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

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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, widelyuse 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 (Mitra 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 use traditional medicines as well as 65% of the whole world make use of it (Zoughagh 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; Sovrić 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-microbial, 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 rhombo (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 from 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 ground 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*, *Providencia 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 were used 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 found 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 compared 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).

**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 (16.6mm) 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 (20mm) 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. 2: Antibacterial activity of D. mucronata and M. philipinesis against B. abortus and E. sakazakii.

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.](image1)

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 (7.6mm) leaves extract showed no significant activity against the P. aeruginosa and S. aureus (Fig 7 and 8).

![Fig 6: Antibacterial activity of D. mucronata and M. philippensis against P. vulgaris and P. stuartii.](image2)

**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. aeruginosa, 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.

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