Pak. J. Biotechnol. Vol. 17 (1) 9 - 15 (2020) pISSN: 1812-1837 eISSN 2312-7791 www.pjbt.org
http://doi.org/10.34016/pjbt.2020.17.1.2

EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF SOME MEDICINAL PLANTS EXTRACT AGAINST SOME HUMAN PATHOGENIC BACTERIA

Anees Ahmad and Sayed Abdullah*

*University of Malakand, Chakdara, Pakistan. Email: *sabdullah.msib05asab@asab.nust.edu.pk*

Article received 29.1.2020, Revised 12.2.2010 Accepted 20.2.2020

ABSTRACT

The need of effective and economical antibiotics and antimicrobials has led to search for novel sources such as medicinal plants, fungi, archaea etc. In the present study, *Daphne mucronata* and *Mallotus philippensis* methanolic extracts were screened as antibacterial agents compared to some antibiotics against eight local isolates of human pathogenic bacteria. The susceptibility test was performed through well diffusion and disc diffusion method. Results reported that *D. mucronata* showed significant antibacterial activity against all the selected strains. The highest zones of inhibition of *D. mucronata* seed extract were recorded against, *Providencia stuartii* (20mm), *Brucella abortus* (19.3mm), *Proteus vulgaris* (19.3mm), *Klebsiella pneumonia* (19mm), followed by *Pseudomonas aeruginosa* (19mm), *Enterobacter sakazaki* (18.3mm), *Escherichia coli* (16.6mm) and *Staphylococcus aureus* (16.6mm). *D. mucronata seed extract* was reported as the most active against *B. abortus* (19.3mm), *E. sakazaki* (18.3mm), *E. coli* (16.6mm) and *K. pneumoniae* (19mm) when compared to Cefepime (8.1mm) and Ciprofloxacin (14.2mm). However, *D. mucronata* methanolic seed extract showed about similar antibacterial activity against *P. vulgaris* (19.3mm), *P. stuartii* (20mm), *P. aeruginosa* (19mm) and *S. aureus* (18.7mm), compared to the following antibiotics Cefepime (19.3mm) Ciprofloxacin (21mm), Moxifloxacin (21mm) and Ceftriaxone (20mm). On the other hand, *M. philippensis* showed insignificant antibacterial activity against the selected bacterial strains, but not significant compared to selected antibiotics. As conclusion, *D. mucronata extract* was considered as high potential antibacterial and recommend to be used as novel and economical source of effective antibacterial agent to combat against human pathogenic bacteria.

Keywords: Antibiotics activity; *Daphne mucronata*; *Mallotus philippensis*; Human Pathogenic bacteria; Antibiotics; medicinal Plants

INTRODUCTION

Antibiotics resistance is one of the most emerging problem associated with global health issues nowadays (Sharif *et al.,* 2018). In Pakistan, resistance against multi antibiotics has been reported for *S. aureus, K. pneumonia, E. coli, Salmonella typhi,* Pseudomonas spp. Enterococcus spp. and some air borne and food borne pathogenic bacteria at alarming stage (Bhatti *et al.,* 2019; Richwagen *et al.,* 2019; Chatham-Stephens *et al.,* 2019; Mehboob and Abbas 2019; Naz & Pasha 2019; Irum *et al.,* 2019). According to the published data, the mortality rate is about 0.7 million worldwide due to antimicrobial resistance pathogens, which will raise 10 million by 2050 (de Kraker *et al.,* 2019) The data reported by Center of Disease and Control showed about 23,000 mortality rates due to infection caused by antimicrobial resistance annually (Lim *et al.,* 2016). In Pakistan, a study reported that 40% infants die every year, due to extensive drug resistance and multi drug resistance bacteria (Hannan *et al.,* 2013; Qamar *et al.,* 2019). The overuse of antibiotics, its open purchase in the market and long-term usage over short interval have led to the major problem of emergence of resistant organisms (Taneja *et al.,* 2019). The widespread usage, self-medication of antimicrobial drugs, drugs prescription with improper susceptibility tests and long duration of hospitalization was suggested to increase the problem of multi drug resistance in developing countries (Malik and Bhattacharyya 2019; Atif *et al.,* 2019). The main issues and challenges reported regarding antibiotics resistance were, misleading advertisement, registered many products unnecessarily, illogical prescribing of antibiotics by physicians, easy access to drugs and antibiotics without prescription, widely used broad spectrum antibiotics with no surveillance systems, lack of experts and lastly, the wide spread use of antimicrobials in dairy farms, agriculture and poultry (Saleem *et al.,* 2018; Khan 2018).

There is a need of the development of new antimicrobials which has been discussed by many analysts (Mittra *et al.,* 2019; Jensen 2019; Sutcliffe 2011). The one of important natural source of antimicrobial agents is medicinal plants, which have great efficiency against variety of microorganisms including bacteria, fungi, and yeasts, insects, nematodes and other plants (Navarro *et al.,* 2019; Rasool 2008). Approximately 2,600

vascular plants were used by American people as traditional medicines (Frey and Meyers 2010). According to World Health Organization (WHO), up to 80% of developing countries' people uses traditional medicines as well as 65% of the whole world make use of it (Zougagh *et al.,* 2019). While country wise of the developed countries distribution, use of medicinal plants base medicine is 48% in Australia, 42% in the USA, 40–50% of the population in Germany and 49% in France depends upon plants for different health issues (Sharif *et al.,* 2018; Kanwal and Sherazi, 2017). It is reported that about 84% of people of Pakistan depend on herbal medicine and traditional medicinal approaches which has been reduce up to 60% since 1985 (Sharif *et al.,* 2018). Pakistan has reported about 10% of the medicinal plants in the field of medicine and as a source natural antimicrobial. Plants that are used in traditional medicines also called Phytomedicine, are plants derived medicines that contain chemicals or mixture of chemicals which acts individually or in combination for the treatment of diseases (Khan *et al.,* 2019; Van Wyk 2018).

Seeds of *D. mucronata* and leaves of *M. Philippensis* are used traditionally for the treatment of ulcer, rheumatism as a purgative abortifacient and against tooth ache (Khan *et al.,* 2015; Al-Snafi *et al.,* 2018). The leaves are poisonous to animals and applied against abscesses and the bark was used against bone diseases. The fruit can be used for eating and also for dyeing purposes in leather (Shaheen *et al.,* 2017; Baloch *et al.,* 2017). In Pakistan, the *D. mucronata* leaves decoction is given to the patients against inflammatory condition, muscle swelling conditions, in effective arthritis and fluke conditions (Zaidi *et al.,* 2015). Also, it has the anticancer activity against different human leukemic cells and animal models (Guo and Xin 2015; Sovrlić and Manojlović 2017). *D. mucronata* is a source of wide range of secondary metabolites particularly, coumarins, lignin, flavonoids, triterpenoids, daphnecin, coumarinolignans etc. (Yahya and Kazmi 2017).

Similarly, In India, it is reported that various parts of *M. philippensis* (Lam) were used for the treatment of bronchitis, diarrhea and diabetes, tape worm eye disease, as an antifungal, jaundice, malaria, urogenital infection and bacterial infection (Abdullah and Nadeem 2016). *M. philippensis* (Lam) also contains pesticidal, anti-microfilaria, hepatoprotective, anti-lithic and anti-oxidant properties (Gupta *et al.,* 2018; Safavi *et al.,* 2015; Amjad 2015). *M. philippensis* (Lam) fruit extract contain phytochemicals such as rottlerin fixed oil 47.80%, Mallotoxin 5.83-40%, Oleic, kamalin, myristics, palmitic acid, stearic acid, crotoxigenin, tannins, octacosanol, Oxalic acid, citric acid, homorottlerin, rottlerin, iso-rottlerin and rhmnoside (Ghosh *et al.,* 2019; Tripathi *et al.,* 2017; Sharma and Varma 2011). Aim of the study is to screen novel medicinal plants extract against human pathogenic bacteria for the as potential source for antibacterial agents against human pathogenic bacteria.

MATERIALS AND METHODS:

Plants Collection: *Daphne mucronata and Mallotus philippensis* (Lam) plants were collected form the Lower Dir, Northern area of Pakistan. The plants were identified by the Department of Botany at University of Malakand, Chakdara. The methanolic crude extract of seeds of *Daphne mucronata* and leaves of *Mallotus philippensis* were used against antimicrobial resistant bacteria.

Extract Preparation: Seeds of *Daphne mucronata* and leaves of *Mallotus philippensis* were dried for 15-20 days in shade and grounded through grinder under sterile condition. The methanolic extracts were obtained through maceration of 25g powdered seed of *Daphne mucronata* and leaves of *Mallotus philippensis* in 150mL of 100% methanol solvent. The mixture was filtered through Whatman filter paper. The final crude extract 10mg/mL was concentrated by a rotary evaporator (Ahmed *et al.,* 2019; Rahimullah and Shah 2019).

Bacterial Strains: The bacterial strains, named *B. abortus, E. sakazaki, E. coli, Enterobacter aerogenes,* Proteus vulgaris, *Klebsiella, Pneumonia, Providencua stuartii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, were kindly provided by Department of Pathology, Lady Reading Hospital (LRH) Peshawar, Pakistan. It is worth to mention that these strains were isolated from blood stream and urine of the patients.

Antibiotics used: The commercial antibiotics discs were purchased from Oxoid, UK Supplier, and used for comparison with the medicinal plants extract and to find out the sensitivity against the isolated pathogenic bacterial strains. The antibiotics includes Moxifloxacin (12µg), Ceftriaxone (5µg), ciprofloxacin (5µg) Cefepime (12µg).

Antibiotic Susceptibility Assay: Bacterial strains were grown and sub-cultured on Nutrient agar plate at 37°C. The antibiotic sensitivity test was performed through disc diffusion or well diffusion method. The plant extracts were dried over 0.5mm paper disc at sterile conditions and were tested against the selected bacterial strains and placed over inoculated media for 24hr at 37°C. The quantity of the plant extracts was 20µL of 10mg/mL per each disc. The same process was repeated for

selected commercial antibiotics. The zones of inhibition were noted and the process was done in triplicates to ensure the precise results (Ahmed *et al.*, 2019; Elizabeth *et al.*, 2019).

Statistical Analysis: The obtained data were expressed in mean with standard deviation and were statistically analyzed using PRISM version 5.01. The significant difference between the antibacterial activity of plant extracts and commercial antibiotics were find through ANOVA (p value < 0.05).

RESULTS:

Antibacterial Activity: Result showed that, all the tested antibiotics were found active against the *B. abortus* and *E. sakazakii* except the ciprofloxacin, which showed no antibacterial activity against *E. sakazakii*. Ciprofloxacin (28.13mm) Moxifloxacin (30.46) and Ceftriaxone (24.63) were found more active against *B. abortus* as compared to *M. philipinesis* (10mm) and *D. mucronata* (19.3mm) except Cefepime (8.1mm). However, the antibacterial activity of *D. mucronata* seed extract was found at considerable amount. On the other hand, the antibacterial activity of Ciprofloxacin (0.0mm), Moxifloxacin (16.16mm) and Cefepime (9.3mm) against *E. sakazakii* was significantly less as compare to *D. mucronata* seed extract (18.333 mm), except the Ceftriaxone (23.4mm). However, the *M. philipinesis* leaves extract (7.66mm, 10mm) showed no significant antibacterial as compared to selected antibiotics against *B. abortus* and *E. sakazakii* (Fig 1 and 2).

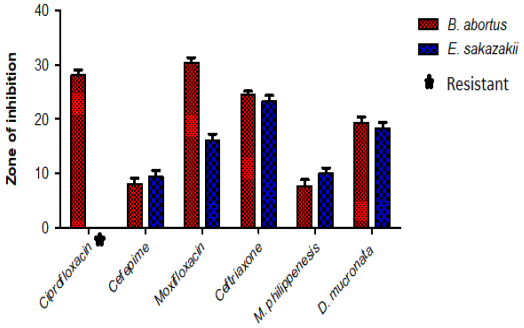

Fig. 1: Antibacterial activity of selected antibiotics and medicinal plants against *B. abortus* and *E. sakazakii.*

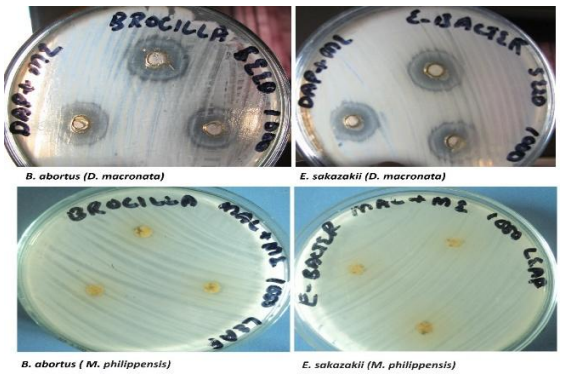

Fig.- 2: Antibacterial activity of *D. mucronata* and *M. philippensis* against *B. abortus* and *E. sakazaki.*

E. coli and *K. pneumoniae* were found to be sensitive to all the selected antibiotics and medicinal plants. The antibacterial activity of Ciprofloxacin (14.2mm) against *E. coli* was found less as compared to *D. mucronata* seed extract (16.6-mm) while the Cefepime (20mm), Moxifloxacin (24mm), Ceftriaxone (24.5mm) showed high activity compared to selected medicinal plants extracts. On the other hand, Cefepime (14mm) showed less activity while the Ceftriaxone (20-mm) showed similar activity against *K. pneumoniae* as compared to *D. mucronata* (19.5mm) seed extract. *However, M. philipinesis* (9.3mm) leaves extract showed no significant activity against *E. coli* and *K. pneumoniae* (Fig 3 and 4).

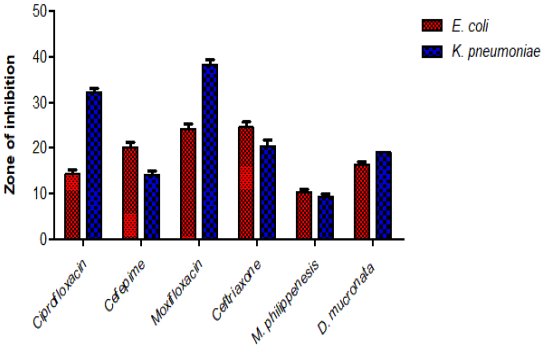

Fig -3: Antibacterial activity of selected antibiotics and medicinal plants extracts against *E. coli* and *K. pneumoniae.*

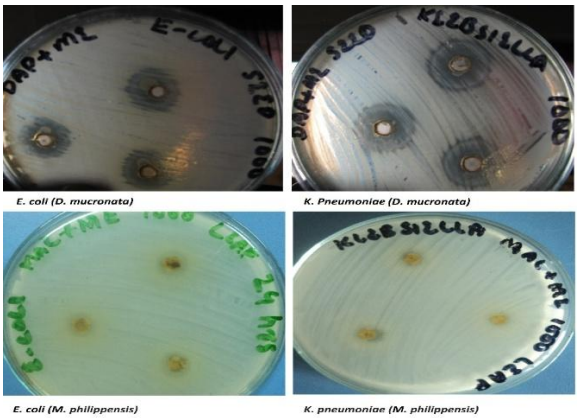

Fig -4: Antibacterial activity of *D. mucronata and M. philippensis* against *E. coli* and *K. pneumoniae*

Regarding *P. vulgaris* and *P. stuartii*, these pathogens were found to be sensitive against all the selected antibiotics and medicinal plants extracts. Cefepime (22mm) and Ceftriaxone (19mm) showed about similar activity against *P. vulgaris* as compared to *D. mucronata* (20mm) seed extract while the Ciprofloxacin (28.4mm) and Moxifloxacin (32.3mm) showed higher activity against

P. vulgaris. On the other hand, all selected antibiotics except Ciprofloxacin (29.2mm), showed about similar activity against *P. stuartii* as compared to *D. mucronata* (20mm) seed extract. However, *M. philipinesis* (7.1mm) leaves extract showed no significant activity against *P. vulgaris* and *P. stuartii* compared to selected antibiotics (Fig 5 and 6).

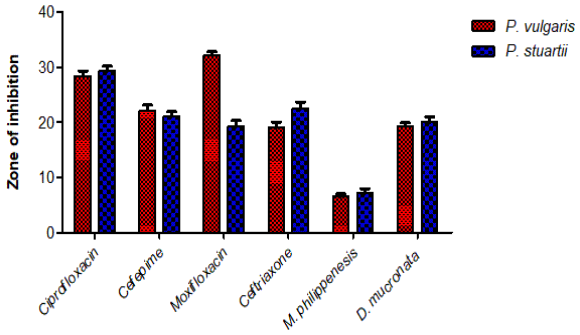

Fig-5: Antibacterial activity of selected antibiotics and medicinal plants extracts against *P. vulgaris* and *P. stuartii*.

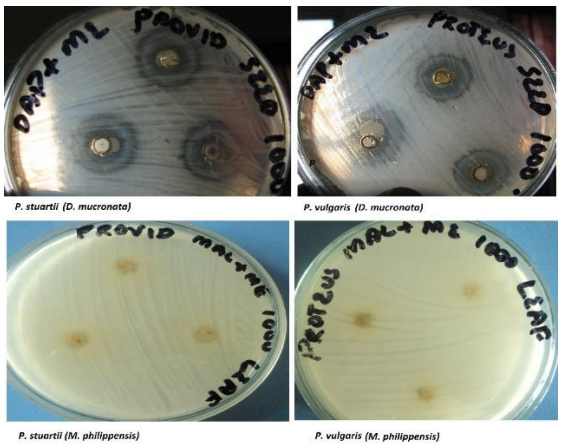

Fig 6. Antibacterial activity of *D. mucronata and M. philippensis* against *P. vulgaris* and *P. stuartii*.

In case of *P. aeruginosa* and *S. aureus*, the strains were found sensitive against both the selected antibiotics and medicinal plants extracts. The Ciprofloxacin (22mm) and Ceftriaxone (20 mm) showed about similar activity against the selected *P. aeruginosa* compared to *D. mucronata* (19.5mm) seed extract, while the Cefepime (34.8mm) and Moxifloxacin (27.3mm) showed more activity. On the other hand, the Ciprofloxacin (19.3mm) and Cefepime(19.4mm) showed about similar activity against the *S. aureus* as compared to *D. mucronata* (18.7mm) while the Moxifloxacin (23.4mm) and Ceftriaxone (33.3mm) showed higher activity. The *M. philipinesis* (7.6-mm) leaves extract showed no significant activeity against the *P. aeruginosa* and *S. aureus* (Fig 7 and 8).

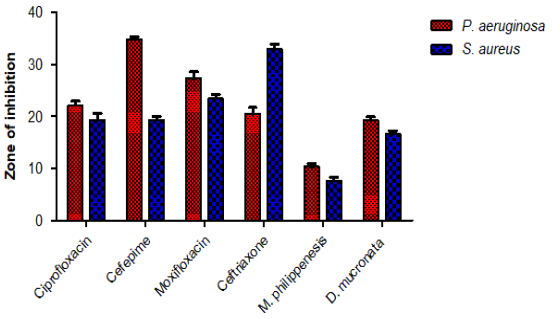

Fig.-7: Antibacterial activity of selected antibiotics and medicinal plant extracts against *P. aeruginosa* and *S. aureus*.

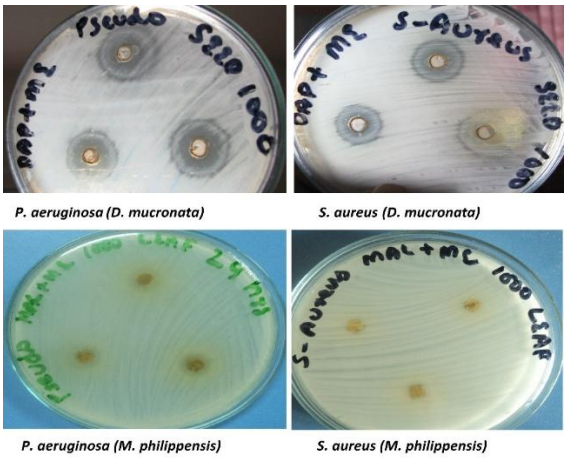

Fig 8: Antibacterial activity of *D. mucronata* and *M. philippensis* against *P. aeruginosa* and *S. aureus*.

DISCUSSION

Recent studies showed that pathogenic bacteria are found resistant to many antibiotics, and become less effective against many infections and diseases leading to high morbidity and mortality rate. The need of searching of novel and economical source of antibiotics is the main focus of the scientist nowadays. Medicinal plants have been screened for many years as a novel and economical source of new antimicrobial agents with effectiveness, fewer side effects and ecofriendly. In this study the *D. mucronata* and *M. philippensis* local medicinal plants in Pakistan was found active against human pathogenic bacteria. The current selected commercial antibiotics comparison with the selected medicinal plants extracts was done to show the effectiveness of the medicinal plants extracts against the selected pathogenic bacteria.

The data in the literature showed that there are few reports available regarding the antibacterial effect of *Daphne Ssp.* against human pathogenic bacteria in Pakistan. In a previous study, a significant antibacterial activity of Daphne spp. against *S. aureus*, *B. subtilis* and *E. coli* was reported which correlates with our present study (Javidnia *et al.*, 2003). Another study reported the antimicrobial activity

of Daphne spp. stem and leaves extract against *Bacillus subtilis, E. coli* and *P. aeruginosa*, similar to our findings (Tayoub *et al.,* 2012). Similarly, our study confirms the results of a recent study reported the significant antibacterial activity of *D. mucronata* against *S. aureus*. *P. aeruginosa*, *Streptococcus mutans*, *Neisseria sicca* and *Staphylococcus epidermidis* (Karamolah *et al.,* 2017). On the other side the antibacterial activity of *M. philippensis* is widely observed and many reports are found. Recent study reported significant antibacterial activity of *M. philippensis* phenolic extract against multi drug resistance bacteria and meticillin-resistant *S. aureus*, which showed some correlation with our results (Oyedemi *et al.,* 2016). Another study reported that the bark and leaf extract of *M. philippensis* showed effective antibacterial activity against *K. pneumoniae*, *E. coli*, *Enterobacter cloacae*, *Bacillus subtilis*, *Serratia marcescens*, *Salmonella typhi* and *Micrococcus luteus* (Sharma *et al.,* 2017). Similarly, a study reported significant antibacterial activity of *M. philippensis* fruit extract against selected gram-positive and gram-negative bacteria such as Escherichia spp. *P. aeru-ginosa*, *Shigella dysenteriae*, *K. pneumoniae*, *S. aureus*, Streptococcus spp. and *Bacillus subtilis*, which correlated with our findings (Adhav 2015).

Conclusion:

As conclusion, *D. mucronata* plant has the potential to compete with commercial antibiotics and could be use an economical and effective source for new antibiotics, to counter human pathogenic bacteria**.** The selected commercial antibiotics was found to be effective against the selected bacterial strains but recent studies showed resistance against many antibiotics leads to multi drug resistance bacteria. Phytochemical analysis and In-vivo mouse model experimentation of selected medicinal plants will strengthen the study to forward findings and effectiveness.

Acknowledgement:

Authors would like to special thanks to Murad Khan for his guidance and support throughout the study.

REFERENCES

Abdullah, S., Nadeem, M., Screening of local medicinal plant extracts against multi drugs resistance bacteria. International Journal of Bioassays 5(10): 4985-4989 (2016).

Adhav, M., Phytochemical screening and antimicrobial activity of *Mallotus philippensis* Muell. Arg. Journal of Pharmacognosy and Phytochemistry 3(5): 188-191 (2015).

Ahmed, N., Zeshan, B., Naveed, M., Afzal, M., Mohamed, M., Antibiotic resistance profile in relation to virulence genes *fimH*, *hlyA* and *usp* of uropathogenic *E. coli* isolates in Lahore, Pakistan. Tropical Biomedicine 36(2): 559-568 (2019).

Ahmed, S.R., Roy, R., Romi, I.J., Hasan, M., Bhuiyan, M.K.H., Khan, M.M.H., Phytochemical screening, antioxidant and antibacterial activity of some medicinal plants grown in Sylhet region. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14(1): 26-37 (2019).

Al-Snafi, A.E., Traditional uses of Iraqi medicinal plants. IOSR Journal of Pharmacy 8(8): 32-96 (2018).

Amjad, M.S., Ethnobotanical profiling and floristic diversity of Bana Valley, Kotli (Azad Jammu and Kashmir), Pakistan. Asian Pacific Journal of Tropical Biomedicine 5(4): 292-299 (2015).

Atif, M., Asghar, S., Mushtaq, I., Malik, I., Community pharmacists as antibiotic stewards: A qualitative study exploring the current status of antibiotic Stewardship Program in Bahawalpur, Pakistan. Journal of Infection and Public Health (2019).

Baloch, A.H., Baloch, I.A., Ahmed, I., Ahmed, S., A study of poisonous plants of Balochistan, Pakistan. *Pure and Applied Biology (PAB)* 6(3): 989-1001(2017).

Bhatti, P.M., Zeshan, B., Saleem, M.A., Qureshi, M., Riaz, S., Correlation of *blaNDM* to antibiotic resistance patterns in *Klebsiella pneumoniae* isolates from Tertiary Care Hospital in Lahore, Pakistan. *Life Science Journal of Pakistan* 1(2): 03-09 (2019).

Chatham-Stephens, K., Medalla, F., Hughes, M., Appiah, G.D., Aubert, R.D., Caidi, H., Angelo, K.M., Walker, A.T., Hatley, N., Masani, S., Nash, J., Emergence of extensively drug-resistant *Salmonella typhi* infections among travelers to or from Pakistan—United States, 2016–2018. Morbidity and Mortality Weekly Report 68(1): 11 (2019).

de Kraker, M.E.A., Stewardson, A.J., Harbarth, S., Will 10 million people die a year due to antimicrobial resistance by 2050? PLOS Medicine 13: e1002184 (2016).

Elizabeth, A.A., Kola, B.S., Rachael, A.M., Busayo, A.I., Adebobola, O.D., Ugochinyere, I.I., Antibacterial activity of the seed of *Dialium guineense* against selected enteric bacteria. Covenant Journal of Physical and Life Sciences 6(2): (2019).

Frey, F.M., Meyers, R., Antibacterial activity of traditional medicinal plants used by Hauden-

osaunee peoples of New York State. *BMC Complementary and Alternative Medicine* 10(1): 64 (2010).

Ghosh, S., Das, A.K., Sarkar, P., Sil, P.C., Oxidative stress in schistosomiasis, echinococcosis, and trypanosomiasis: a therapeutic approach. In Discovery and Development of Therapeutics from Natural Products Against Neglected Tropical Diseases pp. 219-239 (2019).

Guo, J.J., Li, Y.J., Xin, L.L., Tangeretin prevents prostate cancer cell proliferation and induces apoptosis via activation of Notch signalling and regulating the androgen receptor (AR) pathway and the phosphoinositide 3-kinase (PI3k)/Akt/mTOR pathways. Bangladesh Journal of Pharmacology 10(4): 937-947 (2015).

Gupta, V.K., Kaushik, A., Chauhan, D.S., Ahirwar, R.K., Sharma, S., Bisht, D., Anti-mycobacterial activity of some medicinal plants used traditionally by tribes from Madhya Pradesh, India for treating tuberculosis related symptoms. Journal of Ethnopharmacology 227: 113-120 (2018).

Hannan, A., Qamar, M.U., Usman, M., Waheed. K.A.I., Rauf, K., Multidrug resistant microorganisms causing neonatal septicaemia: In a tertiary care hospital Lahore, Pakistan. African Journal of Microbiology Research 7: 1896-1902 (2013).

Irum, S., Potter, R.F., Kamran, R., Mustafa, Z., Wallace, M.A., Burnham, C.A., Dantas, G., Andleeb, S., Draft genome sequence of a *blaNDM-1* and *blaPME-1* harboring *Pseudomonas aeruginosa* clinical isolate from Pakistan. Microbiol Resour Announc 8(17): e00107-19 (2019).

Javidnia, K., Miri, R., Jahromi R.B.N.N.K., A preliminary study on the biological activity of *Daphne mucronata* Royle. DARU Journal of Pharmaceutical Sciences 11(1): 28-1 (2003).

Jensen, C.S., Governing the consumption of antimicrobials: The Danish model for using antimicrobials in a comparative perspective. In Risking Antimicrobial Resistance Pp. 95-110 (2019).

Kanwal, H., Sherazi, B.A., Herbal medicine: Trend of practice, perspective, and limitations in Pakistan. *Asian Pacific Journal of Health Sciences* 4(4): 6-8 (2017).

Karamolah, K.S., Mousavi, F., Mahmoudi, H., Antimicrobial inhibitory activity of aqueous, hydroalcoholic and alcoholic extracts of leaves and stem of *Daphne mucronata* on growth of oral bacteria. GMS Hygiene and Infection Control 12: (2017).

Khan, A.W., Khan, A.U., Shah, S.M.M., Ullah, A., Faheem, M., Saleem, M., An updated list of neuromedicinal plants of Pakistan, their uses, and phytochemistry. Evidence-Based Complementary and Alternative Medicine (2019).

Khan, E.A., Situation analysis report on antimicrobial resistance in Pakistan-findings and recommendations for antibiotic use and resistance. *Internet. The Global Antibiotic Resistance Partnership (GARP) Pakistan* (2018).

Khan, M.P.Z., Ahmad, M., Zafar, M., Sultana, S., Ali, M. I., Sun, H., Ethnomedicinal uses of edible wild fruits (EWFs) in Swat valley, Northern Pakistan. Journal of Ethnopharmacology 173: 191-203 (2015).

Lim, C., Takahashi, E., Hongsuwan, M., Wuthiekanun, V., Thamlikitkul, V., Hinjoy, S., Day, N.P., Peacock, S.J., Limmathurotsakul, D. Epidemiology and burden of multidrug-resistant bacterial infection in a developing country. Elife 5: e18082 (2015).

Malik, B., Bhattacharyya, S. Antibiotic drug-resistance as a complex system driven by socio-economic growth and antibiotic misuse. Scientific Reports 9(1): 9788 (2019).

Mehboob, A., Abbas, T., Evaluation of microbial quality of street food in Karachi city, Pakistan: An epidemiological study. *Microbiology Research* 10(1): (2019).

Mittra, J., Bruce, A., Scannell, J.W., Tait, J., Regulatory and market influences on innovation pathways for the development of new antimicrobial drugs. Technology Analysis & Strategic Management Pp. 1-13 (2019).

Navarro, M.O., Piva, A.C., Simionato, A.S., Spago, F.R., Modolon, F., Emiliano, J., Azul, A.M., Chryssafidis, A.L., Andrade, G., Bioactive compounds produced by biocontrol agents driving plant health. In Microbiome in Plant Health and Disease Pp. 337-374, Springer, Singapore (2019).

Naz, N., Pasha, T.S., Prevalence of antibiotic-resistant airborne bacteria along roadsides in Rahim Yar Khan, Pakistan. *Polish Journal of Environmental Studies* 28(3): (2019).

Oyedemi, B.O., Shinde, V., Shinde, K., Kakalou, D., Stapleton, P.D., Gibbons, S. Novel R-plasmid conjugal transfer inhibitory and antibacterial activities of phenolic compounds from *Mallotus philippensis* (Lam) Mull. Arg. Journal of Global Antimicrobial Resistance 5: 15-21 (2016).

Qamar, M.U., Rasool, M.H., Jahan, S., Shafique, M., Aslam, B., Antimicrobial resistance. In

Antimicrobials, Antibiotic Resistance, Antibiofilm Strategies and Activity Methods. *IntechOpen* (2019).

Rahimullah, T.G., Shah, S.T., Phytochemical and antibacterial screening of *Cichorium intybus* seeds use in traditional medicine systems in Pakistan. International Journal of Basic Medical Sciences and Pharmacy 8(2): (2019).

Rasool, I., Shayegh, S., Taghizadeh, M., Astaneh, S.D.A., Phytotherapeutic prevention of dental biofilm formation. Journal of Phytotherapy Research 22: 1162–1167 (2008).

Richwagen, N., Lyles, J.T., Dale, B., Quave, C.L., Dale, B.L.F., Antibacterial activity of *Kalanchoe mortagei* and *K. fedtschenkoi* against ESKAPE pathogens. Frontiers in Pharmacology 10: 67 (2019).

Safavi, M., Shams-Ardakani, M., Foroumadi, A., Medicinal plants in the treatment of *Helicobacter pylori* infections. Pharmaceutical Biology 53(7): 939-960 (2015).

Saleem, Z., Hassali, M.A., Hashmi, F.K., Pakistan's national action plan for antimicrobial resistance: translating ideas into reality. The Lancet Infectious Diseases 18(10): 1066-1067 (2018).

Shaheen, S., Ahmad, M., Haroon, N., Edible wild plants: An alternative approach to food security, Springer International Publishing (2017).

Sharif, A., Asif, H., Younis, W., Riaz, H., Bukhari, I.A., Assiri, A.M., Indigenous medicinal plants of Pakistan used to treat skin diseases: a review. *Chinese Medicine* 13(1): 52 (2018).

Sharma, B., Thapa, S., Bajracharya, G.B., Promising antioxidative potentiality and antibacterial activity of *Mallotus philippensis* grown in Nepal. Journal of Pharmacognosy and Phytochemistry 6(3): 629-632 (2017).

Sharma, J. and Varma, R., A review on endangered plant of *Mallotus philippensis* (Lam.) M. Arg. Pharmacology online 3: 1256-1265 (2011).

Sovrlić, M.M., Manojlović, N.T., Plants from the genus Daphne: A review of its traditional uses, phytochemistry, biological and pharmacological activity. Serbian Journal of Experimental and Clinical Research 18(1): 69-80 (2017).

Sutcliffe, H.A. Report on responsible research and innovation. MATTER and the European Commission (2011).

Taneja, N., Sethi, S., Tahlan, A.K., Kumar, Y., Introductory Chapter: Stepping into the post-antibiotic Era—challenges and solutions. In Antimicrobial Resistance-A Global Threat. *IntechOpen* (2019).

Tayoub, G., Abu-Alnaser, A., Shamma, M., Microbial inhibitory of the *Daphne oleifolia* lam. ethanolic extract. Int. J. Med. Arom. Plants 2(1): 161–166 (2012).

Tripathi, I.P., Chaudhary, P., Pandey, P., *Mallotus philippensis*: A Miracle Stick (2017).

Van Wyk, B.E., and Wink, M., Medicinal plants of the world CABI (2018).

Yahya, S., and Kazmi, S.U., Anti-Campylobacter activity of extracts of *Daphne mucronata* and *Symplocos racemosa* against avian isolates. National Journal 2(1): 15 (2017).

Zaidi, A., Bukhari, S.M., Khan, F.A., Noor, T., Iqbal, N., Ethnobotanical, phytochemical and pharmacological aspects of *Daphne mucronata* (Thymeleaceae). Tropical Journal of Pharmaceutical Research 14(8): 1517-1523 (2015).

Zougagh, S., Belghiti, A., Rochd, T., Zerdani, I., Mouslim, J., Medicinal and aromatic plants used in traditional treatment of the oral pathology: The ethnobotanical survey in the economic capital Casablanca, Morocco (North Africa). Natural products and bioprospecting 9(1): 35-48 (2019).