In vitro antibacterial activity of selected medicinal plants from lower Himalayas

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Abstract: The present studies cover antibacterial activity of the crude methanolic extracts of 11 medicinal plants viz. *Adhatoda vasica*, *Bauhenia variegate*, *Bombax ceiba*, *Carrisa opaca*, *Caryopteris grata*, *Debregeasia salicifolia*, *Lantana camara*, *Melia azedarach*, *Phyllanthus emblica*, *Pinus roxburghii* and *Olea ferruginea* collected from lower Himalayas against two Gram positive (*Staphylococcus aureus, Micrococcus luteus*) and two Gram negative (*Escherichia coli, Pseudomonas aureginosa*) bacterial strains. The extracts were applied at four different concentrations (120 mg/mL, 90mg/mL, 60mg/mL and 30mg/mL) in *dimethyl sulfoxide (*DMSO) by using agar well diffusion method. Antibacterial activities against *Staphylococcus aureus* and *Micrococcus luteus* were observed formethanolic extracts of all the above mentioned plants. Greater antibacterial activity against *Pseudomonas aeruginosa* was only exhibited by *Phyllanthus emblica, Pinus roxburghii, Debregeasia salicifolia* and *Lantana camara. Escherichia coli* was highly resistant to all the plant extracts at all concentrations. It is inferred that methanolic crude extracts of the above mentioned plantsexhibitantibacterial activities against pathogenic bacteria, which proved the ethnobotanical importance of the selected plants that indigenous people use for cure against various diseases.

Keywords: Antibacterial activity, plant extracts, inhibition zone, methanolic extract.

INTRODUCTION

Plants play an essential role in a human diet. Additionally, plant species are used for increasing flavour diet and are recognized for their preservative and medicinal values (DeSouza *et al.,* 2005). As the conventional medicines shows some side effects, therefore the use of natural products proves an alternative to cure and treatment of various diseases in the last few decades (Ansari *et al.,* 2006).

Ethnopharmacology is the utilization of the wide range of knowledge assembled from the indigenous people about the plant and animal products, which they conventionally used for the medical treatment (Vanden Berghe *et al.,* 1986). Studies reported that one quarter of the recommended drugs dispensed by community drug stores in the United States contain at least one active ingredient, a derivative of plants (Fransworth and Morris, 1976). The flavonols, phenolic acids are specifically impressive due to having useful pharmacological properties such as vasoprotective, anti-carcinogeics anti-viral, anti-inflammatory, anti-neoplastics as well as anti-allergic and anti-proliferative activity on tumor cells (Carr *et al.,* 2000).

The ethno botanical uses and economic importance of the selected plant species studied for the antibacterial activity are given as:

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Adhatoda vasica Nees related to the Acanthaceae family and locally named as, Arusa, Adulsa and Bhekar. Fresh leaves, root and bark of this plant are applied on wounds relative to bronchial spam and considered as remedy in asthma especially in combination with belladonna (Nadkarni, 1976). The leaves are useful in gonorrhea and commonly used for bleeding caused by idiopathic thrombocytopenic purpura, bleeding by peptic ulcer, piles and menorrhagia (Doshi, 1983).

Bauhenia variegate L. belong to Ceasalpinaceae family and locally named as Kachnar. Its root and bark are acrid, constipating, anthelmintic and anti-inflammatory. They are useful in diarrhea, intestinal worms, ulcer and cough. The genus contains various active chemical compounds (Silva and Cechinel Filho, 2002), that could be useful for biological activities.

Bombax ceiba L. related to Bombacaceae family and called as Simbal, locally. Root powder of this plant is used as a tonic for impotency. Half cup extract of bark and flower is given for 3 days to both man and woman in sexual diseases like hydrocele, leucorrohea, gonorrhea (Jain *et al.,* 2003).

Carrisa opaca Stapf ex Haines is from Apocynaceae family and known as Garanda, locally. Its root is grounded and put in worm-infested sores of animals. Fruit and leaves are known as cardiac stimulants. Leaf

decoction is used in asthma (Shinwari and Khan, 1998; CSIR, 2003).

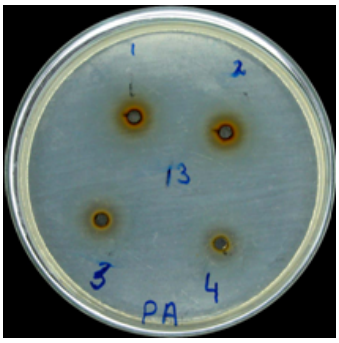

Fig. 1: Antibacterial activity of *Caryopteris grata* against *Pseudomonas aureginosa.*

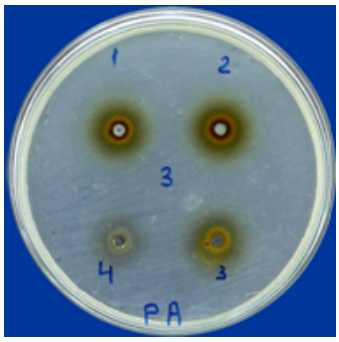

Fig. 2: Antibacterial activity of *Phyllanthus emblica* against *Pseudomonasareginosa*.

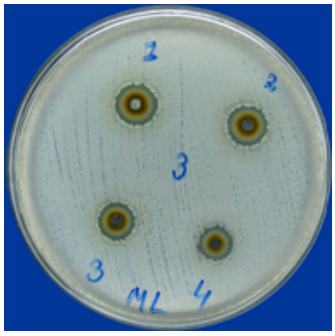

Fig. 3: Antibacterial activity of *Phyllanthus emblica* against *Micrococcus luteus.*

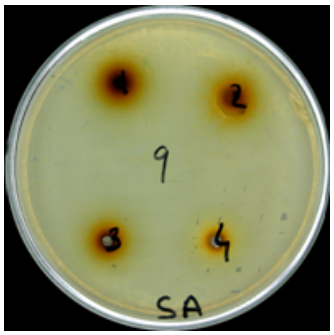

Fig. 4: Antibacterial activity of *Melia azedarach* against *Staphylococcus aureus.*

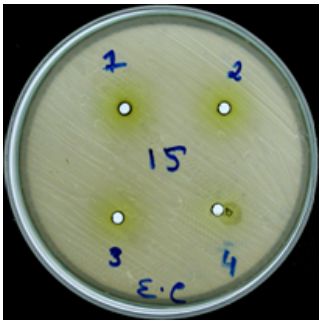

Fig. 5: Antibacterial activity of *Debregeasia salicifolia* against *Escherchia coli.*

Caryopteris grata Benth belongs to the Verbenaceae family. The genus has been used as medicinal plant for the relief of colds, cough and rheumatic pains (Gao and Han, 1997). The decoction of the root and whole plant of *Caryopteris paniculata* are used for diarrhea, skin itch, diminish inflammation, acesodyne (Long and Li, 2004).

Debregeasia salicifolia (D.Don) Rendle related to family Urticaceae and is recognized as Puruni, Siaru, Siharu by the local people. A new triterpene (3β-19α-dihydroxy-30-norurs-12-ene) as well as some other compounds such as lupeol, sitosterol, stigma sterol and oleanolic acid were also reported for the first time from this species (Akbar *et al.*, 2000).

Lantana camara L. is related to the Verbenaceae family and is known as Panch phuli in the local language. The leaves of the plant are boiled like tea and the decoction is a remedy against cough. Leaf decoction is used orally in treating constipation (Watt and Breyer-Brandwijk, 1962).

Melia azedarach L. which is locally known as Derk, belongs to Meliaceae family. Ripe fruits of this plant are used as purgative, insecticides, and antiseptic (Shinwari and Khan, 1998; CSIR, 2003). Oil of the seed is used for the removal of lice from the hairs. The leaf juice is used for intestinal worms, diuretic (Khan *et al.*, 2000).

Olea ferruginea Royle belongs to the Oleaceae family and called as Kahu/Kao in local language. The leaves and bark are bitter, astringent, antiseptic and diuretic. Oil obtained from the fruit is used as rubefacient. Fruit is used as antidiabetic. Root is useful for asthma (Shinwari and Khan, 1998, CSIR, 2003).

Phyllanthus emblica L. from Euphorbiaceae family is locally named as Amla. Powder obtained from the fruit is used as purgative and certain parasitic skin diseases, also employed in hemorrhage, diarrhea, dysentery, anemia and asthma (Shinwari and Khan, 1998).

Pinus roxburghii Sargent related to Pinaceae family and is recognized as Chir by the local people. Wood is used as refrigerant and expectorant. Resin is used in snake bite

and scorpion bite. Crude extract from the needles were found to be inhibitory against several microbes of agricultural importance (Shah, 2006; Khan *et al.,* 2012).

Bacteria have been the worst human diseases causing organism (Ritter *et al.,* 1996) and plant extracts and essential oil have been shown to exert strong biological activities against these microorgainsms (Marinez *et al.,* 1996). Therefore the present investigation was aimed to study the antibacterial activities of eleven plants methanolic extracts (among which some plants that are rarely reported in literature) against gram positive and gram negative bacterial strains.

MATERIALS AND METHODS

Collection and identification of plant material

Plants were collected from Margala Foothills Islamabad Pakistan during 2008 and were identified by the taxonomist. All the plants were provided with voucher numbers and deposited in herbarium of Pakistan in the Department of Plant Sciences, Quaid-e-Azam University, Islamabad. The antibacterial activities of the following plant species were studied.

S. No.	Plant Species	Herbarium vouchers number
1	Adhatoda vasica Nees.	124219
2	Bauhenia variegate L.	124220
3	Bombax ceiba L.	124221
4	Carrisa opaca Stapf ex Haines.	124222
5	Caryopteris grata Benth.	124223
6	Debregeasia salicifolia (D.Don) Randle.	124224
7	Lantana camara L.	124225
8	Melia azedarach L.	124226
9	Phyllanthus emblica L.	124227
10	Pinus roxburghii Sargent.	124228
11	Olea ferrugineaRoyle124229	

The fresh plant materials (200 g) were collected and were rinsed with autoclaved distilled water and kept under shade at room temperature for drying. After dryness, plant parts were ground finely to powder. Fifty grams of each powder was soaked in 500mL of methanol and was blended for vigorous shaking and mixing. The poorly homogenized mixture was kept at room temperature (25±2)°C for 2 weeks. The solutions obtained were filtered by using Whatman filter paper # 41. After filtration, methanol was completely evaporated by using rotary evaporator (Heidolph Germany) and the gummy extract was put in separate bottles and was labeled.

Then, 120 mg of the obtained crude extract of each plant was dissolved in 1mL of DMSO. This stock solution was used for further dilution (90mL, 60mL and 30mM) with DMSO. On the other hand, solution of Ampicillin and Chloramphenicol, 2mg/mL in DMSO, were prepared as standard to compare antibacterial activity or inhibition zone with plant extracts.

ANTIBACTERIAL ASSAY

Test organisms; Bacterial strains

For antibacterial studies, *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 9721) were used as test organisms. The former two are Gram positive and the latter two are Gram negative bacterias. All the four bacterias were obtained from the Department of Microbiology, Quaid-e-Azam University, Islamabad, Pakistan.

Preparation of media for bacteria; Nutrient agar medium

Nutrient broth medium was prepared from 0.13gm/10mL nutrient broth in distilled water at PH 7.0 and was autoclaved. On the other hand 20 gm of the nutrient agar was dissolved in 1L of distilled water to prepareNutrient agar medium, pH was adjusted at 7.0 and was autoclaved.

McFarland BaSO4 turbidity standard

The standard (Barium Sulphate) was prepared by adding 0.5ml (0.048M) of $BaCl_2$ to 99.5mL (0.36N) H_2SO_4. Barium Sulphate turbidity standard (4-6mL) was taken in screwed cap test tube and was used to compare the turbidity (Koneman *et al.,* 1988).

Preparation of inocula

Four colonies of each test organism were cultured into 5ml nutrient broth. The turbid cultures were compared with McFarland standards to obtain 150 x 106 cfu/mL.

Preparation of lawn of bacterial growth

Twenty four hours old Broth culture of the each test organism was then swabbed on the surface of nutrient agar plates with the help of sterile cotton swab to prepare the lawn of test organism. Agar Well Diffusion method was used to study the effectiveness of dye extract against selected gram positive and gram negative bacterias (Saeed and Tariq, 2005).

Pouring of test solution; incubation and measurement of inhibition zone

Using Micropipette, 100µl of test solutions was poured in respective wells. Four concentrations of each plant extract were applied to petri plates. Similar method is used for Ampicillin and Chloramphinicol was used to compare the inhibition zone with plant extracts. These plates were incubated at 37πC. After 24 hour of incubation the diameter of the clear zone, showing no bacterial growth, around each well was measured. Antibacterial activity of eachplant extract dilutions was determined against four bacterial strains.

Table 1: Inhibition zone (mm) after 24 hours at the concentration of 120 mg/ml

Entry	Botanical name	*P. aeruginoa*	*E. coli*	*S. aureus*	*M. luteus*
1	*Adhatoda vasica*	8	-	10	13
2	*Bauhenia vareigata*	-	-	12	15
3	*Bombax ceiba*	-	-	12	15
4	*Carissa opaca*	11	-	12	11
5	*Caryopteris grata*	13	-	17	13
6	*Debregeasia salicifolia*	14	-	11	14
7	*Lantana camara*	10	-	11	15
8	*Melia azedarach*	-	-	10	11
9	*Olea ferruginea*	-	-	-	30
10	*Phyllanthus emblica*	25	-	17	23
11	*Pinus roxburghii*	18	-	13	16

Table 2: Inhibition zone (mm) after 24 hours at the concentration of 90 mg/ml

Entry	Botanical name	*P. aeruginoa*	*E. coli*	*S. aureus*	*M. luteus*
1	*Adhatoda vasica*	8	-	10	10
2	*Bauhenia vareigata*	-	-	9	12
3	*Bombax ceiba*	-	-	12	15
4	*Carissa opaca*	10	-	11	10
5	*Caryopteris grata*	12	-	13	13
6	*Debregeasia salicifolia*	13	-	11	10
7	*Lantana camara*	10	-	11	14
8	*Melia azedarach*	-	-	10	11
9	*Olea ferruginea*	-	-	-	28
10	*Phyllanthus emblica*	22	-	16	22
11	*Pinus roxburghii*	16	-	13	15

Table 3: Inhibition zone (mm) after 24 hours at the concentration of 60 mg/ml

Entry	Botanical name	*P. aeruginoa*	*E. coli*	*S. aureus*	*M. luteus*
1	*Adhatoda vasica*	-	-	-	10
2	*Bauhenia vareigata*	-	-	10	10
3	*Bombax ceiba*	-	-	12	13
4	*Carissa opaca*	9	-	11	10
5	*Caryopteris grata*	6	-	13	13
6	*Debregeasia salicifolia*	12	-	9	8
7	*Lantana camara*	10	-	11	13
8	*Melia azedarach*	-	-	10	11
9	*Olea ferruginea*	-	-	-	28
10	*Phyllanthus emblica*	22	-	15	19
11	*Pinus roxburghii*	14	-	11	15

P.aeruginosa: Pseudomonas aeruginosa, S.aureus: Staphylococcus aureus, E.coli: Eschercia coli, M.luteus: Micrococcus luteus

RESULTS

All the plant extracts exhibited antibacterial activities against *Staphylococcus aureus* and *Micrococcus luteus* (G +Ve) but *Pseudomonas aeruginosa* was susceptible to only few plant extracts while *Escherichia coli* (Gram Negative) was completely resistant to all plant extracts at all concentrations (tables1, 2, 3, 4). Among all the plants studied for the antibacterial activities *Caryopteris grata*, *Debregeasia salicifolia*, *Phyllanthus emblicai* and *Pinus roxburghii* displayed visible zone of inhibition against *Pseudomonas aureginosa*, *Staphylococcus aureus* and *Micrococcus luteus*. Maximum inhibitory concentration was 120 mg/mL at which *Phyllanthus emblica* displayed the greater zone of inhibition among all the plants studied for the antibacterial activity. *Phyllanthus emblica* displayed strong zone of inhibition against *Pseudomonas aureginosa* and *Micrococcus luteus* (figs. 2, 3). *Caryopteris grata*showed minimum inhibition zone against *Pseudomonas aureginosa* (fig. 1). *Melia*

Table 4: Inhibition zone (mm) after 24 hours at the concentration of 30 mg/ml

Entry	Botanical name	*P. aeruginoa*	*E. coli*	*S. aureus*	*M. luteus*
1	*Adhatoda vasica*	-	-	-	10
2	*Bauhenia vareigata*	-	-	8	9
3	*Bombax ceiba*	-	-	8	12
4	*Carissa opaca*	8		11	8
5	*Caryopteris grata*	-	-	11	12
6	*Debregeasia salicifolia*	11	-	9	8
7	*Lantana camara*	10	-	11	13
8	*Melia azedarach*	-	-	8	9
9	*Olea ferruginea*	-	-	-	22
10	*Phyllanthus emblica*	15	-	12	16
11	*Pinus roxburghii*	14	-	11	14

Table 5: Inhibition zone of ampicillin and Chloramphinicol (mm) after 24 hours at the concentration of 2 mg/ml

Entry	Std Antibiotics	*P. aeruginoa*	*E. coli*	*S. aureus*	*M. luteus*
1	Ampicillin	-	15	27	-
2	Chloramphinicol	7	24	25	8

Std: Standard, *P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, E. coli: Eschercia coli, M. luteus: Micrococcus luteus*

azedarach displayed the minimum zone of inhibition against *Staphylococcus aureus* (fig. 4) among all the plants studied for the antibacterial activity. All the plants have no effect on *Escherchia coli* (fig. 5).

Comparing the results with standard antibiotics i.e. Ampicillin and Chloramphinicol, the inhibitory effect of Ampicillin against *Staphylococcus aureus* was about 27mm, 15mm against *Escherchia coli* and had no effect on *Pseudamonas aureginosa* and *Micrococcus luteus*. The inhibitory effect of Chloramphinicol was about 25mm against *Staphylococcus aureus,* 24mm against *Escherchia coli,* 7mm against *Pseudamonas aureginosa* and 8mm against *Micrococcus luteus* (table 5).

All the inhibitory zones of the standards were found to be smaller in size against *Pseudamonas aureginosa* and *Micrococcus luteus* when compared with inhibitory zones of plant extarcts. While the inhibitory effect of the standards were larger against *Staphylococcus aureus* and *Escherchia coli.*

DISCUSSION

Among large number of plants, *Adhatoda vasica* Nees, *Bauhenia variegate* L., *Bombax ceiba* L., *Carrisa opaca* Stapf ex Haines, *Caryopteris grata* Benth., *Debregeasia salicifolia* (D.Don) Randle, *Lantana camara* L., *Melia azedarach* L., *Phyllanthus emblica* L. and *Pinus roxburghii* Royle, appear to have potential for testing as a plant of high medicinal values for various microbial activities as well other medicinal activities. These plants are abundantly found in Pakistan and are easilyaccessible. The present studies revealed that *Phyllanthus emblica* L.has greater antimicrobial activity than rest of the Plants. Ghosh *et al.,* (2008) reported antibacterial activity of *Phyllanthus emblica* L. against five infectious bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris* and *Enterobacter aerogenes*. Extracts from *P. emblica* have shown to possess various pharmacological properties. For example analgesic, anti-inflammatory antioxidant and chemo protective activities.

Antibacterial activity was recorded for methanolic extracts of*Bombax ceiba* L., *Bauhiniavariegata* L., *Caryopteris grata* Benth and *Debregeasia salicifolia* (D. Don) Randle. Dar *etal.,* (2005) reported Mangiferin, 2-β-D-glucopyranosyl-1, 3, 6, 7-tetrahydroxy-9H-xanthen-9-one, from the methanolic extracts of *Bombax ceiba* L. leaves in a considerable amount which showed strong antioxidant activity. Raj*et al.,* (2002) reported anti-microbial activity of the *Bauhiniavariegata* L.against *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus and Vibrio cholerae*. They reported 18 mm zone ofinhibition against *Bacillus subtilis*. Rajkapoor *et al.,* (2006) reported that Oral taking of ethanol extract of *Bauhenia variegata* L. (250 mg/kg) effectively diminish liver tumor. Long and Li *et al.,* (2004) reported that the decoction of root and whole plant of *Caryopteris paniculata* are used for diarrhea, skin itch and diminish inflammation. Akbar *et al.,* (2000) reported a new triterpene (3β-19α-dihydroxy-30-norurs-12-ene), from the methanolic extract of *Debregeasia salicifolia* stem.

Antibacterial activity was also observed for the plant extracts from *Lantana camara* L. Kumar and Chauhan (2006) reported that *Lantana camara* L. exhibit

considerable anti-microbial activity which proves its ethno pharmacological use againstsome infectious diseases. Oliveira *et al.,* (2006) also reported activity of 13 plant extracts against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Strong activity was observed from *Lantana lilacina* and *Phyllanthus tenellus*.

Nearly all the plants we have studied displayed antibacterial activity against Gram Positive bacteria. All the plants have no activity against Gram Negative bacteria. Chandrasekaran and Venkatesalu (2004) also reported more antibacterial activity of *Syzygium jambolana* seeds against Gram Positive strains than Gram Negative strains. Therefore it is explicit why indigenous people use these plants against various infections.

CONCLUSION

Methanolic crude extracts of all the selected medicinal plant species inhibited growth of *Staphylococcus aureus* and *Micrococcus luteus*. *Escherichia coli* was the only bacterial strain exhibiting resistance to extracts of all the plants. *Phyllanthus emblica* extracts showed higher antibacterial activity against *Pseudomonas aeruginosa* as compared with other plant species. It is inferred from the results of present studies that antibacterial activity varies with the plant species and is concentration dependant of the extracts. Methanolic extracts of *Olea ferruginea, Phyllanthus emblica, Lantana camara* and *Pinus roxburghii* have greater potential as antibacterial agents. However, further studies are needed for isolation of some biologically active compounds from these selected medicinal plant species.

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