IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 5, Issue 3 (March 2015), PP. 52-57

Screening of antimicrobial activity of *Balsamodendron myrrha* and *Viola oderata* used in Pakistani Folk medicines

Farzana Gull[1], Fahad Akhtar[1] , Anwar khalid[1], Abdul samad[1], Muhammad Junaid Shah[3], Madiha Bashir[1], Mukhtiar Hassan[1], Fareeha Zahoor[2] , Abdul Hafeez[2], Ayesha Iqbal[2]*.

[1]*Department. of Biochemistry, Hazara University Mansehra, KPK, Pakistan.22060*
[2] *Department of Pharmacy, COMSATS Institute of Information and Technology Abbottabad KPK, Pakistan. 22060.*
[3]*Ayub Medical College Abbottabad KPK, Pakistan. 22060.*

SUMMARY: *The antibacterial activity of aqueous and ethanol extracts of Balsamodendron myrrha and Viola oderata were evaluated for anti-microbial activity using the agar well diffusion method against six pathogenic bacteria (Klebsiella pneumoniae, Escherichia coli, Pseudomonas, Salmonella typhi, Stapylococcus epidermidis and Enterococcus). These plants are commonly used in folk medicine to treat infections of microbial origin. The patterns of inhibition varied with the extracts, the solvent used for extraction and the organisms tested. The studies revealed that the activity was in decreasing order of ethanol extracts>cold-water extract>hot-water extract. The preliminary screening experiment revealed that the ethanol extracts of Balsamodendron myrrha and Viola oderata exhibited a higher antimicrobial activity than the cold and hot water extracts. The most susceptible bacterium was Escherichia coli.*

KEYWORDS: *antibacterial activity, Enterococcus, Escherichia coli, Klebsiella pneumoniae Pseudomonas, Salmonella typhi and Stapylococcus epidermidis*

I. INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008). For centuries plants have been used throughout the world as drugs and remedies for various diseases in virtually all cultures as a source of medicine due to the occurrence of natural products with medicinal properties (WHO, 2002). The widespread use of herbal remedies and healthcare preparations has been described in ancient texts such as the Vedas and the Bible (Dougherty *et al*., 1956). The first generally accepted use of plants as healing agents was showed in the cave paintings discovered in the Lascaux caves in France, which have been radiocarbon-dated to 13,000-25,000 BC (Watta and Hanson., 1992).

In developing countries like Pakistan, low income people, people of small villages and remote communities use folk medicine for the treatment of common infections. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rates than modern medicine. Hakeem's (ttraditional healers) claim that their medicines are cheaper and more effective than modern medicine. Patients of these communities have a reduced risk to get infectious diseases from resistant pathogens than people from urban areas treated with traditional antibiotics (Khalid *et al.*, 2011). For this reason the herbal remedies have become more popular in these societies in the treatment of minor ailments. The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. In fact, approximately 25% of modern drugs used in the United States have been derived from plants (U.S Dept.2002).

Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al*., 2005). The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments.

There are several reports on antimicrobial activity of different herbal extracts (Ihtesham *et al.,* 2011, Momin, 1987. Parekh *et al.,* 2005, Parekh and Chanda., 2007., Khare 2004 & 2007., Fabricant & Farmsworth, 2001). Making antibacterial drug therapy effective, safe and affordable has been the focus of interest during recent years (Shah, 2005).

V*iola oderata* is a species of the genus *Viola* commonly known as Sweet Violet, English Violet, Common Violet, or Garden Violet. The herb is known as *Banafsha* in Pakistan and India, where it is commonly used as remedy to cure. *Balsamodendron Myrrha* or (Commiphora myrrha), also known as Gum Myrrh Tree is part of the Burseraceae plant family. Myrrh is the aromatic gummy substance (resin) used from remote ages as an ingredient in incense, perfumes, etc., in the holy oil of the Jews and the Kyphi of the Egyptians for embalming and fumigations. It is used as astringent, healing, tonic and stimulant.

II. MATERIALS AND METHODS

Plants used in this study consisted of *Balsamodendron myrrha* (stem) and *Viola oderata* (Binafsha flower). (The plants were collected from district Abbottabad and identified by the taxonomists of department of Botany Hazara University Mansehra Kpk. The survey was conducted from April 2014 to May 2014 spring season 200g of each plant part i.e. Flower of Binafsha and stem of B. myrrha were washed with tap water and allow drying for 2 weeks in a dark room and then homogenized to fine powder and stored in airtight bottles.

a) Cold water extraction

In order to obtain the plants extracts, 100 gram of each plant (whole plant) were crushed with the help of mortar and pestle. Ten gram of each plant was soaked in cold water and rotated on a rotary shaker at 150 rpm for 24 hours. The extracts were sieved through a filter paper and then centrifuged at 5000 rpm for 5 min. The supernatant was considered as the 100% concentration of the extract. A series of 80%, 60%, 40%, 20% and 10% dilutions were made diluting the concentrated extract with appropriate volumes of sterile distilled water.

b) Hot water extraction

Ten gram of dried powder was extracted in distilled water for 6 hours at slow heat (40 ºC). After every 2 hours, it was filtered through muslin cloth and centrifuged at 5000 rpm for 5 min. The supernatant was collected. The process was repeated twice and after 6 hours, the supernatant was concentrated to make the final volume one-fourth of the original volume (Parekh et al., 2005). The sample was autoclaved at 121 ºC and 15 lbs. pressure and stored.

c) Ethanol extraction

Ten gram of dried powder was extracted with 100 ml of ethanol keeping on a rotary shaker at 150 rpm for 24 hours. It was filtered through of muslin cloth and centrifuged at 5000 rpm for 5 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Parekh et al., 2005). It was stored at 4 ºC in airtight bottles for further studies.

Determination of antimicrobial activity
Microorganisms Used:

Antimicrobial activity was tested against *E.coli, Salmonella typhi, and Staphylococcus epidermidis, Pseudomonas, Klebsiella and Enterococcus*. The cultures were obtained from National Institute of Health (Islamabad) and Ayub Medical Institute (Abbottabad). The cultures were sub-cultured fortnightly and subsequently incubated aerobically in incubator for 24 hrs. on 37ºC.

Culture media

The agar used was Mac Conkey MUG Agar, Product No. 63014 made by Sigma. Nutrient agar was used for growing *S.epidermidis* and *Klebsiella pneumoniae*, MacConkey agar for *Salmonella typhi, Pseudomonas, E.coli,* and *Enterococcus.* Bacterial cultures were maintained on nutrient and Mac Conkey agar media.23g of Nutrient agar and 40g of Mac Conkey Agar were separately mixed with 100ml deionized water in two separate beakers. The both mixtures were stirred until the solids were completely disappear, then both beakers were placed in an autoclave for 15min at 121^0C (15lbs of pressure).Allow the solution to cool to 45^0C to 50^0C and then poured into sterilized Petri plates for further use

Agar diffusion assay

The Antibacterial Activity of *Balsamodendron myrrha* (Murmuki) *and Viola oderata* (Binafsha) against different bacteria was evaluated by agar diffusion method (Parekh and Chanda. 2007) in nutrient agar medium and Mac Conkey agar medium for the assay. The microorganisms were activated by introducing a roomful of the strain in the nutrient broth (30 ml) and incubated on a rotary shaker at 37ºC. The microbial culture was inoculated into the molten medium and after proper homogenization it was poured aseptically into the petri plates. For agar well diffusion method, a well was made in the seeded plates with the help of a cork-borer (0.8mm). The test compound was introduced into the well with the help of sterilized micro pipette and the plates were incubated at 37ºC for 24 hours.

Microbial growth was determined by measuring the diameter of zone of inhibition in millimeters using a scale. For each bacterial strain, controls were maintained where pure solvents were used instead of extract. The zones of inhibition produced by control were subtracted from the test zones and the resulting zone diameter put in the table. Triplicates were kept for each experiment and the experiment was repeated thrice.

III. RESULTS AND DISCUSSION

The crude extracts of *Balsamodendron myrrha* (Murmuki) and *Viola oderata* (Binafsha) showed good antimicrobial activity against different bacteria. Both cold water and ethanol extracts of these plants were effective against bacterial strains whereas, hot water extracts were weakly effective against the bacteria as judged by zones of inhibitions (Tables 1-6). The ethanol extracts of these plants exerted greater antibacterial activity than corresponding water extracts at same concentration (Tables 3 and 6).

Cold water extract of *Balsamodendron* was effective against all organisms but most activity was against Enterococcus and *S.epi.* Hot water extract showed activity lower than cold extract and only against Enterococcus, E.Coli and *Salmonella typhi*. Ethanol extract was active against all test organisms except *S.epi.* Maximum activity was observed against Enterococcus at the concentration of 0.8 gm/lt. Results are slightly matching the findings of the study conducted by

Cold extract of *Viola oderata* at the conc. of 0.8 gm/ it is effective against *E.Coli and Enterococcus, whereas* there is no activity against all other microorganisms tested. The hot water extract showed activity only against *E.Coli*. The ethanol extract of *Viola oderata* showed activity against all microorganisms except K. pneumoniae and S.epi. Maximum activity was shown against Pseudomonas.

IV. CONCLUSION:

These observations may be attributed to two reasons; firstly, the nature of biologically active components (Alkaloids, anthraquinones, saponins and tannins) which could be enhanced in presence of ethanol. Secondly, the stronger extraction capacity of ethanol could have produced greater active constituents responsible for anti-microbial activity. Traditionally, these plants are soaked in water for days and the large quantities of these extracts, which lack specific concentration, are usually administrated to patients. The results therefore support the traditional claim that these medicinal plants have antibacterial activity.

V. ACKNOWLEDGMENT:

The current study was conducted by the students of Biochemistry department and sincere thanks to the faculty of Botany department Hazara University for their help and support. We are also thankful to the locals of the study area for providing us traditional uses run through their families for generations.

Table 1: Sensitivity pattern of *Escherichia coli, Salmonella typhi, Enterococcus, K. pneumonia, Staphylococcus epidermidis and Pseudomonas* to cold-water extract of *Balsamodendron* (Murmuki)

Conc. (gml^{-1})	Zone of inhibition (mm)					
	Pseudomonas	*Enterococcus*	*S.typhi*	*K. pneumoniae*	*E.coli*	*S.epi*
0.1	12	22	10	13	12	17
0.2	13	25	12	15	15	20
0.4	15	27	16	19	17	23
0.6	18	30	20	23	20	25
0.8	22	33	22	25	21	28

Table 2: Sensitivity pattern of *Escherichia coli, Salmonella typhi, Enterococcus, K. pneumoniae, Staphylococcus epidermidis and Pseudomonas* to hot-water extract of *Balsamodendron* (Murmuki)

Conc. (gml^{-1})	Zone of inhibition (mm)					
	Pseudomonas	*Enterococcus*	*S.typhi*	*K.pneumoniae*	*E.coli*	*S .epidermidis*
0.1	NI	10	12	NI	13	NI
0.2	NI	13	15	NI	15	NI
0.4	NI	15	18	NI	17	NI
0.6	NI	17	21	NI	20	NI
0.8	NI	18	21	NI	21	NI

N I= No-inhibition

Table 3: Sensitivity pattern of *Escherichia coli, Salmonella typhi, Enterococcus, K. pneumoniae, Staphylococcus epidermidis and Pseudomonas* to ethanol extract of *Balsamodendron* (Murmuki)

Conc. (gml^{-1})	Zone of inhibition (mm)					
	Pseudomonas	*Enterococcus*	*S.typhi*	*K. pneumonia*	*E.coli*	*S.epi*
0.1	20	25	15	27	17	NI
0.2	21	28	17	30	20	NI
0.4	22	30	20	30	23	NI
0.6	24	32	24	32	25	NI
0.8	24	35	25	33	28	NI

NI= no-inhibition

Table 4: Sensitivity pattern of *Escherichia coli, Salmonella typhi, Enterococcus, K. pneumoniae, Staphylococcus epidermidis and Pseudomonas* to cold-water extract of *Viola oderata (*Binafsha)

Conc. (gml^{-1})	Zone of inhibition (mm)					
	Pseudomonas	*Enterococcus*	*S.typhi*	*K. pneumoniae*	*E.coli*	*S.epi*
0.1	N.I	11	N.I	N.I	12	N.I
0.2	N.I	12	N.I	N.I	15	N.I
0.4	N.I	14	N.I	N.I	18	N.I
0.6	N.I	17	N.I	N.I	20	N.I
0.8	N.I	20	N.I	N.I	21	N.I

N.I=No inhibition

Table5: Sensitivity pattern of *Escherichia coli, Salmonella typhi, Enterococcus, K. pneumoniae, Staphylococcus epidermidis and Pseudomonas* to hot-water extract of *Viola oderata* (Binafsha)

Conc. (gml^{-1})	Zone of inhibition (mm)					
	Pseudomonas	*Enterococcus*	*S.typhi*	*K.pneumoniae*	*E.coli*	*S .epi*
0.1	NI	NI	NI	NI	12	NI
0.2	NI	NI	NI	NI	15	NI
0.4	NI	NI	NI	NI	18	NI
0.6	NI	NI	NI	NI	20	NI
0.8	NI	NI	NI	NI	21	NI

N I= No-inhibition

Table 6: Sensitivity pattern of *Escherichia coli, Salmonella typhi, Enterococcus, K. pneumoniae, Staphylococcus epidermidis and Pseudomonas* to ethanol extract of *Viola oderata* (Binafsha)

Conc. (gml^{-1})	Zone of inhibition (mm)					
	Pseudomonas	*Enterococcus*	*S.typhi*	*K. pneumoniae*	*E.coli*	*S.epi*
0.1	20	17	11	NI	18	NI
0.2	22	20	15	NI	20	NI
0.4	24	24	19	NI	22	NI
0.6	26	25	22	NI	24	NI
0.8	30	28	23	NI	24	NI

NI= No-inhibition

REFERENCES

[1]. Doughterty, R.W. 1956. Bloat in ruminants. U.S. Department of Agriculture Yearbook of Agriculture 1956: 108-1113. [20781]

[2]. Edeoga H O, Okwu D E, Mbaebie B O.,2005 Phytochemical constituents of some Nigerian medicinal plants. African. J. Biotechnol, 4, 685–688.

[3]. Ihtesham B, Mukhtiar H, Fida M. A, Ghulam M, Nasir M, Noshin, Anees F, Muhammad Afzal, Mujadid-ur-Rehman, Farzana Parveen and M. Tariq Khan., 2011. "A study on comparative pharmacological efficacy of *Berberis Lycium* and Penicillin G*."*. African Journal of Microbiology Research, 5(6), pp725-728.

[4]. Mann, A., Banso, A., and Clifford, L.C., 2008. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*, Tanzania J. Health Res., 10 , 34-38.

[5]. Mahesh, B, S. Satish., 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens, World J. Agric. Sci., 4, 839-843.

[6]. Momin A., 1987. Role of indigenous medicine in primary health care. 1st International seminar on Unani medicine, New Delhi, pp. 54.

[7]. Polombo E. A,Semple S J.,2001 . Antibacterial activity of traditional Australian medicinal plants, J. Ethnopharmacol., 77 (2-3), 15-17.

[8]. Parekh J, Jadeja D, Chanda S., 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk J Biol, 29, 203-210.

[9]. Parekh J, Chanda S., 2007. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J. Biol, 31, 53-58.

[10]. Khalid, A., Rehman, U., Sethi, A., Khilji, S., Fatima, U., Khan, M.I., Waqas, M.K., Saqib, Q.N., Hassham, H.A., Farzana, K., Mahmood, S., Waseem, A., Ismail, T., and Murtaza, G., 2011. Antimicrobial activity analysis of extracts of Acacia modesta, Artimisia absinthium, Nigella sativa and Saussurea lappa against Gram positive and Gram negative microorganisms. *African Journal of Biotechnology*. 10(22), 4574-4580.

[11]. Rose M X., Sorna L V, Sivagama S M.,2008. Antimicrobial activity of Selective native medicines. *Indian Journal of Botanical Research*, 4 (2), 213-222.

[12]. Erdogrul OT., 2002. Antibacterial activities of some plant extracts used in folk medicine. Pharmaceutical Biol., 40,269–273.

[13]. Farhat A K, Asfandyar K, Javed H, Muneeb-ur-Rehman K, Mukarram S,Mukhtiar H .,2011. "Assessment of antioxidant and antibacterial activities of *Mentha longifolia*". Journal of Pharmacy Research, 4(7), 2338-2339.

[14]. Khare C P., 2004. Indian Herbal Remedies; Rational Western Therapy, Ayurvedic and other traditional usage, Botany. Springer-Verlag, Berlin Hedeilberg, NewYork

[15]. Khare, C .P, 2007. *Indian Medicinal Plants (Ed. 1st*) Springer Verlag: Heidelburg, Berlin.PP. 622-23.

[16]. Fabricant D S, Farnsworth N R .., 2001 the value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives Supplements*, 109, 69-75.

[17]. Shah P M., 2005. The need for new therapeutic agents: what is in the pipeline? *Clinical Microbiology and Infection*, 11, 36-42.

[18]. U.S Department of Agricultural Research Services, 2005 USDA National Nutrient Database for Standard References, Release 16. Nutrient Data Laboratory Home Page

[19]. WHO. , 2002 World health organization traditional medicinal strategy ,2002-2005; Geneva

[20]. Watta W.A; Hanson BCS; Grimm EC., 1992. Camel Lake; A 40000-yr record of vegetation and forest history from northwest Florida Ecology, 73 (3), 1056-1066.