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ANTIMICROBIAL SCREENING OF SOME PLANTS OF MEDICINAL IMPORTANCE

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Abstract

Methanolic extracts of *Solanum nigrum* (leaves and seeds of both black and red varieties), *Elettaria cardamomum Cuscuta reflexa* and *Cinnamomum camphora* were tested *in vitro* for their antibacterial and antifungal activities. Antibacterial study performed against six bacteria viz., *Escherichia coli, Citrobacter, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Yersinia aldovae* indicated that investigated plants have potent activity against all microorganisms. The antifungal activity of these extracts was performed against six fungi, viz., *Saccharomyces cereviciae, Aspergillus parasiticus, Trichophyton rubrum, Macrophomina, Fusarium solani* and *Candida albicans*. The extracts showed moderate as well as significant activity against different fungal strains.

Introduction

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc., (Zachariah *et al.,* 2009). In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. There is a permanent and vital need to discern new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens (Diamond, 1993; Perez *et al.,* 1990; Rajendran & Ramakrishnan, 2009).

Large scale evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary first step in the isolation and characterization of the active principle and further leading to drug development. Pakistan has rich flora in which 2,000 plant species are used for medicinal purposes but out of these only 400 to 600 plant species are documented and studied for medicinal purposes (Hazrat *et al.,* 2007). However still a major portion of this plant wealth remains unexplored. In this context as part of our continuous studies on exploring the hidden potential of indigenous flora of Pakistan (Zia-ul-Haq *et al.,* 2007a, b; 2008, 2009), we have screened the extract of *Cuscuta reflexa* Roxb. (*Convolvulaceae*)
locally known as Amar bel; *Elettaria cardamomum* (*Zingiberaceae*) locally known as chhoti elachi ; *Solanum nigrum* (*Solanaceae*), locally known as Mako and *Cinnamomum camphora* (*Lauraceae*) locally known as Camphor for their antibacterial and antifungal activities to evaluate their phytomedicinal potential. The present investigation will provide a broad base for the possibility of further detailed biological studies on these medicinal plants along with its biological standardization.

Material and Methods

Plant material and preparation of crude extract: *Solanum nigrum* (leaves and seeds of both black and red varieties,designted as SNBL, SNBS, SNRL and SNRS respectively), *Elettaria cardamomum, Cuscuta reflexa* and *Cinnamomum camphora* (homeopathic mother tincture Schwabe) were identified by Prof. Dr. Mansoor Ahmad, Department of Pharmacognosy, University of Karachi and voucher, specimen 001116-01, 001116-02,001116-03 and 001116-04 were deposited in Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi, Karachi, Pakistan. The plant materials were grounded and extracted with MeOH at room temperature. The methanolic extract was filtered and evaporated under vacuum to obtain a thick 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 our drugs and crude extracts (Baqir *et al*., 1985).0.1mL of diluted culture was poured on each plate and the plates were dried for thirty minutes at 37 °C. Disc of 8 mm diameter were used and soaked with different concentration of drug solutions and standard drugs Gentamicin 20µg, Ampicillin 1mg, Amoxicillin 1mg and Gatifloxacin 20µg. The discs were placed on plates and incubated for twenty-four hours at 37°C. At the end of incubation period, the inhibition zones were measured. The determination of the minimum inhibitory concentration (MIC) was carried out as described previously (Ndukwe *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 MIC i.e. minimum inhibitory concentration, (Gopal, 2009).

Antifungal bioassay: The petri plates of sabouraud dextrose agar (SDA) were prepared and 0.1mL of diluted culture was poured on each plate as described earlier (Baqir *et al.,* 1985). The discs of 8 mm diameter (approximate) were used. The plates were incubated for twenty-four hours at 27°C. At the end of 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). The lowest concentration of the test solution that led to an inhibition of growth was taken as MIC i.e. minimum inhibitory concentration (Gopal, 2009).

Results and Discussions

Medicinal plants have contributed immensely to health care in Pakistan. This is due in part to the recognition of the value of traditional medical systems and the identification of indigenous medicinal plant which are cheap, easily available and which have significant healing power. These established a good support to the use of this plant in herbal medicines and as base for the development of new drugs and phytomedicine. Plant extracts have been studied against bacteria for years, but in a more intensified way in the last three decades (Suffredini *et al.,* 2004).

Antibacterial activity was observed against both gram negative and gram positive organisms i.e., *Pseudomonas aeruginosa, Citrobacter, Shigella flexneri, E. coli* and *Staphylococcus aureus*. Interestingly *Cuscuta reflexa* (oxidized) has greater antibacterial potential than *Cuscuta reflexa* (unoxidized) .Similarly *Solanum nigrum black seeds* (SNBS) indicated greater antibacterial potential as compared to other *Solanum nigrum* varieties.The antifungal activity was observed against *Saccharomyces cereviciae, Candida albicans, Aspergillus parasiticus, Macrophomina, Yersinia aldovae, Fusarium solani* and *Trichophyton rubrum*. *Elettaria cardamomum* was observed to possess highest antifungal activity. The observations confirm the folk uses of these crude drugs and justify the ethnobotanical approach in the search for novel bioactive compounds. The present status of medicinal plants and their products provide opportunity for the developing countries to benefit from the emerging marks as the developing countries possess most biodiversity of medicinal plants. It is concluded that in coordinance of the chemical literature finding resistant strains of organism plant biodiversity may lead to unexpected research findings (Mahmud *et al.,* 2009).

Table 1. Antibacterial bioassay.

Plant material and standard	*E. coli*	*S. flexri*	*S. aureus*	*P. aerugenosa*	*Citrobacter*	*Y. aldovae*
C. camphora	5d	4d	4d	4d	4d	5d
C. reflexa unoxidized	>10mg	25mg	>10mg	>25mg	>25mg	>10mg
C. reflexa oxidized	>10mg	>10mg	>50mg	>50mg	>25mg	>5mg
E. cardamomum	>10mg	>10mg	>10mg	>5mg	>25mg	>1mg
S. nigrum SNRL	>10mg	>5mg	>25mg	>25mg	>10mg	>5mg
S. nigrum SNRS	1mg	>10mg	>5mg	>10mg	>5mg	>5mg
S. nigrum SNBL	>10mg	>25mg	>10mg	>50mg	>25mg	>5mg
S. nigrum SNBS	>5	>1mg	>5mg	>5mg	>10mg	>1mg
Gentamicin 20µg	19 ± 0.84	20 ± 0.14	19 ± 0.65	19 ± 0.2	19 ± 0.2	17 ± 0.55
Ampicillin 1mg	23 ± 0.11	15 ± 0.11	21 ± 0.55	15 ± 0.09	21± 0.67	16 ± 0.62
Amoxicillin 1mg	24 ± 0.78	20 ± 0.03	20 ± 0.06	22 ± 0.07	20 ± 0.91	18 ± 0.13
Gatifloxacin 20µg	19 ± 0.77	25 ± 0.68	20 ± 0.14	16 ± 0.07	27 ± 0.95	20 ± 0.23

Table 2. Antifungal bioassay.

Dose	*S. cerevisiae*	*A. parasiticus*	*T. rubrum*	*M. haseolinia*	*C. albican*	*F. solani*
C. camphora	0	5drops	0	0	5drops	0
C. reflexa unoxidized	>5mg	>1mg	>1mg	>25mg	>25mg	>10mg
C. reflexa oxidized	>10mg	>1mg	>5mg	>25mg	>5mg	>10mg
E. cardamomum	>1mg	>10mg	>1mg	>1mg	>1mg	>1mg
S. nigrum SNRL	>1mg	>25mg	>1mg	>10mg	>1mg	>5mg
S. nigrum SNRS	>10mg	>10mg	>5mg	>5mg	>1mg	>25mg
S. nigrum SNBL	>1mg	>25mg	>5mg	>10mg	>1mg	>25mg
S. nigrum SNBS	>1mg	>25mg	>10mg	>25mg	>1mg	>25mg
Itraconazole 2mg	19 ± 0.67	16 ± 0.88	21 ± 0.63	16.5 ± 0.31	14 ± 0.66	12 ± 0.34
Amphoteracin B 2mg	14 ± 0.91	13 ± 0.71	11 ± 0.97	15 ± 0.54	14 ± 0.54	12 ± 0.44

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