Research Journal of Chemical and Environmental Sciences
Res J. Chem. Environ. Sci. Vol 7 [4] August 2019: 64-67
Online ISSN 2321-1040
CODEN: RJCEA2 [USA]

Website: www.aelsindia.com/rjces.htm

RJCES

ORIGINAL ARTICLE

Antibacterial activity and Minimum Inhibitory Concentration (MIC) of *Prunus cerasoides* leaves extracts

[1]Naveen Kumar, [2]J. S. Jangwan
[1]Department of Chemistry, KL DAV PG College, Roorkee, 247 667 UK, INDIA
*Email: n.naveen699@gmail.com
[2]Department of Chemistry, H.N.B.Garhwal University Campus, Badshahi thaul,Tehri, UK, India

ABSTRACT
Column chromatography of petroleum ether extract of leaves of Prunus cerasoides gave triacontane, n-pentacosane, octacosanol, ursolic acid and β-sitosterol. Petroleum ether, acetone, and ethanolic extract were screened for the antibacterial activity as well as Minimum Inhibitory Concentration. The maximum antibacterial activity was shown by ethanol extract against the Escherichia coli with zone of inhibition (zone size 6mm). Minimum Inhibitory Concentration (MIC), 0.857 mg/ml shown by acetone extract (vol. of stock sol. 0.6ml) is more active against Staphylococcus aureus and 1.00 mg/ml by ethanolic extract (vol. of stock sol. 0.7ml) active against Pseudomonas aerugenosa.
Keywords: *Prunus cerasoides, Escherichia coli, antibacterial activity, Minimum Inhibitory Concentration (MIC)*

Received 13.05.2019 Accepted 30.07.2019

INTRODUCTION

Prunus cerasoides (syn. *Prunus puddum*) belong to the family Rosaceae, and locally known as Payu Padam, Padmakha, and Himalayan cherry. Plant wildly growing in sub Himalayan tracts to montane zone 2400 mtr high, Sikkim, Nepal, Bhutan, Myanmar, West China, and also cultivated in Dhanolti region in Tehri Garhwal (Uttarakhand), India. Morphologically the plant is a deciduous tree to 10 m high; bark reddish-brown, exfoliating in thin circular strips. Leaves centuplicate in bud, elliptic or ovate-lanceolate, flowers pinkish white, 1.5-2.5 cm across, appearing before the leaves in umbellate fascicles, pedicels 0.5-2 cm long. Calyx bell shaped 5 lobed, lobes ovate-acute [1, 2, 3]. In traditional medicine, the plant is used in Leprosy, Asthma and shown antipyretic activity. According to the earlier investigation, much work has been carried out on stem bark, sapwood, seed of the plant due to the presence of high concentration of flavonoids and flavonoid glycosides[4-8], but leaves are scantly studied. This prompted us to carry out chromatographic resolution of petroleum ether extract and screened petroleum ether, acetone, and ethanol extracts for their antibacterial efficacy against various bacterial strains.

MATERIAL AND METHODS

Clean and healthy leaves were collected from Chamba, Tehri Garhwal (Uttarakhand), and authenticated by Dr. J.K.Tiwari Taxonomists, Botany department, HNB Garhwal University Campus Srinagar Garhwal (Uttarakhand) India. Bacterial cultures, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aerogenosa were obtained from Department of Microbiology, Hill campus Ranichauri and checked for purity by conventional biochemical methods. Leaves obtained were dried in tray drier for 35 hours at 50^0 C and powdered. Powder thus obtained was extracted with various solvents like petroleum ether, acetone, and ethanol using Soxhlet apparatus. The extracts were concentrated under reduced pressure and tested for various phytochemical constituents and screened for their antibacterial efficacy against various pathogenic cultures by Agar disc diffusion and Minimum Inhibitory Concentration (MIC) methods.

ANTIBACTERIAL ACTIVITY STUDIES

(1) AGAR DISC DIFFUSION METHOD

The extract of leaves of *Prunus cerasoides* was tested for antibacterial activity using agar disc diffusion method on solid media [9-11] Luria agar was used as basal medium for *Escherichia coli* and *Bacillus subtilis*; and nutrient agar was used as basal medium for *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

5 g of luria broth and 4 g of agar powder; 3.25 g of nutrient broth and 4 g of agar powder was weighed and 250 ml of water was added separately. The mixture was heated to dissolve the components. Luria agar and nutrient agar are sterilized in an autoclave [12]. Luria agar and nutrient agar are poured in the sterile Petri plates. Mother culture of each organism as set up 24 h before the assays in order to reach stationary phase of growth.[13] The tests were assessed by inoculating Petri dishes from the mother cultures which had been surface spread with 0.1 ml of each bacteria, with the aim of obtaining microorganism concentration of 10^5 colony forming units (CFU/ml [14] An aliquot of Dimethylsulphoxide (DMSO) was added to the extract in order to obtain 5mg/ml concentration range[15]. Sterile dilutions of essential oil were deposited on the sterile Whatmann filter paper No.1 discs (5mm disc diameter), which were subsequently placed in inoculated Petri plates. Therefore the Petri plates were then incubated at 37° C for 24 h. The antibacterial activity was determined by measuring the diameter of zone of inhibition surrounding bacterial growth [11]

(2) MINIMUM INHIBITORY CONCENTRATION (MIC) METHOD

The determination of minimum inhibitory concentration (MIC), involves semi quantitative procedure which gives an approximation to the concentration of antimicrobial needed to prevent microbial growth. The test bacteria used to determine MIC involves *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureaus*. The broth dilution method was used to measure MIC in order to determine the antibacterial effect of plant extracts [12]. Two fold serial dilutions were prepared in broth media to obtain a concentration range of 0.142 mg/ml, 0.285 mg/ml, 0.571 mg/ml, 1.142 mg/ml, 2.285 mg/ml, 4.571 mg/ml using sterile screw bottles.[12] Bacterial colonies (mentioned above) were suspended in saline solution (0.85%) and turbidity of the saline solution was adjusted to 0.5 Mc Farland standard[13] (9.95 ml of 1% H_2SO_4 and 0.5 ml $BaCl_2$). To each test tube 100 µl of standardized suspension of test bacteria were added and incubated at 37º C for 24 h. The end result of the test was the minimum concentration of antimicrobials which gave clear solution i.e. no visual growth [15-16].

RESULTS AND DISCUSSION

The petroleum ether extract of *Prunus cerasoides* was subjected to column chromatographic resolution by silica gel. It gave triacontane, n-pentacosane, octacosanol, ursolic acid and β-sitosterol. Their identities were confirmed by comparison with authentic sample (co-TLC, co-IR, m.m.p.) and also comparing their spectral data with that of reported in literature [17]. Different extracts of *Prunus cerasoides* leaves were found to be quite effective in inhibiting the growth of various bacterial strains as indicated by zone of inhibition. Among the various extracts, Ethanol extract showed good antibacterial activity and maximum zone of inhibition was obtained for *Escherichia coli* (zone size 6mm), followed by *Pseudomonas aeruginosa* (4mm) results are given in (Table-1) and pictures of Petri dices are shown in (Fig 1). Acetone extract showed antibacterial activity was obtained for *E. coli* (zone size 5mm). On the other hand Petroleum ether extract was found to be totally unaffected against these bacterial strains.

Minimum inhibitory concentration (MIC) was showed by the different extracts of *Prunus cerasoides* leaves (Table-2). Minimum inhibitory concentration (0.857mg/ml) shown by acetone extract (vol. of stock sol. 0.6ml) was more active against *Staphylococcus aureus* (Fig. 2). Maximum concentration (1.286mg/ml) was shown by the petroleum ether extract (vol. of stock sol. 0.9ml) against *Staphylococcus aureus* (Fig. 3). 0.7ml vol. of stock sol. of ethanol extract was active against *E. coli* on the concentration of 1.00 mg/ml (Fig. 4). 0.7ml vol. of stock sol. of Pet ether was active against *E. coli* on the concentration of 1.00 mg/ml (Fig. 5). Thus it could be concluded that ethanol extract of *Prunus cerasoides* leaves has very good antibacterial activity by Agar disc diffusion method and Acetone extract has a good antibacterial activity by Minimum Inhibitory Concentration (MIC) method.

Table 1: Antibacterial activity of Different extracts of *Prunus cerasoides* leaves

S.N.	Bacterial strain	Group	Zone of inhibition Ethanolic Extract	Pet. Ether Extract	Acetone Extract
1.	*Bacillus subtilis*	Gram (+)	-ve	-ve	-ve
2.	*Staphylococcus aureus*	Gram (+)	-ve	-ve	-ve
3.	*Escherichia coli*	Gram (-)	-ve	5mm	6mm
4.	*Pseudomonas aerugenosa*	Gram (-)	-ve	-ve	4mm

Table 2: Minimum Inhibitory Concentration (MIC) of different extracts of *Prunus cerasoides* leaves

S.N.	Bacterial strain	Concentration of Extract mg/ml			Volume of stock sol.(ml.)			Bacterial strain shown activity in +ve and -ve		
		Pet Ether	Acetone	Et OH	Pet Ether	Acetone	Et OH	Pet Ether	Acetone	Et OH
1.	*Bacillus subtilis*	1.143	1.286	1.00	0.8	0.9	0.7	**-ve**	**-ve**	**+ve**
2.	*Staphylococcus aureus*	1.286	0.857	1.143	0.9	0.6	0.8	**-ve**	**+ve**	**-ve**
3.	*Escherichia coli*	1.00	1.429	1.143	0.7	0.9	0.8	**+ve**	**+ve**	**+ve**
4.	*Pseudomonas aerugenosa*	1.286	1.143	1.00	0.9	0.8	0.7	**-ve**	**-ve**	**+ve**

Front disc **Back disc**

Fig 1. Antibacterial activity of ethanol extract, against *E.Coli*

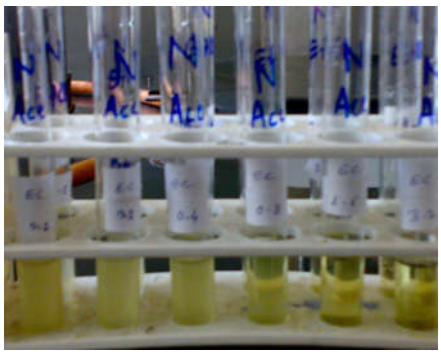

Fig 2. MIC of acetone extract Against *Staphylococcus aureus*

Fig 3. MIC of Pet. Ether extract against *Staphylococcus aureus*

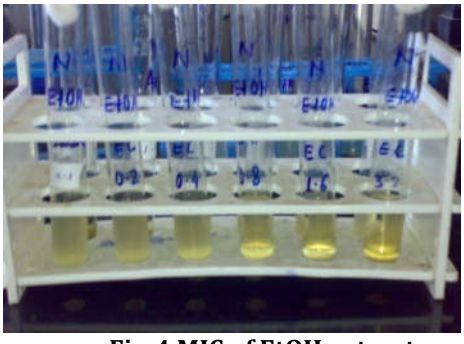

Fig-4 MIC of EtOH extract against *E. coli*

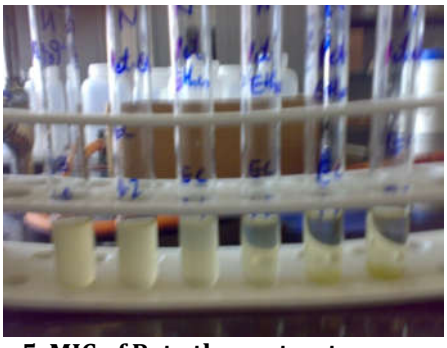

Fig-5 MIC of Pet.ether extract against *E. coli*

ACKNOWLEDGMENTS

Our thanks are due to Dr. J. Kumar, Head, Plant pathology, G.B.Pant University Hill Campus Ranichauri, for providing various bacterial strains.

REFERENCES

1. Gour R.D., (1999), Flora of District Garhwal, 1st ed. Tran's media. Srinagar Garhwal, 226.
2. Wealth of India, (CSIR), New Delhi, Revised Edition.
3. Kirtikar SL, Basu BD., (1976), Indian Medicinal Plant M/S Periodic Expert, Delhi, 959.
4. Kashinath, Yeshwant, Nagar, J., Vikas, (2000) (India) Indian, pp 8.
5. Singh, B.P., Dhyani, S.K Chauhan, D.S., Prasad, R.N., (1995*),* ICAR Meghalya, India, Ind. J. of Agricultural science. 65(5), 345.
6. Jangwan, J.S., Bahuguna, R.P., (1989) International J. of Crude drug research, 27(4), 223-226.
7. Jangwan, J.S., Bahuguna, R.P., (1987), Fitoterapia, 58(2), 140.
8. Bahuguna, R.P., Jangwan, J.S., Kaiya, T., Sakakibara. (1987), Journal of Natural product, 50 (2), 232-234.
9. Milojevi, S., Dimitrijevi,S., Sakala, D.U., (2007), J. Serb. Chem. Soc., 72, 311–320.
10. Mostahara, S., Alam, S., Islam, A., Mostahar, S., (2003), J. Serb. Chem. Soc., 72, 321–329**.**
11. Duramaz, H., Sagun, E., Tarakei, Z., Ozgokce, F., (2006), African Journal of Biotechnology, 15**,** 1795-98.
12. Kim, S.T., Hwang, J.Y., Sung, M.S., Lee, S.H., (2006), Korean Journal of Veteran Survellience, 29, 19-26.
13. Lee, S.B., Cha, K.S., Kim, S.N., (2007), Journal of Microbiology, 45, 53-57.
14. Yuenyongsawad, S., Tewtrakul, S., (2005), Journal of Science & Technology, 27**,** 498-02.
15. Sohel, M., Islam, A., (2006), Journal of Serbian Chemical Society, 72, 321-29.
16. Sacchetti, G., Maietti, S., Muzolli, M., Bruni, R., (2005), Food Chemistry, 91, 621-32.
17. Heilborn, I., Cook, A.H., Jone, E.R.H., (1953), 'Dictionary of organic compounds' vol. IV.

CITE THIS ARTICLE

Naveen Kumar, J. S. Jangwan. Antibacterial activity and Minimum Inhibitory Concentration (MIC) of *Prunus cerasoides* leaves extracts. Res. J. Chem. Env. Sci. Vol 7 [4] August 2019. 64-67

