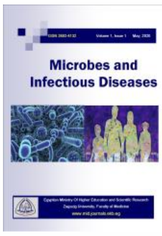
Microbes and Infectious Diseases 2024; Article-In-Press, **DOI:** 10.21608/mid.2024.274553.1833



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Evaluation of the antibacterial activity of a concoction made from *piper guineense* (schumach), *zingiber officinale* (roscoe) and honey on selected pneumonic bacteria in Nigeria.

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ARTICLE INFO

Article history:
Received 4 March 2024
Received in revised form 8 April 2024
Accepted 26 May 2024

Keywords:
traditional concoction
commercial antibiotics
pneumonic Bacteria
antibacterial activities

ABSTRACT

Background: Pneumonic bacteria are bacterial species that cause pneumonia. Although, pneumonia can be treated with antibiotics however because of the problem of antibiotic resistance by many bacterial species, it’s becoming difficult to treat most bacterial infections.Therefore, the need to seek for alternative and more effective therapies is necessary. In this study, one of the traditional concoctions that is used in folk medicine to treat respiratory related diseases was examined for possible antibacterial activity on bacterial species causing pneumonia in Ondo State, Nigeria. **Methods**: The concoction was prepared from the seeds of piper guineense, rhizome of zingiber officinale and honey according to oral tradition. Susceptibility of the bacterial species isolated from pneumonic patients in the study area to the prepared concoction as well as conventional antibiotics was determined using agar diffusion assay.The minimum inhibitory concentration, minimum bactericidal concentration and the phytochemical profile of the prepared concoction were carried out using standard methods. **Results**:The *in-vitro* antibacterial assay of the concoction showed that it has growth inhibitory activity on the test pneumonic bacteria with zone diameter ranging from 31.25 to 37.75 mm which was superior to the one mediated by antibiotics used with zone diameter ranging from 1.25 to 16.00 mm. Phytochemicals such as saponin, tannin, flavonoid, alkaloid, terpenoid and cardiac glycosides were detected in the prepared concoction. **Conclusion**: The findings show that the growth inhibition mediated by the prepared traditional concoction was superior to that of the antibiotics used against the test pneumonic bacteria.Therefore it could be exploited as alternative for treating bacterial pneumonia.

Introduction

Pneumonia is an inflammatory condition of the lung primarily affecting small air sac known as alveoli [1, 2]. Pneumonia can be classified into three types, namely: Community Acquired Pneumonia (CAP) which is acquired outside of a hospital; Hospital Acquired Pneumonia (HAP) which is acquired 48 hrs after being admitted in an hospital and Ventilator Associated Pneumonia (VAP) which is acquired 48 hrs after endotracheal intubation [3]. Symptoms of pneumonia include productive or dry cough, chest pain, fever and difficulty breathing [4]. It can be caused by bacteria, fungi and viruses but bacteria are the most common cause of CAP [5, 6].

DOI: 10.21608/MID.2024.274553.1833

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Earlier work carried out by [7] reported that *Streptococcus pneumoniae*, *Staphylococcus auerus and Klebsiella pneumoniae* are the most frequently encountered bacteria found in the sputum samples of pneumonia patients attending chest clinics of selected government hospitals in Ondo State, Nigeria while *Streptococcus pneumoniae* was the most prevalent caused of CAP in the State. Although, pneumonia can be treated with antibiotics, however, most of these antibiotics are becoming less effective because of the alarming rate of development of resistance to antibiotics by many bacterial species. As such, the issue of bacteria resistance to antibiotics leads to treatment failure [8-10].There is the need to seek for alternative therapy of this infection.

Over the years, plant extracts and plant-derived medicines have been reported to have an immense impact on the overall health and wellbeing of man [11]. For example in most rural communities in Nigeria where orthodox medicine is inaccessible, different kinds of plants, decoctions, concoctions and natural products are explored for the treatment of different health problems such as malaria, diabetes, AIDS, mental disorders, sickle-cell anaemia and microbial infections [12-15]. Moreover, a concoction that is prepared from piper guineense schumach (african black pepper), zingiber officinale roscoe (ginger) and honey is used to treat respiratory related diseases in folk medicine. The individual components of the concoction has been reported to have medical property, for example, piper guineense, has been reported to have antibacterial, antioxidant and anti-inflammatory properties [16], zingiber officinale has been reported for treating upper respiratory tract infections, cough, and bronchitis [17] while honey has been shown to be an effective natural cure for certain infections, such as respiratory diseases and the healing of skin burns and wounds [18-20]. However, there is less information on the medicinal property of the concoction and its antibacterial activity.

This study focuses on the investigation of a traditional concoction used in folk medicine for treating respiratory diseases as a potential alternative therapy for bacterial pneumonia. The central dynamics revolve around the rising challenge of antibiotic resistance and the need for novel treatment options. The study explores the antibacterial activity of the concoction against bacterial species causing pneumonia in Ondo State, Nigeria, comparing its efficacy with conventional antibiotics through in-vitro assays.

Materials and methods

Study design

Seeds of piper guineense (schumach) and rhizome of zingiber officinale (roscoe) were purchased at the Oja Oba market, Akure, Nigeria. The plants were used to prepare a voucher specimen (NO. FHI.113939) which was deposited at Forest Herbarium, Ibadan, Oyo State, Nigeria. The honey used was harvested from a bee farm at Ile-Ife, Osun State, Nigeria. The Pneumonic bacteria used were isolated from volunteered patients attending the chest clinic of selected Government hospitals in Ondo State, Nigeria as describe by [7]. All the laboratory analysis were carried out at the Department of Microbiology, School of Life Sciences, Federal University of Technology, Akure between the period of January and May, 2023. Antibiotics disc and 0.45 µm Millipore membrane filter were purchased at Megababs Scientific Concept, Akure, Nigeria.

Ethical consideration

Ethical clearance for the study was sought for and approval was gotten from the Ondo State Ministry of Health (OSHREC29/12/21/411) and Federal Medical Center, Owo (FMC/OW/380/VOL.CXXXVI/66). The informed consent form was filled before collection of samples from the patients recruited for the study. A well-structured questionnaire was administered to the recruited patients to generate their socio-demography data.

Standardization of bacterial inoculum

The standardization of the bacterial isolates was carried out by diluting a 6 hours old broth cultures of the isolates in test tubes and compared with a 0.5 McFarland standard. The bacterial suspension was adjusted to a density equivalent of approximately 10^8 CFU/ml [21].

Antibiotic susceptibility assay for the isolated bacterial species

Susceptibility of the bacterial isolates to conventional antibiotics was determined using the Kirby-Bauer disc diffusion method of [22].

Preparation of traditional concoction used

The seeds of piper guineense and the rhizome of zingiber officinale were sun-dried for seven days, pulverized using electric grinder and stored in seperate sterile labelled containers at room temperature (30 ± 2^{0}C) until use. The concoction was prepared by mixing 0.5g each of the pulverised *P. guineense* and *Z. officinal* in 9 g of honey as used traditionally. The suspension was then vortexed using vortex mixer XH-C (Searchtech instruments) for 30 minutes to allow for maximum dissolution. It was then sterilized using membrane filter of 0.45 Millipore size. The prepared concoction was refrigerated at 4^{0}C until use.

Determination of phytochemicals in the prepared concoction

Quantitative and qualitative screenings were carried out on the prepared concoction using the method of [23].

***In-vitro* assessment of antibacterial activity of the prepared concoction on the isolated bacterial species**

Susceptibility of the bacterial isolates to the prepared concoction was determined using the agar diffusion method according to [24].

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the prepared concoction

Different grams of the prepared concoction (1g, 2g, 3g, 4g and 5g) was dissolved in 10 ml of sterile distil water to make 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml, 500 mg/ml respectively. The suspension was then vortexed using vortex mixer XH-C (Searchtech instruments) for 30 minutes to allow for maximum dissolution. It was then sterilized using membrane filter of 0.45 Millipore size. The diluted concoctions was used for the determination of the MIC according to [25] while the MBC of the concoction was determined by plating 0.5 ml of the samples from all the tubes with no visible growth on Mueller Hilton Agar and incubated at 37˚C for 18 hrs. Any of the plates on which there was no growth was taken as the MBC of the concoction.

Data analysis

All data generated were subjected to statistical analysis using SPSS 17 version and the data obtained were analyzed by one-way analysis nmof variance (ANOVA). Means were compared by Duncan's new multiple range test and considered statistically significant at $p \leq 0.05$.

Results

Antibiotic susceptibility pattern of the isolated bacterial species

Susceptibility of the bacterial isolates to conventional antibiotics showed that Quinolone had the highest growth inhibitory activity against the two Gram positive organisms *Streptococcus pneumoniae* and *Staphylococcus aureus* used in this study with diameter of 15.50 ± 6.7 mm and 10.90 ± 4.38 mm respectively while Amoxacilin exerted the least growth inhibitory activity of 4.10 ± 1.45 and 2.40 ± 1.51 mm respectively on the two organisms (Table 1). In the case of *Klebsiella pneumoniae*, Fluoroquinolone exerted the highest growth inhibition of 9.20 ± 2.53 mm on the organism while Amoxicillin-clavulanic acid exerted the least growth inhibitory activity of 3.60 ± 1.35 mm on the organism (Table 2).

Growth inhibitory effects of the prepared concoction on bacterial species isolated from pneumonic patients

The traditional concoction that was prepared in this study exerted the highest growth inhibitory activity against all the test pneumonic bacteria with zone diameter which ranged from 31.25 to 37.75 mm. This inhibition was greater than that of all the commercial antibiotics used as control in the *in vitro* assay (Table 3 and 4).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the prepared concoction

The minimum inhibitory concentration of the prepared concoction on the bacterial species isolated from pneumonic patients are 18.95 mg/ml, 75 mg/ml and 37.50 mg/ml for *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Klebsiella pneumoniae* respectively (Table 5) while the MBC was 150 mg/ml, 150 mg/ml, 300 mg/ml of the concoction for *S. pneumoniae*, *S. aureus* and *K. pneumoniae* respectively.

Phytochemical profile of the prepared concoction

Phytochemical profile in the concoction revealed the presence of saponin, tannin, terpenoid, flavonoid, alkaloid and cardiac glycosides (Table 6). The quantitative aspect showed the amount of bioactive components (secondary metabolites) that present in individual components of piper guineense / zingimber officinale and honey used in this study (table 7).

Table 1. Antibiotic susceptibility patterns of the isolated *Streptococcus pneumoniae* and *Staphylococcus aureus* to some commercial antibiotics Mean zone of inhibition (mm) for Gram positive bacteria

Antibiotics	**Disk content µg**	***Streptococcus pneumoniae* (n= 10)**	***Staphylococcus aureus* (n= 10)**	**CLSI STANDARD, 2020**			**INTERPRETATION**
		Mean zone of inhibition in mm	**Mean zone of inhibition in mm**	**S**	**I**	**R**	
Quinolone	10	15.50±6.7	10.90±4.38	≥24	-	≤23	R
Gentamycin	10	10.10±3.87	4.60±1.65	≥14	13-14	≤12	R
Ampicillin-cloxacillin	30	7.40±2.12	5.10±2.56	≥17	-	≤16	R
Cefuroxime	20	5.80±1.48	4.40±1.35	≥20	17-19	≤14	R
Amoxacillin	30	4.10±1.45	2.40±1.51	≥18	14-17	≤13	R
Cenftriaxone Sodium	25	7.50±3.14	5.40±2.41	≥24	17-19	≤16	R
Fluoroquinolone	10	11.50±4.67	8.90±3.81	≥21	16-20	≤15	R
Streptomycin	30	8.50±2.32	4.50±1.51	≥15	12-14	≤11	R
Cotrimoxazole	30	7.90±2.72	6.50±2.76	≥16	11-15	≤10	R
Erythromycin	10	11.40±5.23	6.10±2.02	≥23	14-22	≤13	R

Key

S=Sensitive, I=Intermediate, R=Resistant according to Clinical and Laboratory Standards Institute, 2020 (M100) performance standards for antibacterial susceptibility testing [26]

Table 2. Antibiotic susceptibility patterns of the isolated *Klebsiella pneumoniae* to some commercial antibiotics Mean zone of inhibition (mm) for Gram negative bacteria

Antibiotics	**CON(µg)**	***Klebsiella pneumoniae* n =10**	**CLSI STANDARD, 2020**			**INTERPRETATION**
		Mean zone of inhibition in mm	**S**	**I**	**R**	
Cotrimoxazole	30	4.10±2.08	≥16	11-15	≤10	R
Chloramphenicol	30	6.20±2.97	≥18	13-17	≤12	R
Sparfloxacin	10	3.90±1.10	≥19	16-18	≤15	R
Fluoroquinolone	30	9.20±2.53	≥21	16-21	≤15	R
Amoxacillin	30	4.10±2.18	≥18	14-17	≤13	R
Amoxicillin-clavulanic acid	10	3.60±1.35	≥20	-	≤19	R
Gentamycin	30	6.60±3.03	≥15	13-14	≤12	R
Quinolone	30	4.00±2.11	≥24	-	≤23	R
Ofloxacin	10	6.90±2.38	≥18	15-17	≤14	R
Streptomycin	30	3.60±2.07	≥15	12-14	≤11	R

Key

S=Sensitive, I=Intermediate, R=Resistant according to Clinical and Laboratory Standards Institute, 2020 (M100) performance standards for antibacterial susceptibility testing [26]

Table 3. Growth Inhibitory activity of prepared concoction on Gram positive bacterial species isolated from Pneumonic patients

Treatment	**Concentration**	**Diameter zone of inhibition (mm) on Gram positive Pneumonic bacterial isolates**	
		***Streptococcus pneumonia* (n=4)**	***Staphylococcus aureus* (n=4)**
CONCOCTION	300 mg/ml	**32.25±10.97**	**37.75±8.66**
QUINOLONE	30 μ**g**	16.00±.00	14.00±0.00
Sterile distil water	-	0.00± 0.00	0.00± 0.00

Values are represented as Mean ± SD of duplicates

Table 4. Growth Inhibitory activity of prepared concoction on Gram negative bacterial species isolated from Pneumonic patients

Treatment	**Concentration**	**Diameter zone of inhibition (mm) on Gram negative Pneumonic bacterial isolates**
		***Klebsiella pnuemoniae* (n=4)**
CONCOCTION	300 mg/ml	**31.25±10.80**
FLUOROQUINOLONE	30 μ**g**	10.00±4.08
Sterile distil water	-	0.00± 0.00

Values are represented as Mean ± SD of duplicates

Table 5. Minimum inhibitory concentration (MIC) (mg/ml) and Minimum bactericidal concentration (MBC) (mg/ml) of the prepared concoction

BACTERIAL ISOLATES	MIC	MBC
Streptococcus pneumoniae	18.95	150
Staphylococcus aureus	75	150
Klebsiella pneumoniae	37.50	300

Table 6. Qualitative phytochemical profile of the Concoction and Honey

Active component	CONCOCTION	HONEY
Saponin	+	+
Tannin	+	+
Phlobatannin	-	-
Flavonoid	+	+
Steroid	-	-
Terpenoid	+	+
Alkaloid	+	+
Anthraquinone	-	-
Cardiac glycosides	+	+
Legal test	+	+
Keller kiliani test	+	+
Salkwoski test	+	+
Lieberman test	-	-

Table 7. Quantitative phytochemical profile of the Concoction and Honey

Active component	CONCOCTION	HONEY
Saponin mg/g	15.9091 ± 0.38570	1.4545 ± 0.25713
Tannin mg/g	3.7424 ± 0.00765	3.7424 ± 0.00765
Flavonoid mg/g	0.2673 ± 0.00272	0.0154 ± 0.00544
Phenol mg/g	10.2355 ± 0.72589	1.1775 ± 0.04270
Terpenoid mg/g	44.2287 ± 0.15045	9.2021 ± 0.03761
Glycosides mg/g	15.5466 ± 0.15916	25.0161 ± 0.04547
Alkaloid mg/g	14.3345 ± 0.19870	0.0000 ± 0.0000

Values are represented as Mean± SD of duplicates

Discussion

This study explores the antibacterial activity of the concoction against bacterial species causing pneumonia in Ondo State, Nigeria, comparing its efficacy with conventional antibiotics through *in-vitro* assays. The prepared traditional concoction exerted growth inhibitory activity on all the test pneumonic bacteria with zone diameter which ranged from 31.25 to 37.75 mm. This inhibition was greater than that of the commercial antibiotics used as control. That is, the prepared concoction exerted superior growth inhibition of the test bacteria than that of the selected antibiotics used as control in the *in vitro* assay. The concoction was observed to have broad-spectrum activity against all the test bacteria. This could be a result of the individual components of the concoction that has been documented to possess therapeutic attributes. This findings is in agreement with the previous findings carried out by [16] that piper guineense possessed antibacterial, antioxidant, and anti-inflammatory qualities. Also this findings agrees with previous findings carried out by [17] that zingiber officinale is very effective in treating respiratory tract infection, cough, and bronchitis. Furthermore, this findings agrees with the reports of [18-20] on the effectiveness of honey as a natural remedy for specific infections like respiratory ailments and the recovery of skin injuries such as burns and wounds.

The superior growth inhibitory activity of the prepared traditional concoction over the commercial antibiotics used in this study could be attributed to the secondary metabolites that are present in the concoction. Phytochemicals such as saponin, tannin, flavonoid, alkaloid, terpenoid and cardiac glycosides were detected in the prepared concoction. This findings agrees with previous findings carried out by [27] that these phytochemicals are toxic to microbial cells by exerting both bacteriostatic and bactericidal effects on microorganisms. This findings agrees with the studies of [28-30] who reported that saponin bind with cholesterol inside cell leading to the formation of saponin-cholesterol complex which results in lysing of the cells which disturb the permeability of bacterial cells by binding to the outer membrane. This also is in agreement with previous findings by [31] who reported that tannins are multidentate ligands which may bind to proteins, mainly by hydrophobic interactions and hydrogen bonds and as a result of this, the inhibition of bacteria metabolism is achieved. It also agrees with the previous studies by [32, 33] that flavonoids can suppress nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism which also reduces adhesion and biofilm formation, porin on the cell membrane, membrane permeability, and pathogenicity, all of which are crucial for bacterial growth. Finally, this findings agrees with previous reports carried out by [34, 35] that terpenoid possessed ability to act as adjuvants for antimicrobials and exhibit synergy effects.

This study has showed that traditional concoction that is made from the seeds of piper guineense (african black pepper) and rhizome of zingiber officinale (ginger) and honey exerted superior growth inhibitory activity on the test bacteria over conventional antibiotics and that the inhibition could be attributed to various active bioactive compounds that are present in the two plants and honey. The study has significantly contributed to research knowledge highlighting the potential of a traditional concoction as an effective alternative drug inhibiting the growth of antibiotic resistant pneumonic bacteria and therefore might serve as an effective remedy for treating multiple drug resistant bacterial pneumonia, in the face of antibiotic resistance challenges.

Conclusion

The prepared traditional concoction from the seeds of piper giuneense, rhizome extract of zingiber officinale and honey is very effective in inhibiting the growth of the isolated pneumonic bacteria than conventional antibiotics. Therefore the traditional concoction has novel antibacterial properties that could be harnessed as alternatives for treating bacterial pneumonia.

Disclosure statement

Authors declare that no financial funding received for this research work

Conflict of interest statement

Authors declare no conflict of interest

Acknowledgement

Authors acknowledge the technical assistance rendered by the staff of the following organization:

(i) Microbiology Department, Federal University of Technology, Akure;
(ii) Federal Medical Centre, Owo;
(iii) General Hospital, Akure and

(iv) University of Medical Sciences Teaching Hospital Complex, Ondo Town, Nigeria.

References

1- Leach RE. Acute and Critical Care Medicine at a Glance (2nd ed.). Wiley-Blackwell, 2009; ISBN 978-1-4051-6139-8.

2- McLuckie A. ed. Respiratory disease and its management. New York: Springer, 2009; p. 51. ISBN 978-1-84882-094-4.

3- Vardhmaan J, Rishik V, Gizem Y, Abhishek B. Pneumonia pathology. National Library of Medicine, 2022; August 1.

4- Ashby B, Turkington C. The encyclopedia of infectious diseases (3rd ed.). New York: Facts on File, 2007; p. 242. ISBN 978-0-8160-6397-0 Retrieved 21 April 2011.

5- Sharma S, Maycher B, Eschun G. "Radiological imaging in pneumonia: recent innovations". Current Opinion in Pulmonary Medicine, 2007; 13 (3):15969.

6- Anevlavis S, Bouros D. "Community acquired bacterial pneumonia". Expert Opinion on Pharmacotherapy, 2010; 11 (3): 361–74. doi:10.1517/14656560903508770. PMID 20085502. S2CID 24376187.

7- Komolafe BM, Adebolu TT, Oladejo BO. Etiology of Pneumonia in Ondo State, SouthWest, Nigeria. Microbes and Infectious Diseases, 2024; ():-, doi: 10.21608/mid.2024.253519.1700

8- Mene´ndez R, Torres A. Treatment failure in community acquired pneumonia. Chest 2007; 132:1348–1355

9- Odonkor ST, Addo KK. Bacteria resistance to antibiotics: recent trends and challenges. Int J Biol Med Res 2011; 2(4): 1204-10.

10- Thenmozhi S. "Antibiotic Resistance Mechanism of ESBL Producing Enterobacteriaceae in Clinical Field: A Review". International Journal of Pure and Applied Biosciences 2014 2.3: 207-226

11- Anyanwu CU, Nwosu GC. Assessment of the antimicrobial activity of aqueous and ethanolic extracts of Piper guineense leaves. Journal of Medicinal Plant Research 2014. Vol. 8(10); 436-440

12- Elujoba AA, Odeleye OM, Ogunyemi CM. Traditional medicine development for medical and dental primary health care delivery system in Africa. Africa journal of traditional, complementary and alternative medicines 2004. Vol. 2 No! (2005)/Articles.

13- Morufu EB, Daniel CN, Eghosa EI, Elizabeth EB, Jacinta NO, Serges FA. Djobissie. Antihypertensive effect of methanolic extract from the leaves of Hibiscus Sabdariffa L. in rats. Der Pharmacia Lettre, 2016, 8 (19):473-484. Available online at www.scholarresearchlibrary.com

14- Yogeshwer S, Madhulika S. Cancer preventive properties of ginger. A brief review. Food and chemical toxicology. Vol 45, issue 5, May 2007; 683-690.

15- Basualdo C, Sgroy V, Finola MS, Marioli JM. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Vet Microbiol 2007; 124: 375-81.

16- Boukraˆa L. Honey in traditional and modern medicine. Boca Raton: CRC Press; 2013; 13-20.

17- Bizerra FC, Da Silva PI Jr, Hayashi MA. Exploring the antibacterial properties of honey and its potential. Front Microbiol 2012; 3: 398.

18- John-Isa JF, Adebolu TT, Oyetayo VO. Evaluation of the microorganisms in Nigeria honey for antagonistic activity on selected bacteria causing diarrhea. Bangladesh Journal of Medical Science 2021(3): 533-561.

19- Adeyemi SB, Fatoba PO, Adewole AA, Fatoba MT. Medicinal plants used in treatment of Infant Diseases in South Western Nigeria. Nigeria Journal of Basic and Applied Science 2018; 26(1): 14-22

20- Ojo BA, Adebolu TT, Oladejo BO. Administration of a formulated herbal mixture (DOIB) caused a significant increase in CD4 counts and reduction in HIV RNA of volunterer HIV patients on antiretroviral therapy. European Journal of Pharmaceutical and Medical Research 2023; 9(12): 18-25; ISSN2394-321/EJPMB.

21- Idowu ET, Olusola-Makinde OO, Oladunmoye MK. Antibacterial Efficacy of Garcinia kola (Heckel) Seeds against Bacteria Involved in Throat Infections. International Journal of Advanced Biological and Biomedical Research 2020; 8(3), 253-267.

22- El-Mahmood AM, Isa H., Mohammed, Tirmidhi AB. Antimicrobial susceptibility of some respiratory tract pathogens to commonly used antibiotics at the Specialist Hospital, Yola, Adamawa State, Nigeria. Journal of Clinical Medicine and Research 2010; 2(8); 135-142

23- Daniel I, Ekemini IA, Edidiong CU. Phytochemical Evaluation, Antioxidant and Antimicrobial Activities of Various Extracts from Leaves and Stems of Bryophyllum pinnatum. Nepal J Biotechnol 2020; 8(1):17-28

24- Prescott LM, Klein D, Harley JP. Microbiology 2008; 6th Edition, McGraw Hill, New York.

25- Babajide AB, Adebolu TT, Oladunmoye MK, Oladejo BO. Evaluation of antimicrobial activity of Citrus aurantium L. leaf extracts on bacteria isolated from blood of hepatitis B positive individual in Ondo State, Nigeria. Microbes and Infect Dis 2023; 4(1):304-311.

26- CLSI. Performance standard for antibacterial susceptibility testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical & Laboratory Standard Institute; 2020.

27- Okigbo R, Igwe D. Antimicrobial effects of Piper guineense ‘Uziza’ and Phyllantus amarus ‘ebe-Benizo’ on Candida albicans and Streptococcus feacalis. Acta Microbiologica et Immunologica Hungarica 2007; 353-366. Doi. https://doi.org/10.1556/amicr.54.2007.4.3.

28- Sen S, Makkar HPS, Muetzel S, Becker K. “Effect of Quillaja saponaria saponins and Yucca schidigera plant extract on growth of Escherichia coli,” Letters in Applied Microbiology, vol. 27, no. 1, pp. 35–38, 1998.

29- Winter WP. “Mechanism of saponin induced red cell hemoly-sis: evidence for the involvement of aquaporin CHIP28,” Blood, vol. 84, no. Suppl. 1–10, abstract 445, 1994.

30- Arabski M, Wasik S, Dworecki K, Kaca W. “Laser interfer-ometric and cultivation methods for measurement of colistin/ampicilin and saponin interactions with smooth and rough of Proteus mirabilis lipopolysaccharides and cells,” Journal of Microbiological Methods, vol. 77(2); 178–183, 2009.

31- Theisen LL, Erdelmeier CAJ, Spoden GA, Boukhallouk F, Sausy A, Florin L. Tannins from Hamamelis virginiana bark extract: Characterization and Improvement of the Antiviral Efficacy against Influenza A Virus and Human Papillomavirus. PLoS ONE 2014; 9(1): e88062. https://doi.org/10.1371/journal.pone.0088062

32- Biharee A, Sharma A, Kumar A, Jaitak V. Fitoterapia Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. Fitoterapia 2020, 146, 104720.

33- Donadio G, Mensitieri F, Santoro V, Parisi V, Bellone M, De TN, Izzo V, Piaz FD. Interactions with Microbial Proteins Driving the Antibacterial Activity of Flavonoids. Pharmaceutics 2021; 13, 660.

34- Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo SE, Nabavi SF. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiol. Res. 2017, 196, 44–68.

35- Langeveld WT, Veldhuizen EJA, Burt SA. Synergy between essential oil components and antibiotics: A review. Crit. Rev. Microbiol. 2014, 40, 76–94.

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