Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Study of anticancer and antibacterial activities of *Foeniculum vulgare*, *Justicia adhatoda* and *Urtica dioica* as natural curatives

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Received April 27, 2017; **Accepted** September 10, 2017; **Published** September 30, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.9.19

Abstract: High-throughput technologies, such as synthetic biology and genomics have paved new paths for discovery and utility of medicinally beneficial plants. Bioactive molecules isolated from different plants have significantly higher biological activities. The present study was done to analyze antibacterial potential of some medicinal plants against multi drug resistant (MDR) pathogens and anticancer effect against MCF-7 cell line. Methanolic and ethanolic extracts were tested for their antibacterial activity by disc diffusion method against six MDR bacterial strains and for cytotoxicity evaluation by MTT assay. Ethanolic extracts of the three tested plants exhibited growth inhibitory effect against *Klebsiella pneumonia*, *Serratia marcescens* and Methicillin-resistant *S. aureus. Pseudomonas aeruginosa* was more resistant to all extracts as its growth was least inhibited by the extracts of all tested plants. Ethanol extract of *Foeniculum vulgare* exhibited significant inhibition of cancer cells proliferation. Methanol extract of *Justicia adhatoda* also showed considerable inhibition of cancer cells. Future studies must converge on detailed investigation of modes of action of extracts of tested plants.

Key words: Breast cancer; Multi drug resistant; Medicinal plants; *Justicia adhatoda*; *Urtica dioica*; *Foeniculum vulgare.*

Introduction

In recent years, there has been resurgence in interest in identification of pharmacologically active and efficient natural products (1). Rapidly increasing rate of off-target effects, toxicities and insufficient clinical outcome of different drugs present in our seemingly powerful arsenal of weapons against different diseases is alarming. It was in 1893 when Bartolomeo Gosio reported discovery of first antibiotic, mycophenolic acid from *Penicillium glaucum*. This seminal discovery was brought from shadow to the limelight in 1913 in the United States. Structural details remained unknown until 1952 while its total synthesis was achieved in 1969 (1). Surprisingly, in 1995 it was approved by the FDA not as an anti-bacterial agent but as an immunosuppressant (1).

Drug resistance strains have gained resistance to most antibiotics through a combination of enzymatic inactivation (aminoglycoside modifying enzymes, β-lactamases), chromosomal mutations, impermeability (porin loss) and over expression of active efflux of drugs. Emergence of (MDR) bacteria has significantly reduced the efficiency of our antibiotic armory which subsequently increased the frequency of therapeutic failure (2, 3, 4) . The transmission and spread of drug resistant strains is also due to presence of antibiotic resistance genes on bacterial plasmids and transposons in pathogenic bacteria like enterotoxigenic *Escherichia coli, Salmonella, Streptococcus pneumoniae, Staphylococcus aureus,* and *Mycobacterium tuberculosis*. *P. aeruginosa* is one of the major causes of nosocomial infection and is naturally resistant to a significant number of antimicrobials and this resistance has increased in recent years. *Klebsiella pneumoniae* is responsible for a substantial proportion of community and hospital acquired infections including pneumonia, urinary tract, soft tissue infection and septicemia. Clinical isolates of Methicillin-resistant *S. aureus* (MRSA) are responsible for a broad spectrum of pathogenic bacterial infections and diseases such as pneumonia, endocarditis, toxic shock syndrome and carbuncles. *S. typhi* is a major cause for typhoid fever in humans (2,3,4,5, 6). *S. marcescens* is opportunistic pathogen producing a wide range of human diseases, including wound infections, meningitis, pneumonia, bacteremia, ocular and urinary tract infections and endocarditis (7). Plants are rich source of many secondary metabolite classes, such as flavonoids, terpenoids, alkaloids, and tannins, which possess *in vitro* antimicrobial, anti-viral and anticancers properties. Numerous medicinal plants have been recorded in phototherapy manuals for infectious diseases treatment as having fewer side effects, reduced toxicity and rich source of bioactive substances of chemotherapeutic value (8, 9).

Cancer is a multifaceted and therapeutically challenging disease and rapidly accumulating experimentally verified data has considerable improved our understanding of the underlying mechanisms which are contributory in cancer development and progression (10). Different phytochemicals have been shown to modulate signaling pathways in different cancer cells (11, 12, 13).

The current work was aimed to analyze the antibacterial activity of methanol and ethanol extract of *J.*

adhatoda, *U. dioica* and *F. vulgare* against MDR strains and to investigate the cytotoxic potential of these plants against MCF-7 breast cancer cell line.

Materials and Methods

Collection and processing of plant material

J. adhatoda and *U. dioica* were collected from Balakot while *F. vulgare* was procured from Sargodha, Pakistan. Plants were identified by the taxonomist (Dr. Rizwana Aleem Qureshi, Professor of Taxonomy) at the department of Plant Sciences Quaid-i-Azam University Islamabad, Pakistan. A voucher specimen of each collection was deposited in the Herbarium of Plant Biochemistry and Molecular Biology Laboratory, Quaid-i-Azam University Islamabad (*J. adhatoda* HPBMBL-16-013, *U. diocia* HPBMBL-16-014, and *F. vulgare* HPBMBL-16-015). Plants were thoroughly washed with tap water and then rinsed in distilled water. The plants were dried in air under shadow and were separately ground to homogenous powder.

Preparation of plant extracts

Two types of extracts (methanol and ethanol) were used in the screening programme. 25g of dried powdered material of all the samples were extracted by maceration with 250ml in methanol and ethanol. Powdered plant material was dipped in solvents for seven days, then filtered and again soaked in respective solvents for seven days. This method repeated for three times then filtrates were evaporated by rotary evaporator. Dried extracts were stored at 4ºC until further use.

Preparation of bacterial cultures

Clinical isolates *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *S. marcescens* and MRSA, were collected from tertiary care hospital of Islamabad Pakistan while Two sensitive strains; *Enterobacter aerogenes* ATCC# 13048 and *Staphylococcus aureus* ATCC# 6538 were used as reference strains. Fresh bacterial cultures were prepared by adding a loopful of old bacteria to sterilized nutrient broth. The cultured strains were incubated in shaker at 37 ºC for 24 hours. Then incubated cultures were used for analysis of antibacterial activity.

Strains sensitivity to antibiotics

Microbial strains susceptibility was tested through disc diffusion method according to standard method to various antibiotics. Antibacterial representatives from diverse antibiotics groups were analyzed which encompassed Amikacin, Tazocin, Sulzone, Imipenem, Meropenem, Amoxycillin, Ampicillin, Augmentin, Ciprofloxacin, Ofloxacin, Levofloxacin, Moxifloxacin, Gentamicin, Cephradine, Cefuroxime, Cefixime, Ceftriaxone, Cefotaxime, Cefoperazone, Ceftazidime, Cefipime, Cefpirome, Septran,, Nitrofurantoin, Norfloxacin, Fosfomycin, Pipemedic acid, Septran, Nalidixic acid and Chloroamphenicol (Tertiary care hospital, Islamabad Pakistan).

Antimicrobial activity determination

Antibacterial activity of plants was performed by disc diffusion method as described previously against MDR strains and ATCC strains. Media was prepared, sterilized and poured in plates. After solidification, 0.1ml of each fresh culture of pathogens was swabbed on respective plates. All the extracts were prepared in the concentration of 20mg/ ml in dimethyl Sulfoxide (DMSO) to screen against selected microorganisms. Whatman's filter paper no.1 discs (5mm diameter) impregnated with 5 µl of each extracts was placed on cultured plates using sterilized forceps. Solvents blank including ethanol, methanol and DMSO loaded discs were used as negative controls.

After this the plates were incubated for 24h at 37°C. After the incubation period, each zone of inhibition (ZI) diameter was measured in millimeters (mm) with zone measuring scale. Extracts having an inhibition zone ≥ 10 mm in diameter were screened to find minimum inhibitory concentrations (MICs) at lower concentrations (100, 75, 50 and 25 µg/ml) using broth microdilution technique with slight modifications. Inoculum of each bacterial organism (190 µl) prepared in nutrient broth was inoculated to each well of 96 well microtiter plates while inoculum size and the density was adjusted as per predetermined limits (5×10^2 CFU/ml). After incubating the plate at 37°C for 24 h, absorbance was recorded with microplate reader at 600nm and the minimum concentration which inhibited bacterial growth was considered as MIC.

All the experiment repeated three times and calculate means and standard of deviation (±SD (n=3).

Anticancer assay

Cell culture

The breast cancer (MCF-7) cell line was maintained at Institute of Biomedical and Genetic Engineering (IBGE), Islamabad. The cell line was maintained in RPMI-1640 media supplemented with 10 % FBS. Cells were maintained at 37 °C in a humidified atmosphere with 95% air and 5 % CO^2.

MTT assay

Anticancer potential of studied plant extracts on MCF- 7 cells was examined by the MTT colorimetric assay. The anticancer activities of each tested extract were presented as % viability, which was calculated by using concentrations of extracts at 20 µg/µL, 40 µg/µL, 60 µg/µL, 80 µg/µL and 100 µg/µL using MS excel 2010.

Results

The MDR clinical isolates were investigated for antibiotic sensitivity/resistance against commonly available antibiotics. Most of the tested strains were found resistant to these antibiotics (Table 1). The antimicrobial activity of ethanol and methanol extracts of three medicinal plants were investigated using disc diffusion method by measuring the diameter of inhibition zones (Table 2 and 3) against selected MDR isolates i.e; *E. coli, K. pneumoniae, P. aeruginosa, S. typhi, S. marcescens* and MRSA. *Enterobacter aerogenes* ATCC# 13048 and *Staphylococcus aureus* ATCC# 6538 were also used as reference strains. The results of zones of inhibition of extracts were comparatively efficient against resistant strains then ATCC reference strains.

All the medicinal plant extracts used against the

Table 1. Drug activity profile of the test MDR and sensitive reference strains bacterial strains.

Name of Antibiotics	MRSA	*S. marcescens*	*E.coli*	*K. pneumoniae*	*P. aeruginosa*	*S. typhi*	*ATCC S. aureus*	*ATCC E. aerogenes*
Amikacin	R	S	S	S	S	-	S	S
Tazocin	R	S	S	R	S	-	S	S
Sulzone	R	S	S	R	R	-	-	S
Imipenem	R	S	S	S	R	-	_	S
Meropenem	R	S	S	-	R	-	S	S
Amoxycillin	R	R	R	R	-	-	S	S
Ampicillin	-	R	R	R	-	R	-	S
Augmentin	-	R	R	R	-	-	S	S
Ciprofloxacin	-	R	R	R	R	R	S	S
Ofloxacin	R	R	R	S	R	R	-	S
Levofloxacin	R	R	R	S	-	R	S	-
Moxifloxacin	-	R	R	-	-	-	S	S
Gentamicin	R	R	S	S	R	-	S	S
Cephradine	-	R	R	-	-	-	S	S
Cefuroxime	-	R	R	-	-	-	S	S
Cefixime	-	R	R	S	-	S	S	S
Ceftriaxone	-	R	R	S	-	S	S	-
Cefotaxime	R	R	R	R	-	-	S	S
Cefoperazone	R	R	R	-	R	-	S	S
Ceftazidime	-	R	R	-	R	-	S	S
Cefipime	-	R	R	S	R	-	S	S
Cefpirome	-	R	R	-	-	-	S	-
Septran	-	-	R	S	-	R	-	-
Nitrofurantoin	-	-	S	S	-	-	-	-
Norfloxacin	-	-	R	S	-	-	S	S
Fosfomycin	-	-	S	-	-	-	S	-
Pipemedic acid	-	-	R	-	-	-	S	-
Nalidixic acid	-	-	-	-	-	R	S	S
chloramphenicol	-	-	-	-	-	R	S	S

S=sensitive, R=resistance.

pathogenic organisms have showed varied degree of antimicrobial activity. In this experiment, the extracts generating a growth inhibitory zone ≥ 10 mm in were considered active and were further assessed for MIC determination through broth microdilution method. MIC is defined as the lowest concentration of each sample that prevented this change and demonstrated complete inhibition of microbial growth.[34] Ethanolic extract of *U. dioica* showed maximum Zone of inhibition (ZI) against *K. pneumoniae with* 16 ± .76 (MIC: 75 µg/ml) followed by *S. marcescens* 13 ± 1.00 (MIC: 75 µg/ml) while its methanolic extract showed maximum ZI against *E. coli* 16 ± 0.76 (MIC: 75 µg/ml). Similarly ethanolic extract of *F. vulgare* showed highest ZI against *K. pneumoniae* with 25± 1.89 (MIC: 50 µg/ml) followed by MRSA 19 ± 0.50 (MIC: 50 µg/ml), *S. marcescens* 17 ± 0.76 (MIC: 50 µg/ml) and *S. typhi* 17 ± 1.55 (MIC: 75 µg/ml) while its methanolic extract remained resistant to these strains with highest ZI against *S. typhi* 11 ± 1.60 (MIC: 75 µg/ml) and ATCC strain *S. aureus* 11 ± 1.00 (MIC: 75 µg/ml). Ethanolic root extract of *J. adhatoda* exhibited good resistance against *K. pneumoniae* 17 ± 1.89 (MIC: 75 µg/ml), while its methanolic extract was efficient against *S. marcescens* 18 ± 1.00 (MIC: 50 µg/ml), *K. pneumoniae* 17 ± 1.89 (MIC: 75 µg/ml) and MRSA 17 ± 1.60 (MIC: 75 µg/ml).

Cytotoxic evaluation

The cytotoxic potential of three studied plants was evaluated by monitoring MTT assay. Methanol and ethanol extracts of these plants were analyzed against Estrogen Receptor positive breast cancer, MCF-7 cell line. The cells viability was determined after 48 h treatment with indicated concentrations of each extract. The anti-proliferative effect on MCF-7 cells to increasing concentrations of extracts is shown in fig 1. Almost all the extracts inhibited the proliferation of MCF-7 cells by increasing the dose concentrations but strongest effect was exerted by ethanol extract of *F. vulgare*. Cells viability decreased sharply even at lower concentration (20 and 40 µg/ml) of *F. vulgare* (Fig 1A). Both extracts (methanol and ethanol) of *F. vulgare* were more effective against breast cancer cells as compared to other extracts in this study. The methanol and ethanol extracts of *F. vulgare* at a concentration of 40 µg/ml inhibited MCF-7 cell line growth by 51% and 54% respectively. This inhibitory effect was enhanced by increasing concentrations. Methanol extract of *J. adhatoda* was also found to be effective at 60 µg/ml against breast cancer cells as compared to its ethanol extract where it exhibited less

Table 2. Antibacterial activity of methanolic extracts of studied plants with zone of inhibition in mm against multiple drug resistant (MDR) bacteria.

Plant species	*K. pneumoniae*	MIC µg/ml	*P. aeruginosa*	MIC µg/ml	*S. typhi*	MIC µg/ml	*S. marcescens*	MIC µg/ml	MRSA	MIC µg/ml	*E. coli*	MIC µg/ml	ATCC *S. aureus*	MIC µg/ml	ATCC *E. aerogenes*	MIC µg/ml
U. diocia	8 ± 0.50	75	8 ± 0.76	----	8 ± 1.50	----	9 ± 1.04	----	7 ± 0.50	----	16 ± 0.76	75	----	----	8 ± 1.32	----
F. vulgare	----	----	----	----	11 ± 1.60	75	8 ± 1.32	----	----	----	7 ± 0.50	----	11 ± 1.00	75	8 ± 1.04	----
J. adhatoda	17 ± 1.89	75	----	----	----	----	18 ± 1.00	----	17 ± 1.60	75	10 ± 0.76	----	12 ± 1.90	75	13 ± 1.52	50
DMSO	----	----	----	----	----	----	----	-- --	----	----	----	----	----	----	----	----

Results are presented as mean ± SD (n=3), ----: no zone of inhibition.

Table 3. Antibacterial activity of ethanolic extracts of studied plants with zone of inhibition in mm against multiple drug resistant (MDR) bacteria.

Plant species	*K. pneumoniae*	MIC µg/ml	*P. aeruginosa*	MIC µg/ml	*S. typhi*	MIC µg/ml	*S. marcescens*	MIC µg/ml	MRSA	MIC µg/ml	*E. coli*	MIC µg/ml	*ATCC S.aureus*	MIC µg/ml	*ATCC E. aerogenes*	MIC µg/ml
U. diocia	16 ± .76	75	----	--	----	----	13 ± 1.00	75	10 ± 1.04	----	8 ± 0.50	----	9± 0.76	----	10 ± 0.50	75
F. vulgare	25 ± 1.89	50	----	----	17 ± 1.55	75	17 ± 0.76	50	19 ± 0.50	50	----	----	16 ± 1.00	50	12 ± 1.9	75
J. adhatoda	17 ± 1.89	75	----	----	----	----	18 ± 1.00	----	17 ± 1.60	75	10 ± 0.76	----	12 ± 1.9	75	13 ± 1.52	50
DMSO	----	----	----	-----	----	----	----	----	----	----	-----	-----	----	----	-----	-----

Results are presented as mean ± SD (n=3), ----: no zone of inhibition.

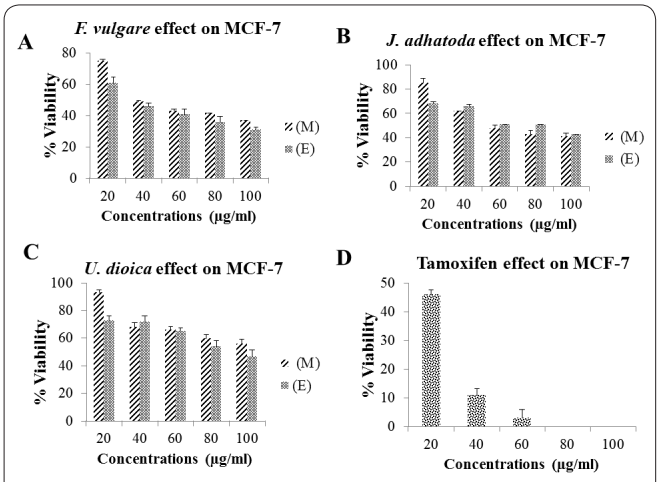

Figure 1. Percentage (%) viability of MCF-7 cells on treatment by (A) *F. vulgare* (B) *J. adhatoda* (C) *U. dioica* and *(D)* Tamoxifen. Key: M; methanol, E; ethanol. Data values are presented as mean ± SEM (n=3).

than 50% cell viability (Fig 1B). While both extracts of *U. dioica* remained least effective to decrease cells viability (Fig 1C). Tamoxifen was also used a positive control and it showed less than 50% cells viability at 20 µg/ml against MCF-7 cells (Fig 1D).

Discussion

The emergence of multidrug resistant pathogenic bacteria has created medical challenges for their treatment. *In vitro* pharmacological screening of traditionally used medicinal plants offers an incredible opportunity to explore and investigate capacity of novel therapeutic agents from a wide range of plant to certify claims related to their safety and efficacy. Plants used in present study are medicinally important. Extract of *U. dioica* has anti-inflammatory, antioxidant, antimicrobial (14, 15), anti- diabetic (16) and anti-cancer (17) properties. The *F. vulgare* is used to recover many diseases, predominantly pain in the digestive system and also in the treatment of cancer, bronchitis, diabetes, chronic cough and kidney stones (18).

Bioactive compounds are commonly extracted by organic solvents such as ethanol, acetone, and methanol as most of the polar compounds are easily eluted by these solvents which is bioactive responsible for their activity.

A fraction of *U. dioica* exhibited MIC values from 7.81 to 250 µg/ml against the human pathogens *P. aeruginosa*, *S. aureus*, *S. typhi*, *K. pneumoniae* and *Enterococcus faecalis* (19). A study demonstrated that *F. vulgare* extract has antibacterial potential on all *Acinetobacter baumannii* strains so can be employed to control MDR bacteria (20). These reports were in accordance with our findings as evidenced by notable antibacterial activity of ethanol and methanol extracts of all studied plants against MDR bacterial strains. Wide zones of growth inhibition in the disk diffusion assay were noted with the lowest concentrations of the extract. On the basis of present study it can be concluded that *K. pneumoniae* was most sensitive to ethanol extracts of all three studied plants. *F. vulgare* (ethanol) was found active against *S. typhi, S. marcescens and* MRSA. Likewise methanol extract of *J. adhatoda* was also found successful against *K. pneumoniae, S. marcescens* and MRSA. However, for characterizing resistance further studies are required using current methods elements to assess the effects of *U. dioica*, *F. vulgare* and *J. adhatoda*, extracts upon clinically significant strains of microorganisms.

The MTT assay applied to determine the cytotoxic effect of the methanolic and ethanolic extracts of three studied plants on breast cancer, MCF-7 cell line revealed that the extracts of *F. vulgare* and *J. adhatoda* were cytotoxic to MCF-7 cells in a dose dependent manner. Their cytotoxic effect increased by increasing the concentrations of doses. Highest cytotoxicity was exhibited by ethanol extract of *F. vulgare*.

These findings suggested that active constituents responsible for observed cytotoxicity may be extracted in polar solvents as in methanol and ethanol. Ethanol extract of *J. adhatoda* also possessed considerable antiproliferative effect in estrogen receptor positive cells. A compound "vasicine" isolated from n-butanol fraction of *J.adhatoda* was found potent in inhibiting proliferation of prostate cancer cells (21). However, *U. dioica* did not exert inhibitory effects on proliferation of breast cancer cells. Isolation of bioactive molecules from these plants will prove to be helpful in pre-clinical trials to explore true potential of these extracts/bioactive molecules against different cancers.

Future studies must converge on the modes of action related to extract of different plant extracts. Furthermore, isolation of bioactive molecules from these plants should be tested against different pathogens to demystify how different phytochemicals/extracts target biological mechanisms in pathogens. These extracts and bioactive constituents can be used for inhibition of oncogenic signaling pathways in different cancer cell lines.

Acknowledgements
The authors are grateful to the Institute of biomedical and genetic engineering Islamabad, Pakistan for all the helps and guidance in order to accomplish this project.

Ethical approval
Not applicable

Competing interests
The authors declare that they have no competing interests.

Funding
Higher Education Commission of Pakistan (HEC) is acknowledged for the provision of funds to conduct basic research.

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