

ANTIMICROBIAL SCREENING OF SELECTED FLORA OF PAKISTAN

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Abstract - Ethanolic extracts of *Ferula assafoetida* resin, *Grewia asiatica* leaves, *Ipomoea hederacea* seeds, *Lepidium sativum* seeds, *Nigella sativa* seeds and *Terminalia chebula* fruits were tested *in vitro* for their antibacterial and antifungal activities. The antibacterial study performed against eight bacterial species viz., *Escherichia coli*, *Citrobacter*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Micrococcus luteus*, *Proteus mirabilis* and *Bacillus subtilis* indicated that the investigated plants have potent activity against all the tested microorganisms. The antifungal activity of these extracts was performed against nine fungal strains, viz., *Aspergillus parasiticus*, *Aspergillus niger*, *Yersinia aldovae*, *Candida albicans*, *Aspergillus effusus*, *Fusarium solani*, *Macrophomina phaseolina*, *Saccharomyces cerevisiae* and *Trichophyton rubrum*. The extracts showed moderate as well as significant activity against the different fungal strains.

Key words: Antibacterial, antifungal, medicinal plants, Pakistan

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INTRODUCTION

Infectious diseases are the number one cause of death world-wide, and in tropical countries they account for approximately 50% of deaths (Iwu et al., 1999). Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms (Harbottle et al., 2006). This resistance is largely due to indiscriminate use of antimicrobial

drugs commonly used in the treatment of infectious diseases. Apart from resistance, some antibiotics have serious undesirable side effects which limit their application. Thus there is urgent need to develop new antimicrobial agents that are very effective with minimal unwanted side-effects, and higher plants represent a potential source of novel antibiotic prototypes (Maurer-Grims et al., 1996). Historically, plants have provided a good source of anti-infective agents; emetine, quinine, and berberine remain highly effective instruments in the fight against microbial infections (Iwu et al., 1999). Plant-derived medicines have been a part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as the source of agents to fight microbial

diseases. The reasons for this herbal renaissance include a reduction in the new antimicrobial drugs in the pharmaceutical pipeline, an increase in antimicrobial resistance, and the need of treatments for new emerging pathogens (Mahady, 2005).

Pakistan is well-known for its luxuriant flora, a major portion of which has not been studied. From this perspective, as part of our continuous studies exploring the hidden potential of the indigenous flora of Pakistan (Nisar et al., 2010 a, b, c; 2011; Zia-Ul-Haq et al., 2007a, b; 2008 a, b; 2009; 2010 a, b; Ahmad et al., 2011; Qayum et al., 2011), we have screened the ethanolic extracts of selected plants for their antibacterial and antifungal activities to rationalize their uses by the indigenous community. The present research will provide the needed preliminary observations necessary to select from crude extracts those with potentially useful properties for further biochemical investigations.

MATERIALS AND METHODS

Preparation of crude extract

The plant material of *Ferula assafoetida* resin, *Grewia asiatica* leaves, *Ipomoea hederacea* seeds, *Lepidium sativum* seeds, *Nigella sativa* seeds and *Terminalia chebula* fruits was extracted with 96% EtOH at room temperature. The ethanolic extracts were filtered and evaporated under vacuum to obtain a thick gummy mass. All these extracts were tested for antibacterial and antifungal activities.

Antibacterial bioassay

Soy agar Petri plates were prepared for testing the antibacterial activity of the crude alcoholic extracts by the disc diffusion method (Bauer et al., 1966; Baqir et al., 1985). Diluted culture (0.1 mL) was poured on each plate and the plates were dried for 30 min at 37°C. Discs of 8 mm diameter were used and soaked with different concentrations of drug solutions, and standard drugs, Gentamicin 20µg, Ampicillin 1mg, Amoxicillin 1mg and Gatifloxacin 20µg, were used as a positive control with

a distilled water-soaked disc as negative control. The discs were placed on plates and incubated for 24 h at 37°C. At the end of the incubation period, the inhibition zones were measured. The bacterial strains used are *Escherichia coli*, *Staphylococcus typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Citrobacter*, *Proteus mirabilis*, *Micrococcus luteus* and *Bacillus subtilis*. The determination of the minimum inhibitory concentration (MIC) was carried out as described previously (Ndokwe et al., 2007; Hernandez-Perez et al., 1994). The lowest concentration of the test solution that led to an inhibition of growth was taken as the MIC (Gopal, 2009).

Antifungal bioassay

Antifungal activity was carried out using nine pathogenic strains of fungi, *Aspergillus parasiticus*, *Aspergillus niger*, *Yersinia aldovae*, *Candida albicans*, *Aspergillus effusus*, *Fusarium solani*, *Macrophomina phaseolina*, *Saccharomyces cerevisiae* and *Trichophyton rubrum*. Petri plates of Sabouraud dextrose agar (SDA) were prepared and 0.1 mL of diluted culture was poured on each plate as described earlier (Bauer et al., 1966; Baqir et al., 1985; Jahan et al., 2011). Discs of 8 mm diameter (approximate) were used. The plates were incubated for 24 h at 37°C. At the end of the incubation period, the inhibition zones were observed. The determination of the minimum inhibitory concentration (MIC) was carried out as described previously (National Committee for Clinical Laboratory Standards 2000; Gopal, 2009). Itranex and Fungizone were used as standard drugs.

RESULTS AND DISCUSSION

Medicinal plants are items of commerce in Pakistan and herbal medicines are gaining momentum in Pakistan for a wide variety of human ailments due to the exorbitant cost of treatments by allopathic drugs, their side effects and the development of resistance to antibiotic drugs for infectious diseases. People who were distanced from the traditional systems of medicine are gearing

Table 1. Antibacterial bioassay

Drug used	<i>E. coli</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>M. luteus</i>	<i>Citrobacter</i>	<i>B. subtilis</i>
<i>F. assafoetida</i>	>10mg	>10mg	>10mg	>10mg	>10mg	>10mg	>10mg	>10mg
<i>G. asiatica</i>	>5mg	>5mg	>1mg	>25mg	>5mg	>5mg	>5mg	>5mg
<i>I. hederacea</i>	>0.125mg	>0.25mg	>0.125mg	>0.125mg	>0.25mg	>0.5mg	>0.25mg	>0.05mg
<i>L. sativum</i>	>15mg	>10mg	>10mg	>10mg	>1mg	>10mg	>10mg	>10mg
<i>N. sativa</i>	>1mg	>1mg	>1mg	>25mg	>1mg	>25mg	>10mg	>10mg
<i>T. chebula</i>	>1mg	>10mg	>10mg	>20mg	>1mg	>10mg	>10mg	>10mg
Gentamicin 20µg	19±0.84	20±0.14	19±0.65	19±0.2	20±0.3	19±0.32	19±0.2	18±0.46
Ampicillin 1mg	23±0.11	15±0.11	21±0.55	15±0.09	16±0.76	14±0.41	21±0.67	20±0.31
Amoxicillin 1mg	24±0.78	20±0.03	20±0.06	22±0.07	21±0.75	16±0.31	20±0.91	21±0.39
Gatifloxacin 20µg	19±0.77	25±0.68	20±0.14	16±0.07	23±0.39	15±0.55	27±0.95	22±0.29

Table 2. Antifungal bioassay

Drug used	<i>S. cerevisiae</i>	<i>A. parasiticus</i>	<i>A. effusus</i>	<i>A. niger</i>	<i>T. rubrum</i>	<i>M. phaseolinia</i>	<i>C. albicans</i>	<i>Y. aldovae</i>	<i>F. solani</i>
<i>F. assafoetida</i>	>10mg	>10mg	>10mg	>10mg	>10mg	>10mg	>5mg	>5mg	>10mg
<i>G. asiatica</i>	>10mg	>5mg	>25mg	>10mg	>1mg	>1mg	>10mg	>1mg	>10mg
<i>I. hederacea</i>	>1mg	>1mg	>1mg	>1mg	>1mg	>1mg	>1mg	>1mg	>1mg
<i>L. sativum</i>	>25mg	>10mg	>25mg	>5mg	>10mg	>10mg	>10mg	>25mg	>10mg
<i>N. sativa</i>	>25mg	>25mg	>5mg	>25mg	>25mg	>5mg	>5mg	>5mg	>10mg
<i>T. chebula</i>	>5mg	>5mg	>5mg	>5mg	>5mg	>5mg	>5mg	>5mg	>5mg
Itraconazole 2mg	19±0.67	16±0.88	16±0.34	13±0.71	21±0.63	16.5±0.31	14±0.66	17±0.43	12±0.34
AmphotericinB 2mg	14±0.91	13±0.71	12±0.32	12±0.63	11±0.97	15±0.54	14±0.54	15±0.23	12±0.44

towards green pharmaceuticals as the period of over-romanticizing allopathic drugs has ended, and cures and drugs derived from plants are being integrated in formal health care systems. The present study has been designed to verify the efficacy of some commonly used medicinal plants of Pakistan as antimicrobial agents.

The order of antibacterial activity observed for this flora was *Ipomoea hederacea* > *Nigella sativa* > *Grewia asiatica* > *Terminalia chebula* > *Lepidium sativum* > *Ferula assafoetida*. *Ferula assafoetida* and *Grewia asiatica* showed highest activity against *S. aureus* while *Nigella sativa* showed

highest activity against *E. coli*. *Lepidium sativum* showed highest activity against *Proteus mirabilis* while *Terminalia chebula* showed highest activity against *Citrobacter*. *Ipomoea hederacea* showed highest antibacterial activity against *Bacillus subtilis*.

The order of antifungal activity observed for this flora was *Ipomoea hederacea* > *Terminalia chebula* > *Grewia asiatica* > *Nigella sativa* > *Lepidium sativum* > *Ferula assafoetida*. *Terminalia chebula* and *Nigella sativa* showed highest activity against *S. cerevisiae*. *Grewia asiatica* and *Ferula assafoetida* showed highest activity against *F. solani* and *Y. al-*

dovae respectively. *Lepidium sativum* showed highest antifungal activity against *Trichophyton rubrum*. It can be concluded that finding resistant strains of organism plant biodiversity could lead to unexpected research findings (Mahmud et al., 2009). The present study will help researchers by providing basic data for future research of the potential therapeutic value of these important plants. The antibacterial action of the extracts may be due to the presence of tannins (Djipa et al., 2000; Cavanagh et al., 2003).

Escherichia coli and *salmonella* cause digestive disorders and the extracts of these plants have antimicrobial potential against these pathogens. *P. mirabilis*, *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *M. luteus* have the ability to produce urease which hydrolyzes urea to ammonia, resulting in kidney stones (Toit et al., 1995). This can be prevented by inhibiting the growth of pathogens by these extracts; in addition, ammonia-related bad odor can also be diminished. The broad-spectrum antimicrobial potential confirms the presence of bioactive principles in all the examined crude drugs, justifying the folk uses of these crude drugs, as well as an ethnobotanical approach in the search for novel bioactive compounds.

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