Pak. J. Bot., 42(6): 4281-4284, 2010.

ANTIMICROBIAL SCREENING OF SOME MEDICINAL PLANTS OF PAKISTAN

NOOR JAHAN*[1], MANSOOR AHMAD[2], MEHJABEEN[3], M. ZIA-UL-HAQ[2], S. MEHBOOB ALAM[4] AND MAHMOOD QURESHI[2]

[1]*Department of Pharmacology, Dow University of Health Sciences Karachi, Pakistan*
[2]*Department of Pharmacognosy, University of Karachi, Karachi-75270, Pakistan*
[3]*Department of Pharmacology, Federal Urdu University of Arts, Science & Technology, Karachi-75300, Pakistan*
[4]*Department of Pharmacology, Jinnah Post Graduate Medical Center Karachi*

Abstract

Methanolic extracts of *Thuja occidentalis, Vernonia anthelmintica, Dryopteris chrysocoma* and *Trachyspermum ammi* were tested *In vitro* for their antibacterial and antifungal activities. Antibacterial study performed against six bacteria viz., *Escherichia coli, Citrobacter, Shigella flexenari, Yersinia aldovae, Staphylococcus aureus* and *Pseudomonas aeruginosa* indicated that has 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 significant results against different fungal strains.

Introduction

Medicinal plants, since dawn of civilization, have been used in virtually all cultures as a source of medicine. There is growing interest in medicinal plants as a re-emerging health aid has due to the rising costs of prescription drugs in the maintenance of personal health and well-being, and the bioprospecting of new plant-derived drugs (Hoareau and DaSilva, 1999; Anon., 1996). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (Anon., 1998). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradzki *et al*., 1999).

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 indigenous 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 *Trachyspermum ammi* (*Apiaceae*) locally known as Ajwain; *Vernonia anthelmintica* (*Asteraceae*) locally known as Kali ziri ,*Thuja occidentalis* (*Cupressaceae*),locally known as Morpankh and *Dryopteris chrysocoma* (*Dryopteridaceae*) locally known as Sarkhas for their antibacterial and antifungal activities. 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.

[1*]Corresponding author: jahann7816@yahoo.com

Material and Methods

Plant material and preparation of crude extract: Seeds of *Trachyspermum ammi* and *Vernonia anthelmintica* were purchased from local market, while aerial and ground parts of *Thuja occidentalis* and *Dryopteris chrysocoma* were collected from Karachi University, Karachi, Pakistan, and identified by Prof. Dr. Mansoor Ahmad, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. The sample materials were deposited in the herbarium of the department. The plant materials were was grounded and extracted with MeOH at room temperature. The combined 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 24 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 (Anon., 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 play an important role for the management of different microbial infections because overmedication and long-term side effects of synthetic drugs have assumed alarming range. Effective, safe and cheap medicinal agents from plants may appear as potential alternatives for controlling microbial infections particularly the resistant cases.

Antibacterial activity was observed against both gram negative and gram positive organisms i.e., *Pseudomonas aeruginosa, Yersinia aldovae, Citrobacter, Shigella flexneri, E. coli* and *Staphylococcus aureus*. The order of antibacterial activity observed was *Trachyspermum ammi*> *Vernonia anthelmintica*> *Thuja occidentalis*> *Dryopteris chrysocoma.*Interestingly *Dryopteris chrysocoma* leaf had greater antibacterial potential followed by root and stem of said plant. *T. occidentalis* showed potent antibacterial activity against all bacteria. The antifungal activity was observed against *Saccharomyces cereviciae, Candida albicans, Aspergillus parasiticus, Macrophomina, Fusarium solani*

and *Trichophyton rubrum*. *T. occidentalis* showed antifungal activity against *Saccharomyces cereviciae, Candida albicans, Aspergillus parasiticus, Yersinia aldovae* and *Trichophyton rubrum*. *T. ammi* show highly potent antibacterial and antifungal activity, no growth was observed on plates. The activity was more than the standard drugs i.e. gentamicin, ampicillin, amoxicillin, itraconazole and amphoteracin B. *V. anthelmintica* show potent antibacterial activity while it show antifungal activity only against *Trichophyton rubrum*. *D. chrysocoma* (root) show antibacterial activity against all bacteria but it not show antifungal activity. *D. chrysocoma* (leaf) show antibacterial activity against all bacteria while it shows antifungal activity against *Saccharomyces cereviciae, Candida albicans* and *Trichophyton rubrum*. *D. chrysocoma* (stem) show potent antibacterial activity against all bacteria while it showed antifungal activity against *Trichophyton rubrum*. 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). The present study will help the researchers as a basic data for future research in exploiting the hidden potential of this important plant which has not been explored so far.

Table 1. Antibacterial bioassay.

Crude extract and standards	*P. aeruginosa*	*Citrobacter*	*S. flexneri*	*Y. aldovae*	*E. coli*	*S. aureus*
D. chrysocoma (leaf)	<10mg	<25mg	<25mg	-	<25mg	<10mg
D. chrysocoma (root)	<10mg	<10mg	<10mg	-	<10mg	<5mg
D. chrysocoma (stem)	<10mg	<25mg	<25mg	-	<25mg	<10mg
T. ammi	<1mg	<1mg	<1mg	<1mg	<1mg	<1mg
T. occidentalis	<1mg	<5mg	<1mg	<1mg	<1mg	<1mg
V. anthelmintica	<10mg	<10mg	<10mg	-	<1mg	<1mg
Gentamicin(20µg)	19 ± 0.440	19 ± 0.333	20 ± 0.577	19 ± 0.333	19 ± 0.440	16 ± 0.333
Ampicillin(1mg)	15 ± 0.577	21 ± 0.666	15 ± 0.333	23 ± 0.440	21 ± 0.405	15 ± 0.666
Amoxicillin(1mg)	22 ± 0.533	20 ± 0.305	20 ± 0.577	24 ± 0.666	20 ± 0.333	17 ± 0.440

Table 2. Antifungal bioassay.

Crude extract and standards	*S. cereviciae*	*C. albicans*	*A. parasiticus*	*M. omina*	*F. solani*	*T. rubrum*
D. chrysocoma (leaf)	<25mg	<10mg	-	-	-	<1mg
D. chrysocoma (root)	-	-	-	-	-	-
D. chrysocoma (stem)	-	-	-	-	-	<10mg
T. ammi	<1mg	<5mg	<1mg	<1mg	<1mg	<1mg
T. occidentalis	<75mg	<25mg	<25mg	-	-	<1mg
V. anthelmintica	-	-	-	-	-	<50mg
Itraconazole (1mg)	15 ± 0.577	13 ± 0.333	14 ± 0.577	13 ± 0.166	09 ± 0.333	15 ± 0.577
Amphoteracin B (1mg)	13 ± 0.440	11 ± 0.520	11 ± 0.400	12 ± 0.133	10 ± 0.416	09 ± 0.500

References

Anonymous. 1996. UNESCO. *Culture and Health*, Orientation Texts –World Decade for Cultural Development 1988–1997, Document CLT/DEC/PRO – 1996, Paris, France, pgs. 129.

Anonymous. 1998. UNESCO. FIT/504-RAF-48 Terminal Report:*Promotion of Ethnobotany and the Sustainable Use of Plant Resources in Africa*, pgs. 60, Paris.

Anonymous. 2000. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, NCCLS: Wayne, P.A.

Baqir, S.N.S., S. Dilnawaz and S. Rah. 1985. Screening of Pakistani plants for antibacterial activity. *Pak J. Sci. Ind Res*., 28(4): 269-275.

Hazrat, A., J. Shah, M. Ali and I. Iqbal. 2007. Medicinal value of *Ranunculaceae* of dir valley. *Pak. J. Bot*., 39(4): 1037-1044.

Hoareau, L and E.J. DaSilva. 1999. Medicinal plants: a re-emerging health aid. *Elec. J. Biot*., 2(2): 56-70

Mahmud, S., H. Shareef, U. Farrukh, A. Kamil and G.H. Rizwani. 2009. Antifungal activities of *Vitex negundo* Linn. *Pak. J. Bot*., 41(4): 1941-1943.

Rashid, R., M. Farah and M.N. Mirza. 2009. Biological screening of *Salvia cabulica. Pak. J. Bot*., 41(3): 1453-1462.

Sieradzki, K., R.B. Roberts, S.W. Haber and A. Tomasz .1999. The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. *N. Engl. J. Med*., 340: 517-523.

Zia-ul-Haq, M., M. Ahmad S. Iqbal, S. Ahmad and H. Ali. 2007b. Characterization and compositional studies of oil from seeds of desi chickpea (*Cicer arietinum* L.) cultivars grown in Pakistan. *J. Am. Oil Chem. Soc*., 84: 1143-1148.

Zia-ul-Haq, M., S. Ahmad, M. Ahmad, S. Iqbal and K.M. Khawar. 2009. Effects of cultivar and row spacing on tocopherol and sterol composition of chickpea (*Cicer arietinum* L.) seed oil. *Tar. Bilim. Derg*., 15: 25-30.

Zia-ul-Haq, M., S. Iqbal and M. Ahmad. 2008. Characteristics of oil from seeds of 4 mungbean (*Vigna radiate* L. wilczek) cultivars grown in Pakistan. *J. Am. Oil Chem. Soc*., 85: 851-856.

Zia-ul-Haq, M., S. Iqbal, S. Ahmad, M. Imran, A. Niaz and M.I. Bhanger. 2007a. Nutritional & compositional study of desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan. *Food Chem*., 105: 1357-1363.

(Received for publication 9 March 2010)