



SCREENING OF ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS

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ABSTRACT

Antibacterial agents are the remarkable source against bacterial infection but continuous increases in antibiotics resistant bacteria threaten human health, especially for immunosuppressed patients since last three decades. The selected plants widely used in folklore remedies, showed broad spectrum activity. Methanol and n-hexane extracts of fruits of *Solanum nigrum* L., leaves of *Dodonaea viscosa* Jacq and *Cannabis sativa* L., were screened for antibacterial activities, against different Gram positive and Gram negative bacterial strains. The results showed that n-hexane extracts of plants were inactive against *Pseudomonas aeruginosa* while methanolic extracts of all plants except *Solanum nigrum* L. were active against all the tested organisms. The results indicated that all medicinal plants have effective antibacterial activities and should be a new source of antibiotics. Further work is needed to isolate the active antimicrobial agents.

Keywords: *Solanum nigrum*, *Dodonaea viscosa*, *Cannabis sativa*, Gram positive/Gram negative bacteria.

INTRODUCTION

The rich flora of Pakistan includes medicinal plants that are being used for therapeutic purposes. Developing countries like Pakistan depend on plant resources for food, shelter, fodder, agriculture and herbal medicines. Out of about 258,650 species of higher plants reported from the world; more than 10% are used to cure ailing communities. Majority of the people in Pakistan relies on medicinal plants to find treatment for their minor, even in some cases major diseases.^{1,2}

All over the world, hundreds of plants are rich sources of medicine³, which are used as potent agents for powerful drug discovery in different countries⁴. The therapeutic value of plants is due to the presence of some chemical substances within the plant tissues which produce a definite physiological action on the human body; include alkaloids, flavonoids, glucosides, tannins, gums, resins, essential oils, fatty oils, carbon compounds, hydrogen, oxygen, nitrogen salts of some chemicals etc.⁵. A wide range of medicinal plant parts is used for extract as raw drugs⁶. This awareness is tremendously increased finding of new antimicrobial bioactive^{7,8}.

The control of bacterial infection has been remarkably impressive since the discovery of antibacterial drugs. However, some of the pathogens rapidly become resistant to many of the first discovered effective drugs (WHO, 2002)⁹, hence, actions must be taken to reduce this problem such as controlling the misuse of antibiotics and continuing investigations aimed at the development of drugs from natural sources¹⁰. Efforts are on to promote novel technologies and to intensify research in combating drug resistant microbes^{7,8}. Therefore, researchers are increasingly turning their attention to ethno-medicine,

looking for new leads to develop more effective drugs against microbial infections¹¹.

Solanum nigrum (L., Family Solanaceae) is commonly used in the traditional medicine as a remedy for treating various diseases. The berries possess medicinal properties such as sedative, diaphoretic, diuretic, hydragogue, expectorant and are useful in the disease of liver, heart and eyes and are also effective at piles, fever and dysentery¹². The leaves are used to heal open wounds and are known to possess hypotensive effect¹³.

Dodonaea viscosa (J.) belongs to family, Sapindaceae and similarly, *Dodonaea viscosa* (J.) is a popular medicinal plant. Its leaves *D. viscosa* is used as anti-inflammatory, anti-ulcer, antibacterial and antifungal agents and in the treatment of fractures¹⁴. *Cannabis sativa* L. belongs to Cannabaceae family and used inflammation, nausea, headache, hematochesia, diarrhea, and alopecia^{15,16}.

The present study aims to evaluate the antibacterial activity of methanol and n-hexane extracts of the fruit of *Solanum nigrum* L, leaves of *Dodonaea viscosa* Jacq and *Cannabis sativa* L. The plants were selected on the bases of their importance in traditional medicine, so this study was carried out to check the anti-microbial activities of these important plants growing in Pakistan.

MATERIALS AND METHODS

Plant materials

Solanum nigrum (L.), *Dodonaea viscosa* (J.) and *Cannabis sativa* (L.) were collected from different areas of Batkhela, Malakand Agency, Pakistan. The plants were identified by Madam Zakia Ahmad, Lecturer in the Department of Botany, University of Malakand, Chakdara Dir (L), KPK, Pakistan.



Extraction of plant materials

Prior extraction, plant parts materials were cleaned 2-3 times with running water and once with sterilized distilled water then surface sterilized with 1%mercuric chloride. The materials were dried under shade at room temperature (25-35°C). The air-dried plant materials were grounded into coarse powder form through electric blender.

Three hundred grams of each air-dried plant material were soaked in 70% methanol and 90% n-hexane respectively. They were regularly shaken for maximum extraction at 80rpm for seven days. After 7 days, the extract was filtered using Whattman filter paper (No. 1). The extract's solutions were evaporated to dryness under reduced pressure at temperature of 45°C using a vacuum pump with the rotary evaporator. The paste obtained after rotary evaporation contained some water content which was further dried in water bath at 60°C for one hour. The thick pastes obtained are known as the crude extract. The extracts were kept in sterile bottles at 5°C until use.

Test microorganisms and microbial culture

Five bacterial strains in which two were Gram positive: *Bacillus cereus* and *Bacillus subtilis* and three Gram negative: *E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*, were used in this study. All the bacterial strains were clinical isolates obtained from the Department of Pharmacy, University of Malakand, Chakdara, Khyber Pukhtunkhwa, Pakistan.

Growth media

Nutrient agar media is best growth media for bacteria. The Media was composed of Beef extract 3.0g, Agar 15.0g and Peptone 5.0g. One liter media was prepared by dissolving 40g of nutrient agar in 700ml of distilled water. After complete dissolution, the final volume of the media was raised to 1000ml by adding more distilled water. The media was boiled using a hot plate. The PH was adjusted to 7.0 at 25 °C, using 0.1M NaOH and 0.1M HCl. The needed media and all glassware were sterilized through autoclaving at 15psi at 121 for 20 minutes.

Antibacterial activity

Antimicrobial was checked by antibacterial susceptibility test (AST). AST standard tests can be conveniently divided into diffusion and dilution methods. Common diffusion tests include agar well diffusion, agar disk diffusion and bio-autography, while dilution methods include agar dilution and broth micro/macro-dilution. The broth and agar based methods are the conventional reference methods for AST¹⁷. Agar well diffusion assay is used to check crude extract of different plant parts for antibacterial activities^{18,19}.

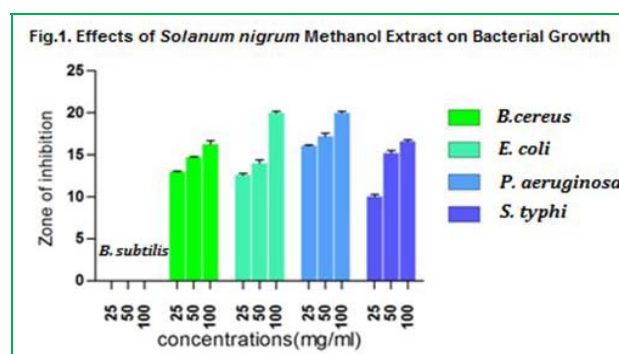
The 20ml of nutrient agar was plated in Petri dishes and allowed to solidify for 30 minutes. Wells of 6mm in diameter and about 2 cm apart were punctured in the

culture media using sterile cork borers to make three to five uniform wells in each Petri dish. A drop of molten nutrient agar was used to seal the base of each well. The wells were filled with 50µl of the extract concentration of 25mg/ml, 50mg/ml and 100mg/ml. In the central well 50µl of 90% n-hexane and 70% methanol was use as negative control for respective extracts. The antibacterial activities were determined after 24 hours at 37°C incubation in the incubator. The diameter of zone of inhibition produced by the extract were measured and compared with standard. Each sample was used in triplicate for the determination of antibacterial activity. The work was carried out in laminar flow.

RESULTS AND DISCUSSION

The methanol and n-hexane extracts of *S. nigrum* L. fruits, leaves of *D. viscosa* Jacq and *C. sativa* L. were screened for antibacterial activity against two Gram-positive bacteria; *B. subtilis*, *B. cereus* and three Gram-negative bacteria; *E. coli*, *P. aeruginosa* and *S. typhi*. The methanol crude extracts showed good antibacterial activity than the n-hexane crude extracts. The n-hexane extracts of all the tested plants were inactive against *P. aeruginosa*. The methanol extracts of the plants showed good antibacterial activities.

The methanol fruit extract of *S. nigrum* L. showed significant antibacterial activity against all the tested strains except *B. subtilis*. The lowest activity was recorded against *B. cereus* while moderate activity with 16.6 mm ZOI was recorded against *S. typhi* at 100mg/ml which is in consensus with Rani and Khullar, (2004)²⁰ that *S. nigrum* L. seeds possess active constituents against *S. typhi*. Our results showed that the extract has active constituents against other bacterial strains like *E. coli* and *P. aeruginosa* with highest ZOI (20mm) at 100 mg/ml. It is shown in table 1 and Fig. 1.

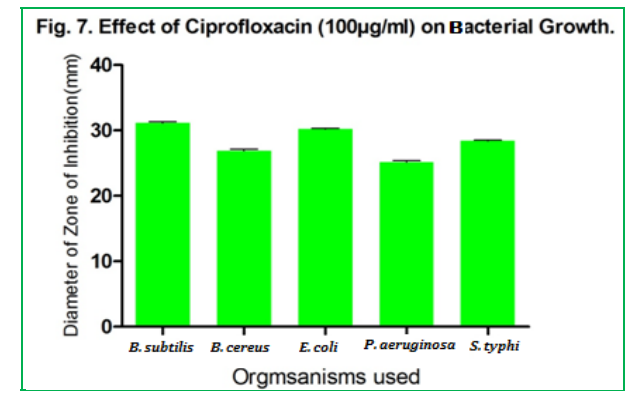
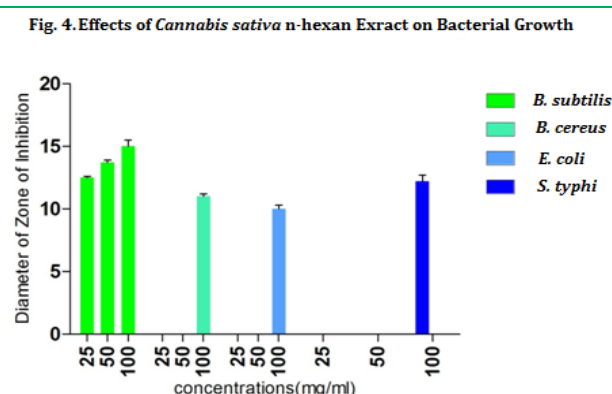
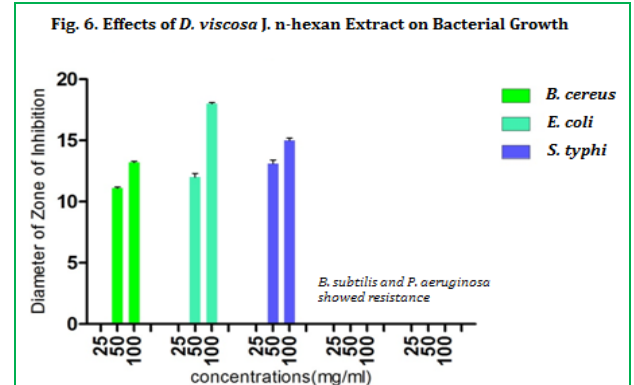
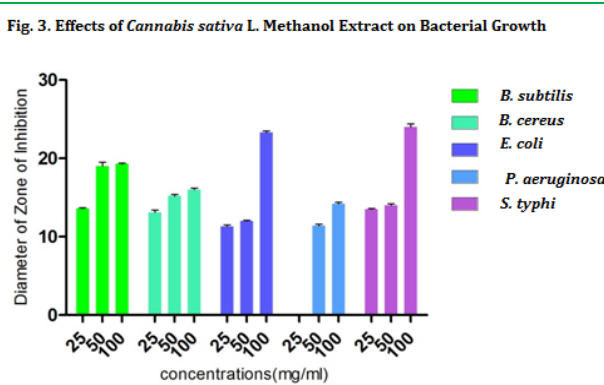
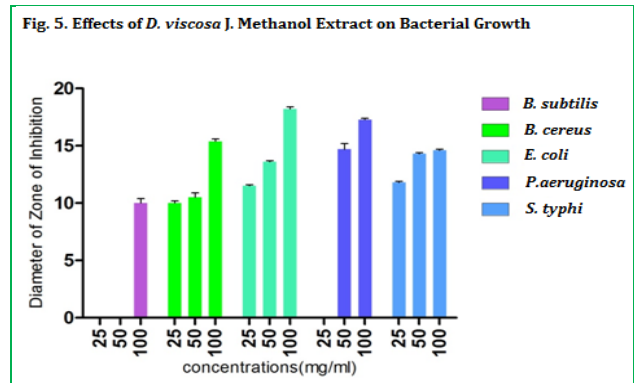
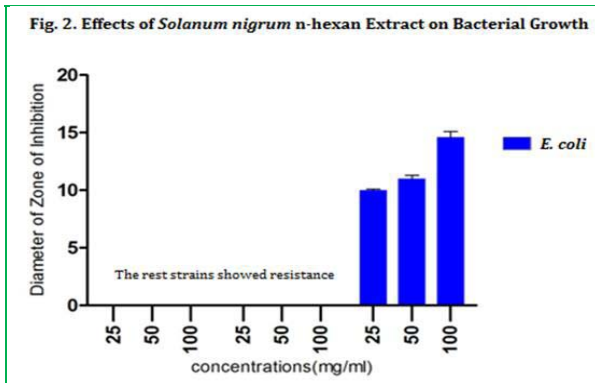


The n-hexane extract of *S. nigrum* L. fruit showed good activity against *E. coli* (14mm) but did not inhibit growth of other strains. This shows that n-hexane have no ability to extract the active constituent from *S. nigrum* L. fruit to inhibit *B. subtilis*, *B. cereus*, *P. aeruginosa* and *S. typhi*. Kaushik *et al.*, (2009)²¹ report is in divergence with our results who checked ethanol extract of fruit of *S. nigrum* against *S. aureus* and *B. subtilis*, and inhibition was recorded at all the tested concentrations (100, 75, 50 and 25mg/ml). It may be due to ethanol use for extraction by Kaushik *et al.*, (2009)²¹. Data is shown in Fig. 2.



Table 1: Antibacterial Activity of Selected Plants

Plants	Solvents	Concentrations (mg/ml)	Diameter of zone of inhibition (mm)				
			<i>B.s</i>	<i>B. c</i>	<i>E.c</i>	<i>P. a</i>	<i>S.t</i>
<i>Solanum nigrum</i> Linn.	Methanol	25	-	13±0.1	12.6±0.2	16.1±0.1	10±0.3
		50	-	14.7±0.1	14±0.4	17.2±0.4	15.2±0.3
		100	-	16.3±0.4	20±0.2	20±0.2	16.6±0.2
	n-hexane	25	-	-	10±0.1	-	-
		50	-	-	11±0.3	-	-
		100	-	-	14.6±0.5	-	-
<i>Cannabis sativa</i> L.	Methanol	25	13.6±0.1	13.1±0.3	11.3±0.2	-	13.5±0.1
		50	19±0.5	15.2±0.2	12±0.1	11.4±0.2	14±0.2
		100	19.3±0.1	16±0.2	23.3±0.2	14.2±0.2	24±0.4
	n-hexane	25	12.5±0.1	-	-	-	-
		50	13.7±0.2	-	-	-	-
		100	15±0.5	11±0.2	10±0.3	-	12.2±0.5
<i>Dodonaea viscosa</i> Jacq.	Methanol	25	-	10±0.2	11.5±0.1	-	11.8±0.1
		50	-	10.5±0.4	13.6±0.1	14.7±0.5	14.3±0.1
		100	10±0.4	15.4±0.2	18.2±0.2	17.3±0.1	14.6±0.1
	n-hexane	25	-	-	-	-	-
		50	-	11.1±0.1	12±0.3	-	13.1±0.3
		100	-	13.2±0.1	18±0.1	-	15±0.2
Ciprofloxacin (100µg/ml)			31.1±0.2	26.9±0.2	30.2±0.1	25.1±0.3	28.4±0.1



The methanol extract of *C. sativa* L. leaves was active against all the bacterial strains except *P. aeruginosa* at 25mg/ml concentration. The extract showed highest activity against all strains which is shown in Fig. 3.

The n-hexane extracts of *C. sativa* L leaves were active against *B. subtilis* at all concentrations and showed activity against *B. cereus*, *E. coli* and *S. typhi* at 100mg/ml. *P. aeruginosa* showed resistance at all concentrations of n-hexane extract. Wasim *et al.*, (1995)²², published that ethanol and petroleum ether extracts of the leaves of *C. sativa* L. exhibited activity both against Gram-positive and Gram-negative bacteria.

B. subtilis showed highest resistance to methanol leaves extract of *D. viscosa* Jacq. and highest lethality was recorded for *E.coli* and *P. aeruginosa*.

The n-hexane extracts (Fig. 4) of *D. viscosa* Jacq. leaves completely failed against *B. subtilis* and *P. aeruginosa* but active against *B. cereus*, *E.coli* and *S. typhi* at 50mg/ml and 100mg/ml concentrations. Previously, Khurram *et al.*, (2009)²³ reported that the n-hexane shoot extracts of *D. viscosa* Jacq. showed inhibition against *E. coli* and *P. aeruginosa*, *B. subtilis* and *S. typhi*. Getie *et al.*, (2003)²⁴ also reported that the crude extracts of the leaves of *D.viscosa* Jacq possess antibacterial activity against *Streptococcus pyogenes* and *staphylococcus aureus*. Our results correlated with Thring *et al.*, (2007)²⁵ previously reported that the aqueous, methanol, ethanol and ethyl acetate leaf extracts of *D. viscosa* Jacq showed activity against *P. aeruginosa*. The data is showed in Fig. 5 and 6.

CONCLUSION

The fruit of *S. nigrum* L, leaves of *D. viscosa* J. and *C. sativa* L. showed antibacterial activity. These plants contained active anti-bacterial constituents. Further work is needed to isolate these active agents which may be use as a good source for antibiotics.

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