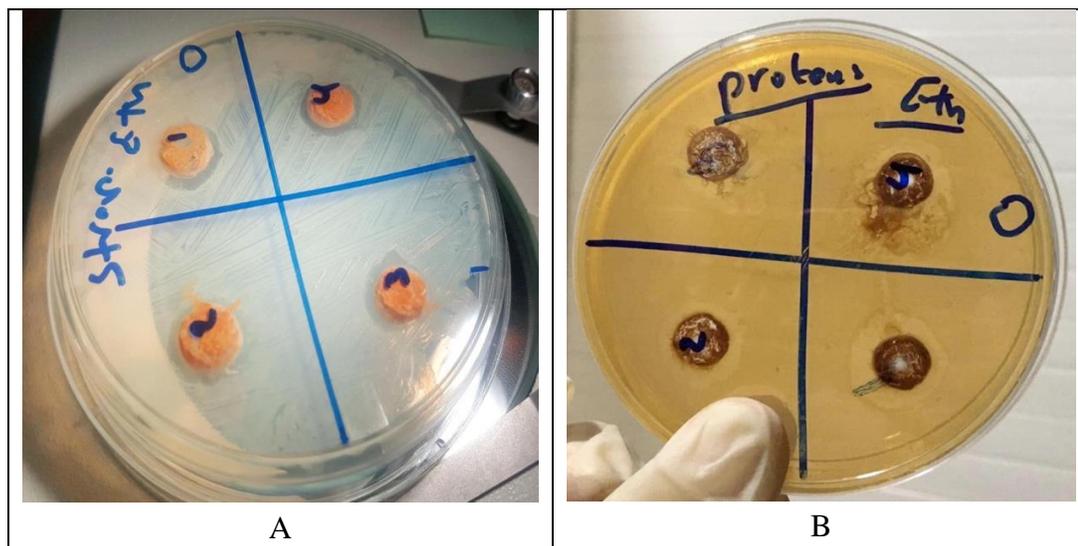
Table 1: Inhibitory activity of plant ethanol and distilled water extract in different concentrations against Test Bacteria.

Concentration of <i>C. zeylanicum</i> ethanolic extract %	Pathogenic bacteria	Average diameter of inhibition zone (mm) by ethanol extract	Average diameter of inhibition zone (mm) by D.W extract
0.1	<i>Pseudomonas spp.</i>	18	9
0.2		17	8
0.3		15	5
0.4		13	2
0.1	<i>Proteus spp.</i>	16	5
0.2		15	4
0.3		14	3
0.4		12	0
0.1	<i>Streptococcus agalactiae</i>	15	4
0.2		12	3
0.3		11	0
0.4		10	0

0.1	<i>Listeria monocytogenes</i>	13	3
0.2		13	3
0.3		10	0
0.4		7	0
0.1	<i>Klebsiella spp.</i>	11	2
0.2		10	0
0.3		8	0
0.4		6	0
0.1	<i>E. coli</i>	0.0	0
0.2		0.0	0
0.3		0.0	0
0.4		0.0	0
0.1	<i>Staphylococcus aureus</i>	0.0	0
0.2		0.0	0
0.3		0.0	0
0.4		0.0	0

The zone of inhibition of bacterial cultures treated with plant material extracted with ethanol ranged from 6 to 18 mm. While, plant material extracted with distilled water showed less significant inhibitory

activity, ranging from 2 to 9 mm. This means that the ethanolic extract showed comparatively higher antimicrobial activity than the aqueous extract, as shown in Figure 1



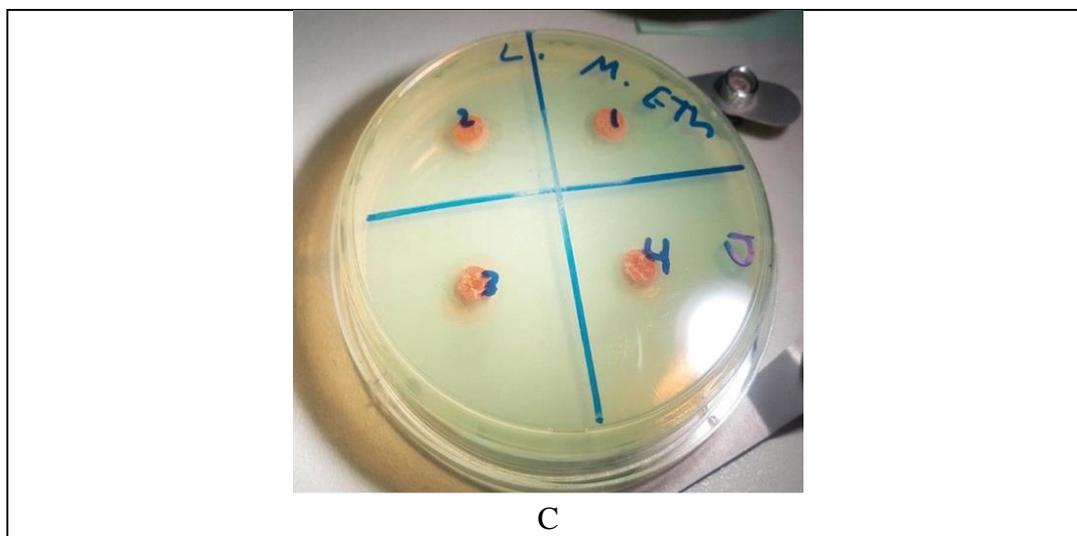


Figure 1: Zone of inhibition (in mm) of ethanolic extract of *C. zeylanicum* against tested pathogenic bacteria. A: *Streptococcus agalactiae*. B: *Proteus spp.* C: *Listeria monocytogenes*

The highest zones of inhibition were produced by ethanolic and aqueous extracts tested against *Pseudomonas aeruginosa* (18 mm for ethanol and 5 mm for aqueous extracts) at the highest concentration. While the lowest zones of inhibition tested against *Klebsiella spp* (6 mm for ethanol extract) at the lowest concentration and *Klebsiella spp.*

(2 mm for aqueous extract) at the highest concentration.

On the other hand, commercial antibiotics sensitivity testing was used as a positive control. All these pathogenic bacteria were examined for their susceptibility against five regularly used commercial antibiotics by the modified Kirby-Bauer method, as shown in Table 2.

Table 2: Antibiotic sensitivity pattern with inhibition zone for each antibiotic disk used against test pathogenic bacteria.

Antibiotics	Disc potency (µg)	pathogenic bacteria	CLSI	Average diameter of inhibition zone (mm)
Imipenem	10	<i>Pseudomonas spp.</i>	S	20
Kanamycin	30		R	0
chloramphenicol	10		R	0
Bacitracin	10		R	0
Tetracycline	10		R	0
Imipenem	10	<i>Proteus spp.</i>	R	0
Kanamycin	30		R	0
chloramphenicol	10		R	0

Bacitracin	10		R	6
Tetracycline	10		R	0
Imipenem	10	<i>Streptococcus agalactiae</i>	S	28
Kanamycin	30		MR	16
chloramphenicol	10		R	8
Bacitracin	10		R	0
Tetracycline	10		R	6
Imipenem	10	<i>Listeria monocytogenes</i>	R	7
Kanamycin	30		R	0
chloramphenicol	10		R	0
Bacitracin	10		R	0
Tetracycline	10		R	0
Imipenem	10	<i>Klebsiella spp.</i>	S	28
Kanamycin	30		s	18
chloramphenicol	10		R	0
Bacitracin	10		R	0
Tetracycline	10		R	0
Imipenem	10	<i>E. coli</i>	S	25
Kanamycin	30		R	0
chloramphenicol	10		R	11
Bacitracin	10		R	0
Tetracycline	10		R	0
Imipenem	10	<i>Staphylococcus aureus</i>	S	31
Kanamycin	30		MR	14
chloramphenicol	10		R	8
Bacitracin	10		R	0
Tetracycline	10		R	11

*S: Susceptible,

*MR: Intermediate,

*R: Resistant.

The results demonstrated that Imipenem showed higher inhibition bacteria than *C. zeylanicum* extract on all tested organisms

(Zone of inhibition ranging from 7 to 31 mm) except for *Proteus* (there was no inhibition zone) as shown in Figure 2.



Figure 2: Zone of inhibition (in mm) of commercial antibiotics sensitivity test against tested pathogenic bacteria.

DISCUSSION:

One of the world's most serious rising health issues is the resistance that bacteria have been acquiring against antimicrobials. This problem worsens and becomes more difficult to fight; such as infections caused by these microorganisms, which are unlikely to be effectively resolved. In this way, rational antibiotic use is recommended, if therapeutic approaches must be based on knowledge of the antibiotic that should be prescribed, without overlooking trends in bacterial resistance profiles, in a more comprehensive manner [16].

Because of the growing number of bacterial species becoming resistant to many different antimicrobial drugs, there is an ongoing search for new treatment strategies, and medicinal plants are an important source of new substances. Cinnamon has demonstrated potent antibacterial activity against methicillin resistant *Staphylococcus aureus* [17].

In the current study, the plant material extracted with ethanol and aqueous showed significant antimicrobial activity on all test

organisms except *E. coli* and *S. aureus*. According to this study, ethanolic *C. zeylanicum* bark extract had significantly higher antibacterial activity than aqueous extract. This supports Abdurashed's [18] and Mukhtar and Ghori's [19] findings that the antibiotic component of *C. zeylanicum* bark is more soluble in ethanol, an organic solvent, than in water, resulting in the release of an active antimicrobial component. It is clear that distilled water is not ideal for extracting *C. zeylanicum*.

According to the results obtained, it was confirmed that ethanolic extract had stronger antibacterial activity than aqueous extract, and the different diameters of the zones of inhibition were proportional to the extract concentration, as shown in table 1. This means that as the concentration of the extracts rises, the zones of inhibition expand. *E. coli* and *S. aureus* were found to confer resistance to all concentrations of both aqueous and ethanolic extracts of cinnamon, with no zones of inhibition for either extract.

On the other hand, Salma [20] reported that both *S. aureus* and *E. coli* have inhibitory activity. In their study, the ethanolic extract was more effective against *S. aureus* than *E. coli*. The first one showed activity from 60% concentration. (zone of inhibition 17 mm) and *E. coli* from 80% concentration. (Zone of inhibition 18 mm).

In consideration of other studies, the results obtained in this particular research are of great importance, especially considering the emerging and ongoing issue of multi-resistant microorganisms to the common synthetic drugs. This work comes with a

large space for research into new sources of drugs, both natural and synthetic.

CONCLUSION:

According to the results obtained in the antibacterial activity tests of ethanolic and aqueous extracts of *C. zeylanicum* bark against bacterial strains isolated and identified from clinical samples, it was possible to conclude that bacterial samples isolated such as *E. coli*, *S. aureus*, and *K. pneumoniae* were multi-resistant to the tested antimicrobials. The extract of *C. zeylanicum* was effective as a bacteriostatic on most tested bacteria.

REFERENCES:

1. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health*. 2015;109(7):309-318.
2. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. *Am J Med*. 2006 Jun;119(6 Suppl 1):S3-10; discussion S62-70.
3. González-Lamothe R, Mitchell G, Gattuso M, Diarra MS, Malouin F, Bouarab K. Plant antimicrobial agents and their effects on plant and human pathogens. *Int J Mol Sci*. 2009 Jul 31;10(8):3400-19.
4. Nabavi SM, Marchese A, Izadi M, Curti V, Daglia M, Nabavi SF. Plants belonging to the genus *Thymus* as antibacterial agents: from farm to pharmacy. *Food Chem*. 2015 Apr 15;173:339-47.
5. Simões M, Bennett RN, Rosa EA. Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. *Nat Prod Rep*. 2009 Jun;26(6):746-57.
6. Nabavi SF, Di Lorenzo A, Izadi M, Sobarzo-Sánchez E, Daglia M, Nabavi SM. Antibacterial Effects of Cinnamon: From Farm to Food, Cosmetic and Pharmaceutical Industries. *Nutrients*. 2015;7(9):7729-7748.
7. Brierley S, Kelber O. Use of natural products in gastrointestinal therapies. *Curr Opin Pharmacol*. 2011;11:604-611.
8. Al-Jiffri O, El-Sayed Z, Al-Sharif F. Urinary tract infection with *E. coli* and antibacterial activity of some plants extracts. *Int. J. Microbiol. Res*. 2011;2:1-7.
9. Saeed S, Tariq P. In vitro antibacterial activity of clove against gram negative bacteria. *Pak. J. Bot. Karachi*. 2008;40:2157-2160.
10. Voukeng IK, Kuete V, Dzoyem JP, Fankam AG, Noumedem JA, Kuate JR, Pages JM. Antibacterial and antibiotic-potential activities of the methanol extract of some cameroonian spices

- against Gram-negative multi-drug resistant phenotypes. BMC Res Notes. 2012 Jun 15;5:299.
11. Al-Mariri A, Safi M. In Vitro Antibacterial Activity of Several Plant Extracts and Oils against Some Gram-Negative Bacteria. Iran J Med Sci. 2014 Jan;39(1):36-43.
 12. Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol. 2001 Sep;91(3):453-62.
 13. Parekh J, Chanda S. In-vitro antibacterial Activity of Crude Methanolic Extract of Woodfordia Fruticosa Kurz Flower (Lythaceae). Brazilian J Microbiol. 2007;38:2–10.
 14. Ibrahim S, Bello AS, Sunusi U, Lere MY, Umar FS, Egbong UD, et al. Phytochemical screening and antimicrobial activities of the leaf, stem bark and root extracts of Combretum sokodense. Bayero J Pure Appl Sci. 2017;10(2):11–5.
 15. Kiehlbauch JA, Hannett GE, Salfinger M, Archinal W, Monserrat C, Carlyn C. Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York state laboratories. J Clin Microbiol. 2000;38(9):3341-3348. doi:10.1128/JCM.38.9.3341-3348.2000.
 16. Kose A, Colak C. Knowledge and Awareness of Physicians About Rational Antibiotic Use and Antimicrobial Resistance Before and After Graduation: A Cross-Sectional Study Conducted in Malatya Province in Turkey. Infect Drug Resist. 2021;14:2557-2568.
 17. Mandal S; Manisha DebMandal, Saha K, Pal NK. In Vitro Antibacterial Activity of three Indian Spices Against Methicillin-Resistant Staphylococcus aureus. Oman Med J. 2011 Sep;26(5):319-23.
 18. Abdulrasheed M, Ibrahim I, Luka A, Maryam A, Hafsta L, Ibrahim S, et al. Antibacterial Effect of Cinnamon (*Cinnamomum zeylanicum*) Bark Extract on Different Bacterial Isolates. JEMAT, 2019, Vol 7, No 1, 16-20.
 19. Mukhtar S, Ghorri I. Antibacterial activity of aqueous and ethanolic extracts of Garlic, Cinnamon and Turmeric against Escherichia coli ATCC 25922 and Bacillus subtilis DSM 3256. Int J Appl Biol Pharm Technol [Internet]. 2012;3(2):131–6.
 20. Salma U, Saha SK, Sultana S, Ahmed SM, Haque SD, Mostaqim S. The Antibacterial Activity of Ethanolic Extract of Cinnamon (*Cinnamomum zeylanicum*) against two Food Borne Pathogens: Staphylococcus aureus And Escherichia coli. Mymensingh Med J. 2019 Oct;28(4):767-772.
 21. Demetrio L. Valle, Jr. Jeannie I. Andrade, Juliana Janet M. Puzon, Esperanza C. Cabrera and Windell L. Rivera . (2015). Antibacterial activities of ethanol extracts of philippine medicinal plants against multidrug-resistant bacteria; Asian pacific journal of tropical, 5(7): 532-540.
 22. Clinical and Laboratory Standards Institute. Wayne: Clinical Laboratory Standards Institute; 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.

23. Kambar Y, Manasa M, Pallavi S, Vivek MN, Swamy SHC, Asha MM, Chaithra M, Kekuda PTR, Mesta SC, Onkarappa R, Mallikarjun N, Inhibitory efficacy of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum* against clinical isolates of *Staphylococcus aureus* and *Streptococcus mutans*, *Pharmanest*, 2013, 4(5), 786-793.
24. National Committee for Clinical Laboratory Standards (NCCLS). performance standards for antimicrobial susceptibility testing. supplement M100. Wayne:PA. Clinical and Laboratory Standard Institute, 3rd .,2020.

