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Studies on Antibacterial Activity of *Verbascum thapsus*

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Abstract
The antibacterial activity of the methanolic and acetone leaf extracts of *V. thapsus* was determined *in-vitro* against medically important pathogens such as *Escherichia coli*, *Yersinia pestis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Staphylococcus aureus* following agar-well diffusion method using different concentrations (25%, 50%, 75% and 100%). Results showed low to significant antibacterial activity against the mentioned bacterial strains. Methanolic leaf extract was found to be more effective against selected pathogenic bacterial *spp*. as compared to acetone leaf extract. Further the leaf extract of both plants inhibited gram- positive bacteria more efficiently than gram-negative bacteria. Therefore, the leaf extracts of these plants can be selected for further investigation to determine their therapeutic potential.

Keywords: *Verbascum thapsus*, leaf extracts, antibacterial activity, agar-well diffusion.

1. Introduction
The use of plants for treating various diseases is as old as the human species. Plants play a vital role in the everyday needs of human life. They are generally used as food, cosmetics, flavours, medicines, and ornaments [1]. Medicinal plants have become part of complementary medicine worldwide because of their beneficial health effects [2]. Plants are traditionally being used for medicinal treatment of numerous human disorders including infectious diseases caused by different microorganisms. Various plant extracts have been widely used for therapeutic purposes including combating infectious diseases [3].
The Scrophulariaceae are members of the Figwort family of herbs and shrubs. They comprise 269 genera and 5100 species, most of them are located in temperate and tropical mountainous areas [4]. Plants within this genus are widely used in folklore medicine [5], and have, therefore, potential pharmacological importance. These plants have inhibitory activities against murine lymphocytic leukemia and influenza viruses A2 and B [6]. Common mullein, also known as Wooly Mullein (*Verbascum thapsus* L.) is represented by 360 global species [7] and has been used as a medicinal herb since time immemorial. The leaves and flowers of this plant are reported to have expectorant and demulcent features which are used to treat various respiratory problems such as bronchitis, dry coughs, whooping cough, tuberculosis, asthma and hoarseness. The plant is also reported to be mildly diuretic and to have a soothing and anti-inflammatory action on the urinary tract, and to act as a mild sedative. *V. thapsus* extract has also been used as a domestic remedy for ailments such as pneumonia, fever, congestion, allergies, migraine, catarrhs and colic [8, 9]. Scrophulariaceae members are a source wide variety of chemical constituents e.g. saponins, monoterpene glycosides, iridoids, phenylethanoid glycosides, neolignan glycosides, flavonoids, steroids, spermine alkaloids, phenolic acids, and fatty acids [10]. *Verbascum thapsus* chemical constituents include: 3% mucilage that transforms to galactose, arabinose and aromatic acids (act as unguent) after hydrolyzes; 4% flovonoeids including rutine and hesperidin as common (cause diuretic effects), triterpene saponins, verbasco saponin(have expectorant activity), iridoid glycoside, tannen: acobin, katapol and related compounds (have anti-inflammatory activity), polysaccharides: galactose, arabinose and phenolic acids that have medical effects in local inflammation [11, 12]. Dried powder and leaves poultice are generally used for severe wounds of any kind. Six spices of this flower are reported to have no side effects and have the following treatment features; pulmonary disease, sominiferous, sore throat, vein constrictor, wound healing and pertussis treatment [13, 14]. A study reveals that a commercial product FO- Com

(Flowers extracted of *V. thapsus* in pure Olive oil), had antibacterial activity against *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This activity was attributed to the presence of saponins [15]. The commercial product obtained from flowers of *V. thapsus* in pure olive oil also exhibited antitumor activity. The saponin fraction was shown to be responsible here [16]. Therefore, in the present work an attempt has been made to analyse the antibacterial potential of methanolic and acetone extracts of *Verbascum thapsus* against selected pathogenic bacterial strains.

Materials and methods

Collection of plant material

Leaves of *Verbascum thapsus* were collected from Devthana-Athrakan area of District Sirmaur, Himachal Pradesh, India. The collected plant materials were brought to the laboratory for further analysis.

Processing of plant material

Leaves of *Verbascum thapsus* were plucked and collected from respective plants, washed thoroughly under tap water and then with 2% Mercuric chloride. After that the leaves were cut into smaller pieces for quick drying. Cleaned leaves were shade dried for 15-20 days. The dried plant materials were crushed into fine powder with the help of pestle mortar. Finally the fine powder was stored in air tight container at room temperature.

Preparation of methanolic and acetone extracts

5gm dried leaves of *V. thapsus* were taken in separate Erlenmeyer flasks to which 50ml of required solvents i.e., methanol and acetone were added. The flasks were covered with aluminium foil and allowed to stand for 3-5 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected and weighed. Finally, stock solution of conc. 50 mg/ml was prepared.

Procurement of bacteria

Bacterial strains used for antibacterial studies were procured from Department of Biotechnology, Himachal Pradesh University, Summer Hill Shimla, India. Pathogens used for the study were *Escherichia coli, Pseudomonas aeruginosa, Yersinia pestis, Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes*.

Revival of pathogen

The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C.

Screening the antibacterial activity of methanolic and acetone extracts of *Verbascum thapsus*

Screening of plant extracts (methanol & acetone) of *V. thapsus* was done using agar-well diffusion method. Nutrient agar medium (Beef extract 1g, Yeast extract 2g, Sodium Chloride 1g, Peptone 5g, Agar 20g, Distilled Water 1000 ml) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and poured into petriplates. Bacteria were grown in nutrient broth for 24 hours. A 100µl of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25%, 50%, 75% and 100% concentration of prepared leaf extracts of *V. thapsus.* The petri plate kept as a control contained pure solvent only. The plates were incubated at 37 ± 2 ^{0}C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition diameter using control as standard [17].

Percentage of growth inhibition= (Control-Test/Control) x100

Control=average diameter of bacterial colony in control.

Test=average diameter of bacterial colony in treatment sets [18].

Results and discussion

In the present study, common plant namely *Verbascum thapsus* was tested for its antimicrobial properties against selected human pathogens. Results obtained revealed that the tested plant extracts possess considerable potential antibacterial activity against *Escherichia coli, Yersinia pestis, Pseudomonas aeruginosa, Bacillus cereus, Listeria monocytogenes and Staphylococcus aureus* (Table 1).

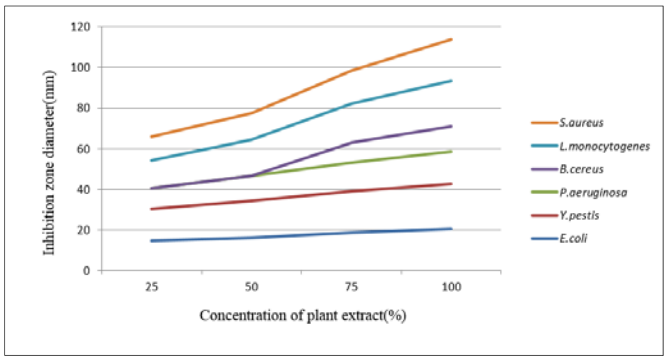
Table: 1 Percent inhibition of growth of pathogenic bacterial *spp.* at different concentrations of methanolic and acetone extracts of V. *thapsus*

Extract	Concentration in %	Inhibition zone diameter (In mm)					
		E. coli	*Y. pestis*	*P. aeruginosa*	*B. cereus*	*L. monocytogenes*	*S. aureus*
Methanolic Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	14.45±0.25	15.80±1.30	10.10±0.00	0.00±0.00	14.00±1.05	11.50±0.78
	50	16.20±1.27	17.95±1.56	12.40±0.59	0.00±0.00	17.70±2.22	13.20±0.44
	75	18.47±1.50	20.50±2.00	14.15±0.28	9.90±1.08	19.00±0.00	16.60±0.67
	100	20.43±0.47	22.00±0.00	16.10±1.82	12.30±0.06	22.45±1.65	20.35±1.33
Acetone Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	9.00±0.89	10.50±1.44	10.15±0.89	0.00±0.00	11.00±0.54	11.60±1.75
	50	11.90±0.70	13.20±0.76	12.85±1.87	0.00±0.00	13.45±0.66	14.65±1.94
	75	14.10±1.08	14.60±0.70	14.05±1.91	0.00±0.00	16.10±0.79	17.20±0.90
	100	16.25±0.76	17.75±2.03	17.10±0.98	10.00±1.56	18.55±0.24	21.50±2.54

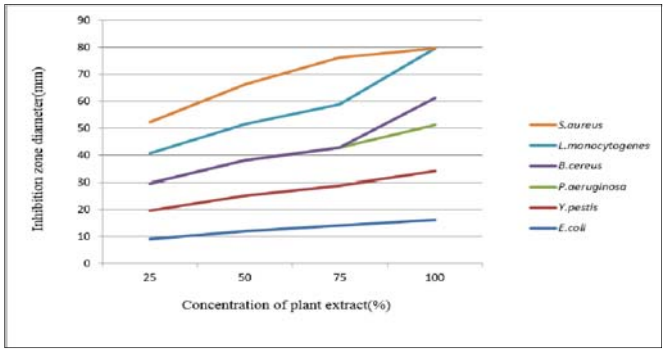
Each data represent mean of three replicates ± S.D.

The screening revealed that the methanolic leaf extract of *V. thapsus* was quite effective against *Listeria monocytogenes* (22.45±1.65mm at 100%, 19.00±0.00mm at 75%, 17.70±2.22mm at 50% and 14.00±1.05mm at 25%) and showed minimum inhibition against *Bacillus cereus* (12.30±0.06mm at 100%, 9.90±1.08mm at 75%, 0.00±0.00mm at 50% and 0.00±0.00mm at 25%) while the acetone leaf extract was found to be most effective against

Staphylococcus aureus (21.50±2.54mm at 100%, 17.20±0.90mm at 75%, 14.65±1.94mm at 50% and 11.60±1.75mm at 25%), and showed minimum inhibition against *Bacillus cereus* (10.00±1.56mm at 100%, 0.00±0.00mm at 75%, 0.00±0.00mm at 50% and 0.00±0.00mm at 25%) as given in table 1.



(A)



(B)

Fig 1: Antibacterial activity of *V. thapsus* against selected bacterial strains:
(A) Methanolic leaf extract; (B) Acetone leaf extract.

Conclusion

It was concluded from the above experimental observations that the plant *Verbascum thapsus* showed potent antibacterial activity against different bacterial strains at all concentrations. Methanolic leaf extract was found to be more effective against pathogenic bacterial *spp*. as compared to acetone leaf extract. Further the leaf extract of both plants showed more inhibition effect in gram- positive bacteria than in gram- negative bacteria. Possible reasons for this antibacterial activity of *V. thapsus* are presence of alkaloids, tannins, saponins, terpenes and flavonoids in their leaves [19, 20]. Findings of present study are preliminary and further investigations are required to determine the exact nature of the bioactive compounds which may be present in the leaves.

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Conflict of interest

The authors hereby declare that there is no conflict of interest regarding the manuscript and experimentation done.

References

1. Chan L, Cheah ELC, Saw CLL, Weng W, Heng PWS. Antimicrobial and antioxidant activities of Cortex *Magnolia officinalis* and some other medicinal plants commonly used in South-East Asia. Chinese Med. 2008; 3(1):15.
2. Zanon SM, Ceriatti FS, Rovera M, Sabini LJ, Ramos BA. Search for antiviral activity of certain medicinal plants from Cordoba, Argentina. Rev Latinoam Microbiol. 1999; 41(2):59-62.
3. Rajbhandari M, Mentel R, Jha PK, Chaudhary RP, Bhattarai S, Gewali MB. Antiviral activity of some plants used in Nepalese traditional medicine. Evid Based Complement Alternat Med. 2009; 6(4):517-22.
4. Rahmatullah Q, Bhatti GR. Taxonomy of Scrophulariaceae from Nara desert, Pakistan. Pak J Bot. 2008; 40(3):973-978.
5. Gvazava LN, Kikoladze VS. Verbascoside from *Verbascum phlomoides*. Chem Nat Comp. 2007; 43(6):710-711.
6. Alper S, Basaran D. Antimicrobial activity of the leaves of *Verbascum sinuatum* L. on microorganisms isolated from urinary tract infection. Afr J Microb Res. 2009; 3(11):778-781.
7. Faik AK, Zeki A. Revision of the Genus *Verbascum* L. (Group A) in Turkey. Bot Res J. 2008; 1(1):9-32.
8. Turker AU, Camper ND. Biological activity of common mullein, a medicinal plant. J Ethnopharmacol. 2002; 82:117-125.
9. Millspaugh CF. American medicinal plants. Dover Publishing Inc, New York, 1974; 430-434.
10. Grigore A, Colceru-Mihul S, Litescu S, Panteli M, Rasit I. Correlation between polyphenol content and anti-inflammatory activity of *Verbascum phlomoides* (mullein). Pharm BioL. 2013; 51(7):925-9.
11. Jafarnia S, Khosroshahi S, Hasemi MG. Textbook and Extensive Medical Plants. Integrated Agricultural Education Sabze Iran, 2006; 36-39.
12. Millspaugh CF. American Medicinal Plants. Dover Publishing Inc. New York, 1974; 430-434.
13. Khosbin S. 100 Magical herbals. Donyaye Noor Company. 2007; 2:667-669.
14. Zargari E. Iran Medical Plants. Iran University of Medical Scinces and Health Services. 1989; 4:230-235.
15. Meurer-Grimes B, Mcbeth DL, Hallihan B, Delph S. Antibacterial activity in medicinal plants of the Scrophulariaceae and Acanthaceae. Int J Pharmacog. 1996; 34:243-248.
16. Paszkiewicz G, Turker AU, Camper ND. Antitumoractivity of common mullein,a medicinal plant. J Ethnopharmacol. 2002; 82:117-125.
17. Hemashenpagam N, Selvaraj T. Antibacterial potential of different extracts of *Solanum xanthocarpum* Chard and Wendt. Plant Arch. 2010; 1:387-390.
18. Kannan P, Ramadevi SR, Hopper W. Antibacterial activity of *Terminalia chebula* fruit extract. Afr. J. Microbiol. 2009; 3:180-184.
19. Lin MT, Chen LC, Chen CK, Liu KC, Lee SS. Chemical constituents of *Verbascum* L. species. J. Nat. Prod. 2001; 64:707.
20. Panchal MA, Murti K, Lambole V. Pharmacological properties of *Verbascum thapsus*. Journal of Pharmaceutical Sciences Review and Research. 2010; 5(2):73-77.