El-Sayed et al., International Current Pharmaceutical Journal, December 2014, 4(1): 336-339
http://www.icpjonline.com/documents/Vol4Issue1/01.pdf

International Current Pharmaceutical Journal

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Microbiological evaluation of antibacterial potentiality of some edible plant extracts against multidrug resistant (MDR) human pathogens

*Mohamed Helal El-Sayed[1,2], Bahgat Mohamed Refaat[2] and Mohamed Hamed Sharaf[2]

[1]*Biology Department, Faculty of Science and Arts, Northern Border University (Rafhaa), Kingdom of Saudi Arabia*
[2]*Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt*

ABSTRACT
Antimicrobial resistance is a subject of great concern in public health and also in the designing of strategies for current therapeutic protocols all over the world. Plants used for traditional medicine contain a wide range of substances which can be used to treat various infectious diseases. Hence, antibacterial activities of aqueous extracts of 10 plant species were studied against 25 multidrug resistant (MDR) clinical isolates using the agar well diffusion method. The most resistant organisms were *Acinetobacter baumanii (A. baumanii)* (resistant to 16 different antibiotics), *Enterococcus faecium (E. faecium)* (resistant to 15 different antibiotics), *Pseudomonas aeruginosa (P. aeruginosa)* (resistant to 15 antibiotics), *Gemella morbillorum (G. morbillorum)* (resistant to 14 different antibiotics), *Enterobacter cloacae (E. cloacae)* (resistant to 13 different antibiotics) respectively. Among the tested plant extracts, only extracts of *Allium cepa, Allium sativum, Foeniculum vulgare, Matricaria chmomilla, Salvia offcinalis* and *Thymus Vulgaris* showed strong antibacterial activity against MDR isolates with inhibition zones ranging from 8.33 to 26 mm. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the most active plant extracts; *Allium cepa* and *Foeniculum vulgare* were ranged from 0.062 to 0.25 mg/ml and 0.031 to 0.125 mg/ml, respectively. *Foeniculum vulgare* extract was bactericidal for all bacteria while *Allium cepa* extract was bacteriostatic. Hence, the discovered compounds from these plants can use as templates for the development of new antibacterial agents.

***Key Words*:** Antibacterial activity, clinical isolates, drug-resistant, medicinal plants.

INTRODUCTION

In the last many years the commercial antimicrobial drugs were used to control the microbial pathogenecity and other infectious diseases. Excess use of antibiotics results in multiple drug resistance (MDR) in many bacterial pathogens. Successful treatment of infectious diseases and control of microbial pathogenicity results in increasing the drug resistance (Fu *et al.*, 2007). Similarly, preservatives like sulfites, nitrates, nitrites and antibiotics, are harmful for human health and have many side effects, including headache, nausea, weakness, mental retardation, seizures, cancer and anorexia (Rangan and Barceloux, 2009).

Although production of new antibacterial compounds by the pharmaceutical companies had been increased in the last years, the resistance of the microbial pathogens to these drugs was also increased (Adwan and Mhanna, 2008). The global emergence of multi-drug resistant (MDR) bacteria significantly causing treatment failure due to increasingly limiting the effectiveness of current drugs (Hancock, 2005). Bacterial resistance to chemically unrelated antimicrobial agents is a public health concern (Sharma *et al.*, 2005) and may be caused by over-expression of MDR efflux pumps (Li and Nikaido, 2004).

Emergence of antimicrobial resistance is due to losing the activity of numerous classes of antimicrobial agents, often as a result of the selective pressure of antimicrobial usage. Among the important emerging resistance problems are penicillin resistance in streptococci, vancomycin resistance in enterococci (and eventually staphylococci), oxacillin resistance in staphylococci, resistance to extended-spectrum Enterobacteriaceae, and carbapenem resistance in *P. aeruginosa* (Pfaller *et al.*, 1998), and carbapenem resistance in *P. aeruginosa* (Pfaller *et al.*, 1998), aminopenicillins, ureidopenicillins, first and second-generation cephalosporins, cephamycins, most aminoglycosides, chloramphenicol, and tetracyclines resistance in *A. baumannii* (Murray and Moellering, 1979).

Because the resistance to antibiotics had been increased, the need to discover new and innovative antimicrobial agents has also increased. For thousands of years, using of natural products for treatment of infectious diseases and controlling of microbial pathogenicity all over the world is the major alternative way to predate the introduction of antibiotics and other modern drugs. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists (Kafaru, 1994). The popular use of the plants as remedies for treatment of many infectious diseases, searches for plants containing antimicrobial substances are frequent (Betoni *et al.*, 2006).

Plants contain a variety of important secondary metabolites as tannins, alkaloids and flavonoids, which possess *in vitro* antimicrobial properties (Lewis and Ausubel, 2006). Phytotherapy manuals recorded a various medicinal plants used for infectious diseases treatment because of their availability, fewer side effects and reduced toxicity (Lee *et al.*, 2007).

Several studies discussed the antimicrobial activity of different plant extracts (Bonjar, 2004; Islam *et al.*, 2008; de Boer *et al.*, 2005). Some plants exhibited a good treatment for the urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections (Brantner and Grein, 1994; Somchit *et al.*, 2003). Using plants for medicinal purposes is an important part of the culture and the tradition in Egypt. Therefore, this in vitro

***Corresponding Author**:
Dr. Mohamed Helal El-Sayed Ahmed, Assistant Professor
Biology Department, Faculty of Science and Arts
Northern Border University (Rafhaa)
Kingdom of Saudi Arabia
E-mail: *m_helal2007rm@yahoo.com*
Contact No.: +966 0506862801 & +966 0545905650

Table 1: List of the studied plants.

No	Studied plants			Family	Plant part
	Local name	English name	Botanical name		
1	Bassal	Onions	*Allium cepa*	Amaryllidaceae	Derna
2	Thoum	Garlic	*Allium sativum*	Amaryllidaceae	Lobes mature
3	Shamar	Fennel	*Foeniculum vulgare*	Apiaceae	seeds
4	Moghat	Calotropis	*Glossostemon bruguieri*	Steculiaceae	root
5	Bapong	Chamomile	*Matricaria chmomilla*	Asteraceae	Blooming flowers
6	Habbat al-barakah	Black Cumin	*Nigella sativa*	Ranunculaceae	seeds
7	Ikleel Al Jabal	Rosemary	*Rosmarinus officinailis*	Lamiaceae	Leaves, stems
8	Maramia	Sage	*Salvia offcinalis*	Lamiaceae	Leaves, flowering heads
9	Zatar	Thyme	*Thymus Vulgaris*	Lamiaceae	Flowering branches
10	Zanjabil	Ginger	*Zingiber officinale*	Zingiberaceae	Alrizumat

study was aimed at screening selected plants for their antibacterial activity and evaluating their potential use in treating infections caused by multi-drug resistant clinical bacteria.

MATERIALS AND METHODS

Plant materials and preparation of the aqueous extracts

Ten edible plants used in this work were purchased from the local Egyptian herbal. The plant parts used were) Derna, Lobes mature, seeds, root, Blooming flowers, Leaves, stems, flowering heads, Flowering branches, Alrizumat), data are recorded in table 1. The above plant parts were cut, washed with distilled water, dried and then powdered finely using electric blender. 10 grams of ground, air-dried plant material was soaked in 100 ml of distilled water in conical flasks, and then incubated at room temperature for 72 hours with shaking at 120 rpm. Centrifugation of the crude extracts was carried out at 3000 rpm for 10 minutes at 25°C then evaporated at 80°C in a rotary evaporator. The dried extracted samples were dissolved in distilled water separately to the concentration of 100 mg/ml and further centrifuged at 10,000 rpm to remove the undissolved residues. The extract solutions were stored at 4°C for further experiments.

Bacterial strains

The bacterial strains used in this study included the following clinical isolates; *A. baumannii* (Tetracycline-, vancomycin-, piperacillin-, clindamycin-, trimethoprime/sulfomethoxazole-, oxacillin-, azactam-, ciprofloxacin-, flucloxacillin-, ampicillin-, gentamycin-, bacitracin-, chloramphenicol-, erythromycin-, tobramycin- and rifampicin-resistant, and amikacin-sensitive), *E. faecium* (Tetracycline-, vancomycin-, piperacillin-, clindamycin-, trimethoprime/sulfomethoxazole-, oxacillin-, azactam-, ciprofloxacin-, flucloxacillin-, ampicillin-, gentamycin-, bacitracin-, chloramphenicol-, erythromycin and rifampicin-resistant, tobramycin-intermediate,and-, Amikacin-resistant), *P. aeruginosa* (Tetracycline-, vancomycin-, piperacillin-, clindamycin-, trimethoprime/ sulfomethoxazole-, oxacillin-, ciprofloxacin-, flucloxacillin-, ampicillin-, gentamycin-, bacitracin-, chloramphenicol-, erythromycin-, tobramycin- and rifampicin-resistant-and azactam- intermediate, and Amikacin-sensitive), *G. morbillorum* (Tetracycline-, vancomycin-, piperacillin-, clindamycin-, trimethoprime/ sulfomethoxazole-, oxacillin-, azactam-, flucloxacillin-, ampicillin-, gentamycin-, chloramphenicol-, erythromycin-, rifampicin- and ciprofloxacin-resistant, and tobramycin- and bacitracin-intermediate, and amikacin-sensitive), *E. cloacae* (Tetracycline-, vancomycin-, piperacillin-, clindamycin-, oxacillin-, flucloxacillin-, ampicillin-, gentamycin-, bacitracin-, chloramphenicol-, erythromycin-, rifampicin- and tobramycin-resistant, and Azactam- and trimethoprime/Sulfomethoxazole-interme-diate, and amikacin- and ciprofloxacin-sensitive). Also, Gram-positive *S. aureus* ATCC 29213 and Gram-negative *E. coli* ATCC 25922 were used as reference strains for comparison of MIC and inhibition zones.

Culture preparation

The bacterial strains were inoculated in 1 ml Mueller-Hinton Broth (MHB) and grown overnight at 37°C separately before performing antimicrobial assay. Each bacterial strain was refreshed by inoculation of 50µl of overnight culture into 5 ml of MHB (pH 7.2) under aseptic conditions then shaken for 16 hours in a water bath at 37°C. The bacterial cells were harvested by centrifugation at 4°C for 15 minutes with 3000 rpm then washed twice with phosphate buffer saline (pH 7.4) and resuspended in MHB. The inoculum concentration was adjusted to 10^7 CFU/ml.

Antimicrobial assay using the disc diffusion method

The disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Zaidan *et al.* (2005) to assess the presence of antibacterial activities of the plant extracts. Plant extracts were screened for antibacterial activity against the highest five species of multi-drug resistant bacteria; *E. faecium, A. baumanii, P. aeruginosa, G. morbillorum, E. cloacae,* and two reference strain *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. Nutrient agar mixed with bacteria at a concentration of $1x10^6$ CFU/ml was poured in Petri dishes and allowed to cool. The plant extracts equivalent to 1000 µg, dissolved in distilled water, was applied to sterile paper discs (6 mm diameter).

To facilitate the dissolution of extracts under study, 5% (v/v) dimethyl sulfoxide (DMSO) was added which not affected the growth of microorganisms (control experiment). The paper discs were allowed to be free from any water content by evaporation and deposited on 96-well plates at room temperature, then transferred to the surface of the agar plates inoculated with the tested bacterial strains. Plates were incubated for 24 h at 37°C and antibacterial activity was evaluated by measuring the diameter of inhibition zone observed around the discs. In addition amikacin (10µg/ml) was used as a positive control to determine the sensitivity of the strains by the disc diffusion method (Bauer *et al.*, 1966). Zones of inhibition were measured in mm after 24 h of growth. The experiment was performed in triplicate.

Determination of minimum inhibitory concentrations and minimum bactericidal concentrations

The microplate method of Eloff (1998) was used to determine the minimum inhibitory concentrations (MIC) values for plant extracts with antibacterial activity. Residues of plant extracts were dissolved in 25 mg/ml using the extracting aqueous solvent. All extracts are tested at 1000 µg/ml (Al-Fatimi *et al.*, 2007) and serially diluted twofold to 15.6 µg/ml in a 96-multiwell polystyrene flat-bottomed microplate (Sigma-Aldrich, St. Louis, MO,USA) after which 100 µl (1x10^6 CFU/ml) of bacteria are added to each well. The antibiotic ampicillin was added as reference antibiotic in each assay. Extract-free solution was used as the negative control. Pre-incubation absorbance values were read from an ELISA reader (Biokinetic Reader EL 350, Bio-Tek™ Instruments, Winooski, VT, USA). The microplates were then incubated overnight at 37°C and absorbance values were read after 24 h and MIC values were recorded. The experiment was performed in duplicate. Bacterial cells were transferred from the MIC plate and subcultured on solid nutrient agar by streaking on the surface of the agar. The plates were incubated overnight at 37°C and the minimum bactericidal concentrations (MBC) were determined after 24 h. Plates that did not show growth were considered to be the MBC for the extract or drug used. The experiment was carried out in duplicate.

Statistical analysis

The antibacterial activity of the tested extracts comparing with standard antibiotic discs was evaluated by applying a two tailed-unpaired *t*-test. All values are expressed as the mean ± standard deviation and $P>0.05$ values were considered to indicate statistically significant differences. Numerical data were analyzed using the Student's *t*-test using statistical Package for the Social Sciences (SPSS), SPSS Statistics versions 16.0 and later runs under Windows, Mac, and Linux.

RESULTS AND DISCUSSION

Antibacterial activity of plant extracts

In the present study ten plants belonging to seven botanical families were tested in vitro for their antibacterial activity against both five drug-resistant clinical isolates and other two standard bacterial strains. Among 10 aqueous extracts that were tested for antibacterial activity against multidrug resistant isolates, only 6 extracts (60%) exhibited variable antibacterial activity against multidrug resistant isolates. The aqueous extracts of all the potent plants resulted in a variable zone of inhibition ranging from 8.33 to 26 mm for all bacteria tested (table 2).

Extracts were not strain specific and showed antibacterial activity for all seven bacterial species. Exceptions were observed for the extract of *Glossostemon bruguieri, Nigella sativa, Rosmarinus officinailis, Zingiber officinale* that showed no activity at all for all tested bacteria. *Matricaria chmomilla* and *Thymus vulgaris* extracts showed antibacterial activity against only *P. aeruginosa* and *E. faecium* with diameter of inhibition zone 14.33 and 9.66 mm respectively. *Allium sativum* extract showed no activity against both *A. baumanii* and *P. aeruginosa*, but resulted in a zone of inhibition ranged from 8.33 to 12 mm against other isolates. *The Salvia offcinalis extract* showed antibacterial activity against *A. baumanii* and *E. faecium* with zone of inhibition 8.66 and 11.66 mm respectively. *Allium cepa* and *Foeniculum vulgare* extracts were shown strong antibacterial activity with the zone of inhibition diameter ranging from 15.38 to 26 mm and were selected to be the most potent extracts for all test bacteria in all the assays carried out in this study (figure 1).

Activity of plant extracts under study on the multi-drug resistant isolates were compared to that of standard strains and it was found that only *Allium cepa, Allium sativum* and *Foeniculum vulgare* showed antibacterial activity with the zone of inhibition diameter ranging from 8.33 to 26 against the tested strains.

Table 2: Zones of inhibition (mm) induced by aqueous extracts from the selected plants against multi-drug resistant bacteria and the reference bacteria.

Plant species	Bacterial test strains						
	Clinical isolates					Standard strains	
	E. faecium	*A. baumanii*	*P. aeruginosa*	*G. morbillorum*	*E. cloacae*	*E. coli* ATCC 25922	*S. aureus* ATCC 29213
*Allium cepa****	20	15.38	20	19.66	19	18.66	22
*Allium sativum***	10.33	0.0	0.0	10.66	12	10.33	8.33
*Foeniculum vulgare****	17	19.66	18.33	20.33	21	23	26
*Glossostemon bruguieri**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
*Matricaria chmomilla***	0.0	0.0	14.33	0.0	0.0	0.0	0.0
*Nigella sativa**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
*Rosmarinus officinailis**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
*Salvia offcinalis***	11.66	8.66	0.0	0.0	0.0	0.0	0.0
*Thymus Vulgaris***	9.66	0.0	0.0	0.0	0.0	0.0	0.0
*Zingiber officinale**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Amikacin (10 µg/disc)	15	17	21	20	20	23	21
Negative control (DMSO, 100 µl)	0.00	0.00	0.00	0.00	0.00	0.00	0.00

***: most potent **:median potent *:least potent

Values are expressed as mean ± standard deviation (n=4).

Table 3: A summary table for the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) assays.

No.	Microorganism	Plant species	MIC[a] (µg/ml)	MBC[b] (µg/ml)
1	*E. faecium*	*Allium cepa*	250	500
		Foeniculum vulgare	125	250
2	*A. baumanii*	*Allium cepa*	250	500
		Foeniculum vulgare	125	250
3	*P. aeruginosa*	*Allium cepa*	250	500
		Foeniculum vulgare	125	250
4	*G. morbillorum*	*Allium cepa*	62.5	125
		Foeniculum vulgare	31.25	63
5	*E. cloacae*	*Allium cepa*	250	500
		Foeniculum vulgare	125	250
6	*S. aureus* ATCC 29213	*Allium cepa*	125	250
		Foeniculum vulgare	125	250
7	*E. coli* ATCC 25922	*Allium cepa*	125	250
		Foeniculum vulgare	62.5	125

a: Minimum inhibitory concentration

b: Minimum bactericidal concentration

Minimum inhibitory concentrations and minimum bactericidal concentrations

The two most potent extracts *Allium cepa* and *Foeniculum vulgare* showing considerable good antibacterial activity for each test organism were selected to determine MIC.

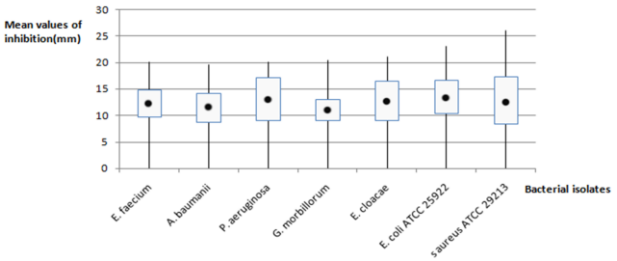

Figure 1: Mean values of inhibition (mm) of bacteria in relation to their susceptibility to the plant extracts (means are indicated by solid circles).

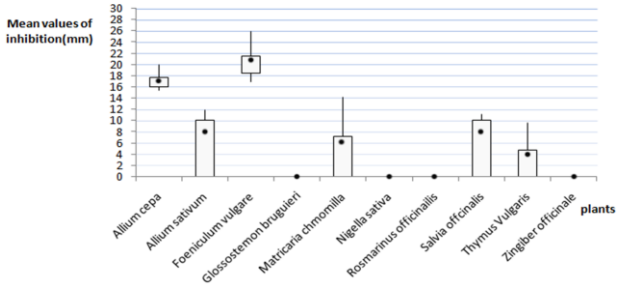

Figure 2: Mean values of antibacterial activity of plants against isolated bacteria (means are indicated by solid circles).

Values for MICs were dependent on the bacterial species. Generally, the MIC values were low 0.031-0.250 mg/ml showing that the extracts are potent (table 3). However, amikacin was a more potent antibacterial than all the extracts with MIC values ranging from 0.002-0.008 mg/ml. *Allium cepa* and *Foeniculum vulgare* were the most potent extracts against all the test bacteria. *Foeniculum vulgare* was the most potent showing the same trend of inhibition against all bacteria 0.031-0.125 mg/ml followed by *Allium cepa* with MIC values ranging from 0.062-0.250 mg/ml.

The results of the MBC assays indicate the results of the disc diffusion assay and also the MIC determination. These results further confirmed that *Allium cepa* and *Foeniculum vulgare* were the most potent extracts. These two extracts exhibited a bactericidal nature as observed from their MBC values that ranged from 0.063-0.5 mg/ml (table 3). From the MIC and MBC assays, Gram-negative species seemed to be more resistant to plant extracts than Gram-positive species as indicated by their high MIC and MBC dvalues (table 3). Other studies carried out have also shown Gram-negative strains to be less sensitive to antibiotics than Gram-positive bacteria (Stavri *et al.*, 2007; Doughari and Manzara, 2008). Gram-negative bacteria and mycobacteria both possess thick outer membranes that are highly hydrophobic, providing these organisms with a permeability barrier, especially towards hydrophilic compounds such as macrolide antibiotics such as erythromycin. This in part explains the observed less sensitivity to antimicrobials by Gram-negative bacteria than by Gram-positive organisms (Stavri *et al.*, 2007). Some of the extracts used in this study have shown antibacterial activity in other studies as well.

CONCLUSION

In conclusion, in this study the extracts from *Allium cepa* and *Foeniculum vulgare* were found to have potent antibacterial activities against the clinical and reference strains. Our results support the use of these plants in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search for new drugs.

REFERENCES

Adwan, G. and Mhanna, M. (2009). Synergistic effects of plant extracts and antibiotics on Staphylococcus aureus strains isolated from clinical specimens. Journal of Scientific Research, 3: 134-139.

AL-Fatimi, M., Wurster, M., Schwoder, G. and Lindequist, U. (2007). Antioxidant antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. Journal of Ethnopharmacol, 111: 657-666. [DOI]

Bauer, A., Kirby, W., Sherris, J. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45: 493-496.

Betoni, Jec., Mantovani, Rp., Barbosa, Ln., Di-Stasi, Lc., Fernandes, A. (2006). Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Mem. Inst., Oswaldo Cruz. 101 No. 4. [DOI]

Bonjar, (2004). Evaluation of antibacterial properties of some medicinal plants used in Iran J. Ethnopharmacol, 94: 301-305.

Brantner, A. and Grein, E. (1994). Antibacterial activity of plant extracts used externally in traditional medicine. J. Ethnopharmacol, 44: 35-40. [DOI]

De Boer, H., Kool, A., Broberg, A., Mziray, W., Headberg, I., Levenfors, J. (2005). Antifungal and antibacterial activity of some herbal remedies from Tanzania. j. Ethophormacol, 96: 461-469. [DOI]

Doughari, J. and Manzars, S. (2008). In vitro antibacterial activity of crude leaf extract of Mangifera indica Linn. African Journal of microbiology Research, 2: 67-72.

Eloff, J. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica, 64: 711–713. [DOI]

Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S. and Efferth, T. (2007). Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother Res, 21: 989-999. [DOI]

Hancock, Ew. (2005). Mechanisms of action of newer antibiotics for Gram-positive pathogens. Lancet Infect Dis., 5: 209218. [DOI]

Islam, B., Khan, S., Haque, I., Alam, M., Mushfiq, M., Khan, Au. (2008). Novel Anti-adherence activity of Mulberry Leaves: inhibition of Streptococcus mutans, biofilm by 1-deoxynojirimycin isolated from Morus alba. J. Antimicrob. chemother, 62 (4): 751-757. [DOI]

Kafaru, E. (1994). Immense Help from Natives Workshop, 1st Ed, Elizabeth Kafaru, Lagos, Nigeria. Pp, 11-14.

Lee, S., Cha, K., Kim, S., Altantsetseg, S., Sarangerel, O., Nho, C. (2007). The Antimicrobial activity of essential oil from Dracocephalum foetidum against pathogenic microorganisms. J. Microbiol, 45: 53-57.

Lewis, K. and Ausubel, F. (2006). Prospects for plant-derived antibacterials Nature biotechnology, 24: 1504-7. [DOI]

Li, X., Nikaido, H. (2004). Efflux-mediated drug resistance in the bacteria. Drugs, 64: 159204. [DOI]

Murray, B. and Moellering, R. (1979). Aminoglycoside-modifying enzymes among clinical isolates of Acinetobacter calcoaceticus subsp. anitratus: explanation for high-level aminoglycoside resistance. Antimicrob Agents Chemother, 15: 190–9. [DOI]

Pfaller, M., Ronald, N., Gary. V., Kari, K. and the Sentry Participants Group. (1998). Bacterial pathogens isolated from patients with blood-stream infection: frequencies of occurrence and antimicrobial susceptibility patterns of the sentry antimicrobial surveillance program (United States and Canada). Antimicrob. Agents Chemother, 42: 1762-1770.

Rangan, C. and Barceloux, D. (2009). Food additives and sensitivities. Dis. Mon., 55: 292311. [DOI]

Sharma, R., Sharma, C. and Kapoor, B. (2005). Antibacterial resistance: current problems and possible solutions, 59: 120129.

Somchit, M., Reezal, I., Nur, I. and Mutalib, A. (2003). In vitro antimicrobial activity of ethanol & water extracts of Cassia alata. J. Ethnopharmacol, 84: 4-1. [DOI]

Stavri, M., Piddock, L. and Gibbons, S. (2007). Bacterial efflux pump Inhibitor from natural sources. Journal of antimicrobial chemotherapy, 59: 1247-1260. [DOI]

Zaidan, M., Noor, A., Badrul, A. and Aldin, A. (2005). In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Tropical Biomedicine, 22(2): 165–170.