Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Phytochemistry

ISSN 1819-3471
DOI: 10.3923/rjphyto.2021.51.57

Research Article
Activity of *Terminalia chebula* and *Erythrina variegata* Extracts Against Pathogen Causing Respiratory Tract Infection

[1]Chandrika Das, [1]Dil Umme Salma Chowdhury, [2]Abdul Mannan and [1]Nazneen Naher Islam

[1]Department of Genetic Engineering and Biotechnology, University of Chittagong, Chattogram-4331, Bangladesh
[2]Department of Chemistry, University of Chittagong, Chattogram-4331, Bangladesh

Abstract

Background and Objective: Respiratory Tract Infections (RTIs) are one of the leading causes of morbidity and mortality worldwide. As the bacterial resistance towards commercially available antibiotics indicated for microorganisms associated with RTIs is increasing, the need of finding an appropriate alternative antimicrobial agent is also on the rise. Therefore, the present study was executed to explore the antibacterial activity of the acetone extracts of two medicinal plants, *Terminalia chebula* and *Erythrina variegata* against antibiotic-resistant *Staphylococcus aureus.* **Materials and Methods:** For this study, the presence of *S. aureus* in the human sputum samples was ensured by growing culture on Mannitol Salt Agar (MSA) and performing a battery of biochemical tests. Culture sensitivity tests were done to determine the antibiotic susceptibility pattern of *S. aureus.* MAR index of *S. aureus* isolates was also calculated to determine if they are resistant to more than one antibiotic. The antimicrobial activity of the plant extracts was determined using a disc diffusion assay. **Results:** The isolates were found highly resistant to Erythromycin (68.97%) and least resistant towards Chloramphenicol (13.79%). The high MAR index (>0.2) has signified the exposure of *S. aureus* to multiple antibiotics. Acetone extracts collected from the two plants showed the highest activity at 5 mg mL^{-1} among the three different concentrations used (1.25, 2.5 and 5 mg mL^{-1}) in the study. It was also observed that the extracts from *T. chebula* exhibited better antibacterial activity than that of *E. variegata* against *S. aureus* isolates. **Conclusion:** *T. chebula* and *E. variegata* extracts could be further investigated as potential antibacterial agents to treat respiratory tract infection.

Key words: Antibacterial resistance, *Staphylococcus aureus*, *Terminalia chebula*, *Erythrina variegata*

Citation: Das, C., D.U.S. Chowdhury, A. Mannan and N.N. Islam, 2021. Activity of *Terminalia chebula* and *Erythrina variegata* extracts against pathogen causing respiratory tract infection. Res. J. Phytochem., 15: 51-57.

Corresponding Author: Nazneen Naher Islam, Department of Genetic Engineering and Biotechnology, University of Chittagong, Chattogram-4331, Bangladesh Tel: +880-01772-236350

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Respiratory Tract Infections (RTIs), are one of the leading causes of mortality, morbidity, malnutrition, impaired growth and cognitive development in children as well as in older age groups[1]. They are classified into two broad categories based upon which part of the respiratory region is affected: Upper Respiratory Tract Infections (URTIs) and Lower respiratory tract infections (LRTIs). Pharyngitis, nasopharyngitis, otitis media, tonsillitis and sinusitis are the major infections of the upper respiratory tract whereas pneumonia is the formidable one among the lower RTIs since it can easily lead to death[2]. URTIs are generally caused by viruses (adenovirus, rhinovirus, parainfluenza virus and influenza virus). Following the viral invasion, secondary infection ensues by various types of bacteria which results in chronic obstructive lung disease and high fever[3].

Several factors can influence the incidence and associated mortality owing to RTI notably, demographic risk profile, irrational antibiotic prescribing, the prevalence of causative agents and antibiotic resistance[4]. The different types of bacteria which are involved in RTIs are *Staphylococcus aureus, Haemophilus influenzae*, *Streptococcus pyogenes*, *Moraxella catarrhalis*, *Streptococcus pneumonia*, *Klebsiella pneumonia* and *Mycobacterium tuberculosis*. All of these bacteria liable for respiratory infections have been reported to become resistant towards different antibiotics including Amoxicillin, Cephalosporin (Ceftriaxone), Trimethoprim, Sulfamethoxazole, Erythromycin, Chloramphenicol, Ciprofloxacin, Penicillin, Medemycin, Aztreonam, Streptomycin, Ampicillin and Tetracycline[5-8]. Increased fatality in patients with serious infections has been linked with excessive and improper initial antibiotic therapy which is a potentially modifiable factor[9].

The main reason for increased resistance to antibiotics in bacteria is due to the mobile genes present on the plasmids which spread and transfer through bacterial populations[10]. Another reason for antibiotic resistance of bacteria has been considered to be the production of biofilms during the quorum-sensing-regulated mechanism which releases beta-lactamase responsible for the degradation of various antibiotics[5]. Nowadays, antibiotic resistance developed by microorganisms is recognized as a far-reaching health concern by the global research community[11]. Researchers around the world have been focusing on obtaining knowledge of bacterial etiology and drug-resistance pattern of evolving microorganisms and employ necessary strategies to decrease the development of resistance and produce a better therapeutic outcome. So, there is an urgent need for alternative antibacterial agents which can target these drug-resistant pathogenic microbes. Many attempts have been made to investigate the potential role of plant extracts containing active compounds to combat the problem of antibiotic resistance in bacteria. Various essential oils (EOs) have been shown to exhibit noteworthy antimicrobial actions against a wide range of both gram-positive and gram-negative bacteria[12]. The Essential Oils (EOs) and their components cause permeabilization of the cell wall and cytoplasmic membrane of bacteria which results in ion leakage and reduction of the membrane potential and ATP pool[13]. EOs have been traditionally used for treating respiratory tract infections and are currently used as alternative medicines for the treatment of colds[14]. Moreover, to treat acute and chronic bronchitis, acute sinusitis and inhalation therapy using essential oils have previously been used. It has been reported that inhalation therapy using volatile essential oil vapours is effective in reducing asthma-associated respiratory distress and inflammation of the trachea as well as increasing the respiratory tract fluid output[1,15].

Some plants have been used in folk medicines for a long period by traditional medicine practitioners in the South Asian region to treat cold and chronic asthma among which *Terminalia chebula* (seed) and *Erythrina variegata* (leaf) are included[16,17]. The claim of therapeutic efficacy of these plants has not yet been validated adequately through *in vitro* or *in vivo* methods which is crucial to give them a secure place in modern health care. Hence, this study has been conducted to explore primarily the effectiveness of the crude extracts of these medicinal plants against drug-resistant bacteria isolated from a human sputum sample.

MATERIALS AND METHODS

Study area, population and sample collection: Sputum samples of patients (N = 30) with respiratory tract infections were collected from different diagnostic centres of Chattogram Metropolitan area. Samples were collected in sterile sealed plastic vials and stored in the refrigerator at 4°C for further analysis. This study was carried out from February, 2019 to February, 2020. Ethical clearance was obtained from the ethical review committee of Chattogram Maa-O-Shishu Hospital, Chattogram. The patient consents were also taken as forms of the questionnaire.

Isolation of *Staphylococcus aureus*: Luria Bertani (LB) broth media was prepared in the sterile conical flask and sterilized by autoclaving. After that, the media was cooled to 45°C and poured into sterile test tubes. Then, a loopful of freshly

collected sputum sample was inoculated in LB broth. The same step was followed for the rest of the samples. All of the culture tubes were then kept in an incubator at 37°C for 24 hrs. The growth of bacterial culture was determined by measuring optical density in a spectrophotometer at a wavelength of 600 nm. Then, Mannitol Salt Agar (MSA) media was prepared, cooled down and poured on sterile Petri plates and kept to solidify. One loop of liquid culture was streaked on the MSA plate and the same procedure was followed for each plate. The plates were then incubated for 24 hrs at 37°C in an incubator in the inverted position. Yellow coloured colonies that grew on MSA plates were identified as *S. aureus*[18,19].

Biochemical identification of *Staphylococcus aureus*: Isolated *S. aureus* colonies were identified by performing the Coagulase test, Catalase test and Gram staining test[18]. Indole test, Methyl-Red (MR) test, Voges-Proskauer (VP) test, Citrate utilization test was also performed[20].

Antibiotic susceptibility test: Kirby-Bauer disk diffusion assay was followed to perform antibiotic susceptibility test[21]. All *S. aureus* positive samples were subjected to disc diffusion assay to determine their antibiotic susceptibility pattern against five antibiotics. The selected antibiotics for assay were Erythromycin (15 µg), Chloramphenicol (30 µg), Amoxicillin (30 µg), Cefepime (30 µg) and Ciprofloxacin (5 µg). About 200 µL of overnight grown bacterial culture in LB media was spread on Mueller Hinton agar plate with a sterile glass spreader. Then after 5-10 min, commercially available standard antibiotic discs were placed on Mueller-Hinton agar (MHA) plates with sterile forceps. The plates were then incubated and then the organisms were categorized as "resistant", "sensitive" or "intermediate" based on their production of the zone of inhibition which was compared with the obtained standard data from Clinical Laboratory Standards Institute.

Multiple antibiotic resistance (MAR) indexes: The MAR index of an isolate is calculated as a/b. Where, a = no. of antibiotics to which the isolates were resistant and b = no. of antibiotics to which the isolates were subjected[22].

Collection and processing of plant sample: Plant samples were collected from two different locations of Chattogram regions namely, Anwara upazila and Botanical garden of the University of Chittagong. Healthy and matured leaf samples of *Erythrina variegata* and seeds of *Terminalia chebula* were collected and transported to the lab on the day of collection. Then the samples were properly washed with sterile distilled water and dried at 45°C in an oven followed by grinding and packing into thimbles of 8-9 cm which were made of clean dry clothes.

Preparation of plant extract: The Soxhlet extraction method was employed using acetone as extraction solvent[23]. The extract was filtered through Whatman no. 1 filter paper. Acetone was evaporated using a vacuum rotary evaporator at 50°C and then dried at room temperature to remove the residual solvent. The collected extracts were again filtered and finally stored in a sterile sealed glass bottle at 4°C before use for antimicrobial assay. Plant extracts obtained from *T. chebula* and *E. variegata* were diluted into 1.25, 2.5 and 5 mg mL^{-1}.

Determination of the antimicrobial activity of plant extract: The antimicrobial activity of the acetone extracts of both plants was determined against the test organisms using disc-diffusion assay[24]. Sterilized and dried filter paper discs (6 mm in diameter) were soaked with plant extracts of *T. chebula* and *E. variegata*. About 200 µL of overnight grown LB media culture of *S. aureus* was spread on MHA media plates with a sterile spreader. After that, a disc containing test extracts were placed on MHA plates. The plates were kept in incubation overnight at 37°C. Standard discs of erythromycin (15 µg) were used as the positive control. Clear and distinct zone of inhibitions were visualized around the plant extract impregnated discs.

Statistical analysis: The experiment regarding the determination of the antimicrobial activity of the plant extracts was carried out in triplicate and zones of inhibition were calculated as mean ± SD (mm).

RESULTS

Prevalence of *S. aureus*: The results of the study revealed that out of thirty (30) collected samples, the prevalence of *S. aureus* was 96.6% (29) as shown in Fig. 1.

Bacteriological analysis: Among the 29 positive *S. aureus* isolates of this study, 24 (82.76%) isolates were Coagulase-positive and 5 (17.24%) isolates were coagulase-negative. All of the 29 isolates (100%) were found positive for the catalase production test, Methyl Red, Voges Proskauer, Citrate utilization test and Gram staining but negative for Indole production test (Table 1).

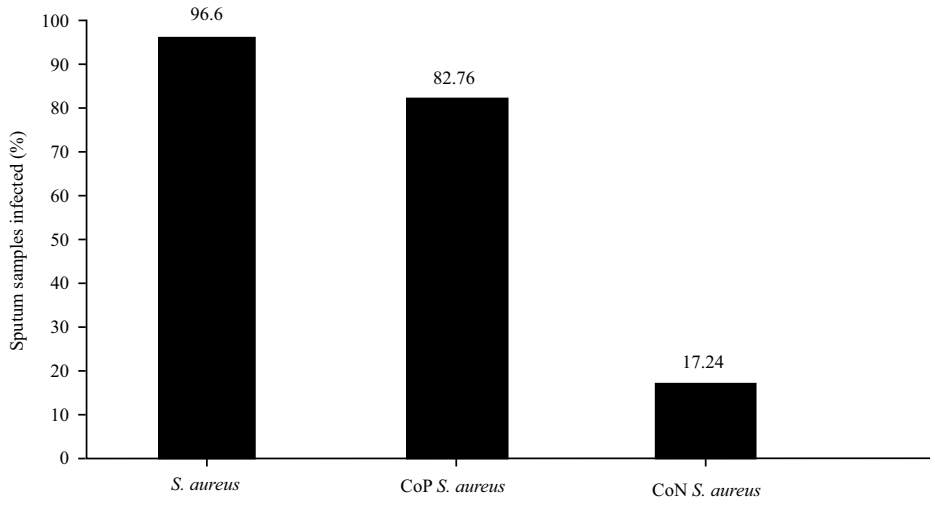

Fig. 1: Percentage of sputum samples infected with *Staphylococcus aureus*
Co P: Coagulase positive and Co N: Coagulase-negative

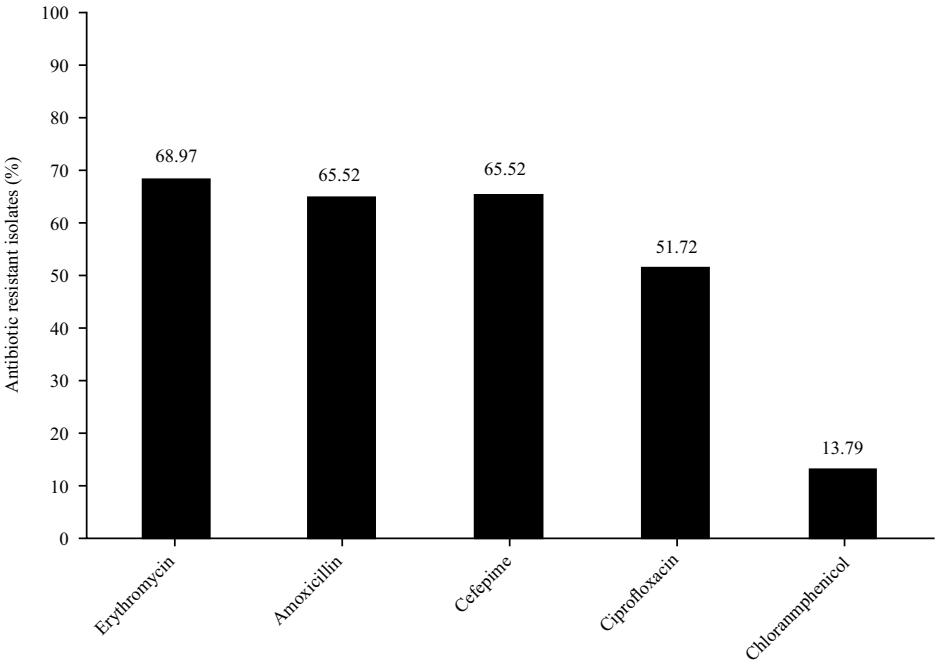

Fig. 2: Antibiotic resistance profile of *S. aureus* isolates

Table 1: Different biochemical tests of *S. aureus* isolated from a sputum sample

Total positive samples	Catalase test	Coagulase test	MR test	VP test	Indole test	Citrate utilization test	Gram staining
29	29	24	29	29	-	29	29

Antimicrobial susceptibility pattern: The antimicrobial susceptibility pattern was examined for 29 *S. aureus* isolates against 5 commercially available standard antibiotics by using disc diffusion assay. The whole analysis was done by following the standard data available from National Committee for Clinical Laboratory Standards. According to the result of culture sensitivity, the isolates were found to show the highest resistance towards erythromycin (68.97%) which was followed by ampicillin and Cefepime (65.52%). The resistance was moderate towards ciprofloxacin (51.72%) and least for chloramphenicol (13.79%) which meant that the highest susceptibility of *S. aureus* was found against chloramphenicol (75.86%). The results of the antibiotic resistance profile of *S. aureus* are displayed in Fig. 2.

Table 2: Multiple antibiotic resistance (MAR) indexes

No. of isolates	No. of antibiotics they are subjected to (b)	No. of antibiotics to which they showed resistance (a)	MAR Index (a/b)
4	5	1	0.2
6	5	2	0.4
11	5	3	0.6
7	5	4	0.8
0	5	0	0

Table 3: Antibacterial activity of plant extract collected from *Erythrina variegata* against *S. aureus* isolates

	Diameter of inhibition zone (mm) of *Erythrina variegata* extract		
Drug resistance pattern	5 mg mL^{-1}	2.5 mg mL^{-1}	1.25 mg mL^{-1}
E, AML, CPM, CIP	7.5±0.408	7.0±0.707	5.83±0.408
E, AML, CPM, CIP	11.5±1.225	11.0±0.408	10.83±0.471
E, C, AML, CIP	16.0±0.408	13.0±0.408	13.00±0.408
E, AML, CPM, CIP	10.0±0.00	7.5±0.408	6.50±0.408
C, AML, CPM, CIP	9.5±0.408	8.0±1.225	6.00±0.408
E, C, CPM, CIP	13.5±0.408	11.0±0.00	9.50±0.408
E, C, CPM, CIP	8.0±0.408	0.0	0

E: Erythromycin, AML: Amoxicillin, CPM: Cefepime, CIP: Ciprofloxacin and C: Chloramphenicol

Table 4: Antibacterial activity of plant extract collected from *Terminalia chebula* against *S. aureus* isolates

	Diameter of inhibition zone (mm) o f *Terminalia chebula* extract		
Drug resistance pattern	5 mg mL^{-1}	2.5 mg mL^{-1}	1.25 mg mL^{-1}
E, AML, CPM, CIP	19±1.080	16.0±1.080	14.0±0.408
E, AML, CPM, CIP	11±0.408	10.5±0.707	9.0±1.225
E, C, AML, CIP	19±1.225	19.0±1.225	18.0±1.225
E, AML, CPM, CIP	14±1.225	13.0±1.225	12.0±0.408
C, AML, CPM, CIP	13±0.816	13.0±0.408	10.0±1.225
E, C, CPM, CIP	17±0.408	14.0±0.408	10.5±0.408
E, C, CPM, CIP	13±0.408	11.0±1.225	8.5±0.408

E: Erythromycin, AML: Amoxicillin, CPM: Cefepime, CIP: Ciprofloxacin and C: Chloramphenicol

Multiple antibiotic resistance (MAR) indexes: In this study, among the 29 isolates, 4 isolates had a MAR index of 0.2 while 24 isolates showed a MAR index value of greater than 0.2 in Table 2.

Antimicrobial activity of plant extracts: It was revealed from the disc diffusion assay that seven *S. aureus* isolates exhibited resistance towards four different antibiotics (out of five antibiotics) used in this study. Then, the antimicrobial activity of extracts obtained from both of the plants was investigated against these seven *S. aureus* antibiotic-resistant isolates. Erythromycin (15 µg) was used as a positive control because the isolates showed maximum resistance (68.97%) against it. In the case of extracts from *E. variegata*, the highest zones of inhibition found for 7 different *S. aureus* isolates in 5 mg mL^{-1} were 7.5±0.408, 11.5±1.225, 16±0.408, 10±0, 9.5±0.408, 13.5±0.408 and 8±0.408, respectively while the highest zones of inhibition for *T. chebula* were 19±1.080, 11±0.408, 19±1.225, 14±1.225, 13±0.816, 17±0.408 and 13±0.408, respectively (Table 3 and 4).

DISCUSSION

In developing countries, RTIs are considered to be the second most common cause of death in children due to pneumonia. In Bangladesh, pneumonia is responsible for around 15% of the deaths of children under five years of age[25]. Bacteria responsible for respiratory infections have been reported to become resistant to different antibiotics. Though pharmaceutical companies are producing several new antimicrobial drugs, the resistance gain rate is pretty high. So, the need of producing alternate pharmacological products cannot be overlooked. Medicinal plants having antimicrobial potential can exert a better effect in this regard[12].

The prevalence rate of *S. aureus* (96.6%) found in the current study aligned with the finding of Ullah *et al.*[26] in which the highest occurrence rate was observed for *S. aureus* (57.81%) in sputum samples collected from patients with lower respiratory tract infections. The highest frequency of resistance exhibited by the *S. aureus* was against Erythromycin (68.97%) whereas the least resistance was

observed against Chloramphenicol (13.79%). The reason for developing increased resistance of *S. aureus* isolates against these antibiotics could be their frequent administration. In a different study conducted by Ullah *et al.*[26], it was shown that *S. aureus* showed a high rate of resistance against Amoxicillin (89.2%) which corroborates with the findings of our study (Amoxicillin: 65.52%). Moreover, it was observed that Chloramphenicol was the most effective antibiotic used in this study which is in agreement with the findings (53.3% susceptibility) of Olugbue *et al.*[27]. The MAR index indicates whether the isolates are from high or low antibiotic using region making it an effective tool for health risk assessment. MAR index values higher than 0.2 are considered to have originated from high-risk sources where antibiotics are often used. And MAR index values of less than or equal to 0.2 indicates a strain originated from sources where antibiotics are seldom or never used[22,28]. In this study, it was observed that out of 29 isolates, 24 isolates had a MAR index of greater than 0.2 which suggested that the *S. aureus* isolates have been identified from a high-risk source (human sputum sample). It was seen that extracts of both plants showed maximum activities at the highest concentration used in the study (5 mg mL^{-1}). The antibacterial activities of the extracts were found better compared to the standard antibiotic (Erythromycin). Moreover, between the two acetone extracts used in this study, increased diameter for inhibition zone (mm) was observed for *T. chebula* extract in all three different concentrations which suggested that it has better antibacterial activity against *S. aureus* isolates. It is stated in a report that fruiting bodies of *T. chebula* exhibited antibacterial activity against methicillin-resistant *S. aureus* and the main components responsible for this effect are gallic acid and its ethyl ester. Another study observed that ethanedioic acid and ellagic acid from the fruits of *T. chebula* exhibited moderate inhibitory activity against intestinal *E. coli*, respectively[29].

On the other hand, a study on *E. variegata* and its antibacterial effects against cariogenic oral bacteria revealed that isoflavonoids of *E. variegata* had bactericidal activity against methicillin-resistant *S. aureus* and various other strains among which the most active compounds are erycristagallin and orientanol B[30]. Another study revealed that isoflavonoids extracted from *E. Variegata* had shown substantial anti-MRSA activity[31]. So, it could be said that these two plant extracts could be used in future as alternatives to antibiotics to which *S. aureus* has grown resistant.

CONCLUSION

This study has found the potential effect of *Terminalia chebula* and *Erythrina variegata* plant extracts against antibiotic-resistant bacterial isolates. Moreover, *T. chebula* and *E. variegata* have shown better inhibitory activity than the standard antibiotic (Erythromycin) used in this study to which most of the bacterial isolates were found resistant.

SIGNIFICANCE STATEMENTS

In brief, *T. chebula* and *E. variegata* plant extracts exhibited good antibacterial activity towards *S. aureus* indicating the possibility of further exploration of their potential anti-microbial activity through *in vitro* and *in vivo* study. This study will help the researchers to embark on the further explorative study on the active compounds isolated from these plants which are required to understand their pharmacological effect as well as employ them as an antimicrobial agent in future.

ACKNOWLEDGMENT

This study was fully funded by the research and publication cell, University of Chittagong, Chattogram-4331, Bangladesh.

REFERENCES

1. Cashat-Cruz, M., J.J. Morales-Aguirre and M. Mendoza-Azpiri, 2005. Respiratory tract infections in children in developing countries. Seminars Pediatr. Infect. Dis., 16: 84-92.
2. Eccles, M.P., J.M. Grimshaw, M. Johnston, N. Steen and N.B. Pitts *et al*., 2007. Applying psychological theories to evidence-based clinical practice: Identifying factors predictive of managing upper respiratory tract infections without antibiotics. Implementation Sci., Vol. 2. 10.1186/1748-5908-2-26.
3. El-Radhi, A.S., 2018. Fever in Common Infectious Diseases. In: Clinical Manual of Fever in Children, El-Radhi, A.S. (Ed.)., Springer, Cham, pp: 85-140.
4. Gagneja, D., N. Goel, R. Aggarwal and U. Chaudhary, 2011. Changing trend of antimicrobial resistance among gram-negative bacilli isolated from lower respiratory tract of ICU patients: A 5-year study. Indian J. Crit. Care Med., 15: 164-167.
5. Wilke, M.S., A.L. Lovering and N.C. Strynadka, 2005. β-lactam antibiotic resistance: A current structural perspective. Curr. Opin. Microbiol., 8: 525-533.

6. Karchmer, A.W., 2004. Increased antibiotic resistance in respiratory tract pathogens: PROTEKT US-an update. Clin. Infect. Dis., 39: S142-S150.
7. Kumarasamy, K.K., M.A. Toleman, T.R. Walsh, J. Bagaria and F. Butt *et al*., 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan and the UK: A molecular, biological and epidemiological study. Lancet Infect. Dis., 10: 597-602.
8. Guitor, A.K. and G.D. Wright, 2018. Antimicrobial resistance and respiratory infections. Chest, 154: 1202-1212.
9. Kollef, M.H., Y. Golan, S.T. Micek, A.F. Shorr and M.I. Restrepo, 2011. Appraising contemporary strategies to combat multidrug resistant gram-negative bacterial infections-proceedings and data from the gram-negative resistance summit. Clin. Infect. Dis., 53: S33-S55.
10. Bennett, P.M., 2008. Plasmid encoded antibiotic resistance: Acquisition and transfer of antibiotic resistance genes in bacteria. Br. J. Pharmacol., 153: S347-S357.
11. Aly, M. and H.H. Balkhy, 2012. The prevalence of antimicrobial resistance in clinical isolates from gulf corporation council countries. Antimicrob. Resist. Infect. Control, Vol. 1. 10.1186/2047-2994-1-26.
12. Yap, P.S.X., B.C. Yiap, H.C. Ping and S.H.E. Lim, 2014. Essential oils, a new horizon in combating bacterial antibiotic resistance. Open Microbiol. J., 7: 6-14.
13. Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods: A review. Int. J. Food Microbiol., 94: 223-253.
14. Trombetta, D., F. Castelli, M.G. Sarpietro, V. Venuti and M. Cristani *et al*., 2005. Mechanisms of antibacterial action of three monoterpenes. Antimicrob. Agents. Chemother., 49: 2474-2478.
15. Pasdaran, A., A. Pasdaran and D. Sheikhi, 2016. Volatile Oils: Potential Agents for the Treatment of Respiratory Infections. In: The Microbiology of Respiratory System Infections, Kon, K. and M. Rai (Eds.)., Elsevier Inc., pp: 237-261.
16. Nosalova, G., L. Jurecek, U.R. Chatterjee, S.K. Majee, S. Nosal and B. Ray, 2013. Antitussive activity of the water-extracted carbohydrate polymer from *Terminalia chebula* on citric acid-induced cough. Evidence Based Complementary Altern. Med., Vol. 2013. 10.1155/2013/650134.
17. Kumar, A., S. Lingadurai, A. Jain and N.R. Barman, 2010. *Erythrina variegata* Linn: A review on morphology, phytochemistry and pharmacological aspects. Pharmacogn. Rev., 4: 147-152.
18. Islam, N.N., M. Akter, Z. Farzana, A.J.B. Kader, I. Uddin, A.M.A.M.Z. Siddiki and K.M. Kamaruddin, 2014. Detection of *Staphylococcus aureus* in frozen chicken rinse through bacteriological and NUC gene specific PCR methods and their drug resistance patterns in Southern Chittagong, Bangladesh. Res. J. Microbiol., 9: 251-264.
19. Chakraborty, S.P., S.K. Mahapatra and S. Roy, 2011. Biochemical characters and antibiotic susceptibility of *Staphylococcus aureus* isolates. Asian Pac. J. Trop. Biomed. India, 1: 212-216.
20. El-Hadedy, D. and S.A. El-Nour, 2012. Identification of *Staphylococcus aureus* and *Escherichia coli* isolated from Egyptian food by conventional and molecular methods. J. Genet. Eng. Biotechnol., 10: 129-135.
21. Onwubiko, N.E. and N.M. Sadiq, 2011. Antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. Pan Afr. Med. J., Vol. 8. 10.4314/pamj.v8i1.71050.
22. Odjadjare, E.E., E.O. Igbinosa, R. Mordi, B. Igere, C.L. Igeleke and A.I. Okoh, 2012. Prevalence of multiple antibiotics resistant (MAR) *Pseudomonas* species in the final effluents of three municipal wastewater treatment facilities in South Africa. Int. J. Environ. Res. Public Health, 9: 2092-2107.
23. Azwanida, N.N., 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med. Aromat. Plants, Vol. 4. 10.4172/2167-0412.1000196.
24. Mostafa, M., H. Hossain, M.M. Hasan, M.A. Hossain and S. Parveen, 2014. Antibacterial activity of six medicinal plants used in traditional medicine growing in Bangladesh. Int. J. Pharm. Sci. Res., Vol. 5. 10.13040/ijpsr.0975-8232.5(6).2210-15.
25. Ferdous, F., S. Ahmed, S.K. Das, M.J. Chisti and D. Nasrin *et al*., 2018. Pneumonia mortality and healthcare utilization in young children in rural Bangladesh: A prospective verbal autopsy study. Trop. Med. Health, Vol. 46. 10.1186/s41182-018-0099-4.
26. Ullah, B., S. Ahmed, M. Shahariar and S. Yesmine, 2016. Current trend of antibiotic resistance in lower respiratory tract infections (LRTIS): An experience in a teaching hospital in Bangladesh. Bangladesh Pharm. J., 19: 85-91.
27. Olugbue, V. and S. Onuoha, 2011. Prevalence and antibiotic sensitivity of bacterial agents involved in lower respiratory tract infections. Int. J. Biol. Chem. Sci., 5: 774-781.
28. Adeleke, E.O. and B.O. Omafuvbe, 2011. Antibiotic resistance of aerobic mesophilic bacteria isolated from poultry faeces. Res. J. Microbiol., 6: 356-365.
29. Kim, H.G., J.H. Cho, E.Y. Jeong, J.H. Lim, S.H. Lee and H.S. Lee, 2006. Growth-inhibiting activity of active component isolated from *Terminalia chebula* fruits against intestinal bacteria. J. Food Prot., 69: 2205-2209.
30. Sato, M., H. Tanaka, S. Fujiwara, M. Hirata, R. Yamaguchi, H. Etoh and C. Tokuda, 2003. Antibacterial property of isoflavonoids isolated from *Erythrina variegata* against cariogenic oral bacteria. Phytomedicine, 10: 427-433.
31. Tanaka, H., M. Sato, S. Fujiwara, M. Hirata, H. Etoh and H. Takeuchi, 2002. Antibacterial activity of isoflavonoids isolated from *Erythrina variegata* against methicillin-resistant *Staphylococcus aureus*. Lett. Appl. Microbiol., 35: 494-498.