Pak. J. Pharm. 20-23 (1 & 2)
3-6, 2007-2010

ISSN: 1019-956X

ANTIMICROBIAL AND IRRITANT ACTIVITIES OF THE EXTRACTS OF *Malva parviflora* L., *Malvastrum coromandelianum* L. AND *Amaranthus viridis* L. – A PRELIMINARY INVESTIGATION

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ABSTRACT

Hexane, chloroform and ethanol extracts of *Malva parviflora* L. and *Malvastrum coromandelianum* L. were tested for their antibacterial, antifungal and irritant activities. While the hexane, chloroform, ethanol and aqueous extracts along with the polar mass of *Amaranthus viridis* L. were tested for above activities, except that of the antifungal. The extracts of *Malva parviflora* L. and *Malvastrum coromandelianum* L. showed similar patterns of antibacterial activity against *Escherichia coli* but slight variations in the antibacterial response against *Proteus vulgaris, Bacillus subtilis* and *Staphylococcus aureus*. Chloroform extracts of both the plants displayed prominent antibacterial activity as compared to that of other extracts. Antifungal activities of all the extracts were almost same on *Aspaergillus niger* and *Aspergillus oryzae*. The antibacterial range of ethanol extract of *Amaranthus viridis* L. was more prominent than that of its aqueous extract and polar mass. However, hexane extracts of the plant displayed a greater antibacterial activity against Gram positive and Gram negative microorganisms than that of chloroform extracts. Application of the hexane, chloroform and aqueous extracts of the three plants on inner surface of ear of the male albino rabbits *(Oryctolagus cuniculus)* expressed acute irritant response.

Keywords: *Malva parviflora* L., *Malvastrum coromandelianum* L., *Amaranthus viridis* L., Antimicrobial activity, Irritant activity

INTRODUCTION

Herbal drugs have received greater attention in recent times because of their diversity of curing diseases, safety and being well tolerated remedies when compared to the conventional medicines. Development of resistance against antibiotics has further emphasized the necessity of research for alternative antimicrobial agents (Bax and Mullan 2000). *Malva parviflora* L. and *Malvastrum coromandelianum* L. belong to the family Malvaceae which has been famous for medicinal properties for many years. The plants of this family are well known for their antibacterial and antifungal activities due to the presence of alkaloids, essential oils and phenolic quleoside (Ndunga *et al.,* 1997; Abad *et al.,* 2007).

The plants of the Malvaceae have irritant and allergic compounds (Rohrabach *et. al.,* 1990). It contains wide range of cytokines which are effective in the treatment of inflammation (Shale *et al.,* 2005). The plant *Amaranthus viridis* L. belongs to family Amaranthaceae and is known for various medicinal uses. Chopra and coworker reported its emollient and vermifuge properties (Chopra *et al.,* 1986) while its antioxidant properties have been reported by various workers (Amin *et al.,* 2006).

Leave of *Amaranthus viridis* L. are used for treating eczema, psoriasis and rashes, constipation, inflammation, bronchitis, anemia and leprosy. It inhibits enzymes, plays regulatory role on different hormones and is used for

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anticancer, antihepatotoxic and protection of cardio vascular system (Ashok *et. al.,* 2009; Abbasi *et. al.,* 2010). Present work was aimed at screening extracts of *Malva parviflora* L., *Malvastrum coromandelianum* L. and *Amaranthus viridis* L. for their antibacterial, antifungal and irritant activities.

MATERIALS AND METHODS

Plant materials

The plants *Malva parviflora* L., *Malvastrum coromandelianum* L. and *Amaranthus viridis* L. were obtained from the Herbarium of Government College University, Lahore and were extracted in hexane, chloroform and ethanol (E. Merck, Germany) by Soxhelt extraction method as described by Vogel (Vogel *et al.,* 1978). The extracts were concentrated on vacuum rotary evaporator (Rikakikai co. Ltd., Tokyo) and their percentage yields were calculated.

Procurement and maintenance of animals

Six male albino rabbits *(Oryctolagus cuniculus)* weighing 1.0-1.3 Kg were purchased from Veterinary Research Institute, Lahore and housed in the animal house of University College of Pharmacy, University of The Punjab, Lahore in steel cages. Before experiments they were fed on animal fodder provided with tap water *ad libitum* and kept under the semiconstant temperature conditions.

Microorganisms

The following microorganisms, were obtained from the Drug Testing Laboratory, Government of the Punjab, Lahore, Scheiring Pharmaceutical industries, Lahore and Schazoo Laboratories, Lahore; *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 293737), *Escherichia coli* (ATCC 10536), *Proteus vulgaris* (ATCC 838), *Bordetella bronchiseptica* (ATCC 4617), *Bacillus cereus* (ATCC 6051), *Bacillus pumilus* (ATCC 8241), *Micrococcus flavus* (ATCC 10240), *Sarcina lutea* (ATCC 9341), *Aspergillus niger* (NRRL 1510), *Aspergillus oryzae* (NRRL 1560) The bacteria were revived in nutrient broth and harvested after 18 hours of growth. The fungid cultures were maintained in 3.9% DPA (pH 5.6) slopes. Spore suspension prepared by adding 10ml of sterilized 0.05% (w/v) monoxal OT, a 3-5 days old slant of *Aspegillus niger* and *Aspergillus oryzae* were used as inoculums in the present study. Then 1ml of the spore suspension was cultivated in 25ml of cultivation medium. A haemocytometer was used for counting the spores.

Determination of antibacterial activity

For determining antibacterial activity concentrated extracts of whole plants of *Malva parviflora* L. in hexane (5.53%), chloroform (6.67%) and ethanol (6.37%), *Malvastrum coromandelianum* L. in hexane (10%), chloroform (6.67%) and ethanol (12.61%) and extracts of *Amaranthus viridis* L. in hexane (8%), chloroform (5.90%), ethanol (11.80%), aqueous extract (12.08%) and polar mass were used. Bacteria and fungi were inoculated in molten nutrient agar plates separately. Inoculums introduced 10^5 to 10^6 cells or spores/ml. Following solidification 6 wells of 8.00 mm diameter were made in each Petri dish. Then 5, 50 and 100 mg/ml of extracts of *Malva parviflora* L., *Malvastrum coromandelianum* L. and *Amaranthus vridis* L. along with standard antibacterial agents (ampicillin and streptomycin, each of 1mg/ml) were introduced into the wells. Aqueous Gum acacia (4.5% w/v) was the vehicle and served as control. The plates were incubated at 37°C for 24 hours. The zones of growth inhibition of all extracts were determined as mean of six replicates by using the method of Haavik (Haavik *et al.,* 1973).

Determination of irritant activity

For testing the irritant activity, hairs from the inner surface of rabbit ear were shaved off. One ear was divided into three portions with the help of black marker. 10µl of different concentrations of each extract was applied on the three portions. The other ear was used as vehicle control. The ear was observed for redness after 15 and then every 30 minutes up to 24 hours. Maximum irritancies on rabbit's ear that corresponded to the ++ scale of Hecker (1971) after 24 hours were recorded.

Statistical analysis

All results were expressed as means ± S.D. The significance between means was determined using student's t-test and results were regarded as significant at $P<0.05$.

RESULTS AND DISCUSSION

Hexane, chloroform and ethanol extracts of *Malva parviflora* L., displayed antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris.* Chloroform extract showed statistically greater antibacterial effects than hexane and ethanol extracts. The diameters of growth inhibitions against *Bacillus subtilis* ranged from 10.81 to 15.3mm for the chloroform extracts as compared to the values ranging from 9.34 to 10.56 and 9.67 to 12.87mm for the hexane and ethanol extracts, respectively. Comparable trends appear for the observed test organism including the fungi. The findings of the present study correlated to the earlier reports (Wang *et al.,* 2001; Shale *et al.,* 2005). The antifungal activity might be due to the presence of alkaloids, essential oils and phenolic quleoside as reported by Abad (Abad *et al.,* 2007). Likewise the

chloroform extract of *Malvastrum coroman-delianum* L. showed statistically, higher antibacterial as well as antifungal activities against *Bacilluus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Aspergillus niger* and *Aspergillus oryzae* than hexane and ethanol extract showing values of growth inhibition zones in the range of 11.67 to 15.58, 10.96 to 14.32, 13.03 to 16.00, 10.38 to 15.37, 10.19 to 14.25 and 10.97 to 16.58mm, respectively. These findings agree with those found in the previous studies (Jimenez *et. al.,* 2003; Abad *et al.,* 2007). Hexane extracts of *Amaranthus viridis* L. exhibit antibacterial activity against the gram positive bacteria *Bordetella bronchiseptica, Bacillus cereus, Micrococcus flavus, Staphylococcus aureus* and *Sarcina lutea* having values of growth inhibition zones in the ranges of 23.21 to 35.61, 13.40 to 13.60, 10.80 to 19.80, 16.60 to 13.80 and 10.04 to 17.40mm respectively. Our result correlates to the findings of Sharma (Sharma, 1993) who attributed the antibacterial effects to 16-hentriacintanone and sterols.

Chloroform extract of *Amaranthus viridis* L. showed antibacterial activity against *Bordetella bronchiseptica, Bacillus pumilus, Staphylococcus aureus* and *Proteus vugaris.* Both, hexane and chloroform extracts showed antibacterial activities against the test organism, though the activity from the chloroform extract demonstrated lesser activity. Ethanol extracts of *Amaranthus viridis* L. displayed antibacterial activity against *Bordetella bronchiseptica, Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Micrococcus flavus, Staphylococcus aureus, Sarcina lutea, Escherichia coli* and *Proteus vulgaris.* Aqueous extract and polar mass of *Amaranthus viridis* L. exhibited antibacterial activity against all above mentioned microorganisms but have notable antibacterial activity against pathogenic bacterium *Bordetella bronchiseptica.*

The acute and chronic irritant response was exhibited by the chloroform extracts of *Malva parviflora* L. and *Malvastrum coromandelianum* L. Hexane and ethanol extracts also showed acute irritant response, which was in accordance with the work already reported (Rohrabach *et a1.,* 1990). Further detailed analysis of the extracts is required to identify the presence and magnitudes of specific components in the extracts responsible for the antibacterial, antifungal and irritant activities.

CONCLUSION

The extracts of *Malva parviflora* L. and *Malvastrum coromandelianum* L. showed almost the similar pattern of antibacterial activities against *Escherichia coli* but slight variations in antibacterial response against *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus*, The chloroform extracts of above plants exhibited prominent antibacterial activity as compared to that of other extracts. The antibacterial range of ethanol extract of *Amaranthus viridis L.* was more prominent than that of its aqueous extract and polar mass.

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