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Antimicrobial potential of various extract and fractions of leaves of *Solanum nigrum*

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Abstract

This study was carried out to evaluate the antimicrobial activity of methanolic extract and different fractions (n-butanol, ethyl acetate, chloroform and n-hexane) of S.nigrum leaves. The antimicrobial activity was determined by the disc diffusion method and minimum inhibitory concentration (MIC) against a panel of microorganisms (four bacterial strains, i.e P. multocida, E. coli, B. subtilis and S. aureus and three fungal strains, i.e A. flavus, A. niger and R. solani).The results indicated that leaf extract and fractions of S. nigrum were mildly potent as antibacterial agent. While Antifungal activity of S. nigrum leaves extract/fractions was poor.

Keywords: Solanum nigrum, antimicrobial activity, Resazurin, A. flavus, Minimum inhibitory concentration.

Introduction

The chemical constituents of plant play an important role in modern medicine after profiling against different biological activities. Antibiotics brought about a revolution to control pathogenic diseases and infections. But these synthetic drugs are out of reach to millions of people. Those people who live in remote places depend on traditional healers, whom they know and trust [2]. About three quarters of population of the world are estimated to be dependent mainly on plants and plant extracts for the care of their health [6].Medicinal plants possess potent medicinal value that is due to the presence of a variety of phytochemical constituents in the plant tissues which cast a definite physiological action on the human body. Very few of these chemicals are toxic also [10]. The plant Solanum nigrum belongs to Family Solanaceae (Genus, solanum). This family consists of 90 genera and approximately 2000-3000 species. In this family, Solanum constitutes the largest and the most complex genus and it consists of more than 1500 species, many of which are also economically important throughout their cosmopolitan distribution. The generic name Solanum is considered to be derived from the Latin "Solamen" to refer to the quieting or sedative effects associated with many species [3]. S. nigrum elaborated a wide spectrum of medicinal properties such as anticancer, antioxidant, [1] neuroprotective [5], antimicrobial, and antipyretic properties. S. nigrum has been used as the important ingredient for herbal formulations in India, namely Liv. 52, which is mainly used for treating liver diseases [4].It has been found that

the antifungal activity of fractions from ammonium sulphate dialyzed was greater than those from crude extracts of S. nigrum [6]. In vitro antimicrobial assay of some medicinal plants including S. nigrum has been evaluated against plant and food-borne pathogens. S. nigrum seed extract exhibited selective antifungal activity, with strong zone of inhibition against P. nicotianae [9] .We were tried to evaluate the antimicrobial activity of leaves extract and fractions of S. nigrum against four bacterial and three fungal strains. Methanolic extract and four fractions (n-butanol, ethyl acetate, chloroform and n-hexane) were used to test the antimicrobial activity of S. nigrum leaves.

Materials and Methods

Plant material

Leaves of the selected plant Solanum nigrum purchased from the local market of Faisalabad and identified from the Department of botany University of Agricultural Faisalabad, Pakistan where a voucher specimen number has been deposited.

Preparation of extract and fractions

3 Kg fresh leaves of S. nigrum washed with distilled water so that the adhering dust particles must be removed. They were shade dried. The dried leaves were powdered and stored in the clean containers. In the weighed amount of powdered leaves the measured amount of 100% methanol (2×15 L) was added and kept it for 4-5 days at room temperature. The solvent was removed by using rotary evaporator. Methanolic extract (300 g) became viscous which stored at– 4 ^{0}C. The process was repeated three times with intervals of 4 days. Extract was dissolved in distilled water then fractionation was performed by using different polarity based solvents 7and obtained successively n-hexane (125g), chloroform (60g) ethylacetate (55g), and n-butanol (40g) fractions. All samples (dry residue) were dissolved in 10% sterile dimethyl sulfoxide.

Antimicrobial Assay of Plant Extracts and Different Fractions

Microbial strains.

The S. nigrum methanolic extract and its different fractions were individually tested against a panel of microorganisms, including four bacteria, Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Pasturella multocida and three pathogenic fungi, Aspergillus niger, Aspergillus flavus and Rhizopus solani. The pure bacterial and fungal strains were obtained from the Biological Division of the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The purity and identity of the strains were verified by the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured overnight at 37 °C in nutrient agar (Oxoid, Hampshire, UK) while fungal strains were cultured overnight at 28 °C using potato dextrose agar (Oxoid).

Disc diffusion method.

The antimicrobial activity of the S. nigrum methanolic extract and its different fractions were determined by the disc diffusion method [7]. The discs (6 mm in diameter) were impregnated with 10mg/mL extracts/fractions (100µL/disc) placed on the inoculated agar. Rifampcin (100 µL/disc) (Oxoid) and Fluconazole (100 µL/disc) (Oxoid) were used as positive reference for bacteria and fungi, respectively. Disc without samples was used as a negative control. Antimicrobial activity was evaluated by measuring the inhibition zone.

Minimum Inhibitory Concentrations (MIC) of Plant Extracts/fractions

The minimum inhibitory concentration (MIC) of the plant extracts/fractions was evaluated by a modified resazurin microtitre-plate assay reported by Sarker and coworker [8] with modification. Briefly, a volume of 100 µL of extracts/fractions solutions in 10% dimethyl sulfoxide (DMSO, v/v) was transferred into the first row of the 96 well plates. To all other wells, 50 µL of nutrient broth and muller hinton broth for bacteria and fungi respectively were added. Two-fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test

material in serially descending concentrations. To each well 10 µL of resazurin indicator solution (prepared by dissolving 270 mg resazurin tablet in 40 mL of sterile distilled water) were added. Finally, 10 µL of bacterial/fungal suspension were added to each well to achieve a concentration of approx 5 × 105 cfu/mL. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad spectrum antibiotics as positive control, a column with all solutions with the exception of the test samples, a column with all solutions with the exception of the bacterial/fungal solution adding 10 µL of broths instead and a column with 10% DMSO (v/v) solution as a negative control. The plates were prepared in triplicate, and incubated at 37 °C for 24 h and 28 °C for 48 h for bacteria and fungi respectively. The absorbance was measured at 620 nm by micro quant for fungus and at 500 nm for bacteria. The color change was then assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at which color change appeared was taken as the MIC value.

Statistical Analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference $p \leq 0.05$ was considered to denote a statistically significance All data were presented as mean values ± standard deviation (SD).

Results and Discussion

The antimicrobial activity of the methanolic extract and different fractions from *S. nigrum* leaves against a panel of food-borne and pathogenic microorganisms were assessed. The results are presented in Table 1. The results from the disc diffusion method, followed by measurement of minimum inhibitory concentration (MIC), indicated that 100% methanolic extract showed good activity against *S. aureus*, with the inhibition zone (24.5mm) and the lowest MIC value (34.3mg/ml). Least activity was exhibited against *R. solani* and *A. niger* with the smallest inhibition zones (3.02 and 1.10 mm) and the highest MIC values (400 and 500 mg/ml). Ethylacetate fraction showed strong activity against *B. subtilis* and *S. aureus* with inhibition zones (25.5and 24.3 mm) and the lowest MIC values (30.2 and 34.8 mg/ml), respectively. While *A. flavus* and *A. niger* were resistant with small inhibition zones (3.10 and 1.22 mm) and the highest MIC values (400 and 484 mg/ml), respectively. Chloroform fraction showed good activity against *S. aureus* and *E. coli*, with inhibition zones (16.8 and 16.5 mm) and the lowest MIC values (86.4 and 98.2 mg/ml), respectively while *A. flavus* and *A. niger*, were resistent with the smallest inhibition zones (2.7 and 2.0 mm) and the highest MIC values (440 and 450 mg/ml). n-butanol fraction showed strong activity against *B. subtilis* and *E. coli*, with the inhibition zones (23.5 and 17.2 mm) and the lowest MIC values (38.4 and 80.4 mg/ml), respectively. Least activity was exhibited against *S. aureus* and *A. niger*, with the smallest inhibition zones (3.16 and 1.75 mm) and the highest MIC values (400 and 462 mg/ml). n-hexane fraction was potent against *S. aureus*, with the inhibition zone (17.3mm) and the lowest MIC value (80.4mg/ml), respectively. Least activity was exhibited against *P. multocida* and *A. niger*, with the smallest inhibition zones (5.35 and 1.32 mm) and the highest MIC values (299 and 472mg/ml). In general, the antimicrobial activity of the tested extract and fractions is comparable with the standard drugs, rifampcin and fluconazole. The results indicated that plant extract and fractions showed very poor inhibition activity against *A. niger*. Overall, Antifungal activity of *Solanum nigrum* leaves extract/fractions was not significant. While ethylacetate fraction showed good antibacterial activity while other extract/fractions showed mild antibacterial activity. Venkatesan and coworkers [11] reported the antimicrobial activities against 16 types of bacteria and 4 fungus of ethanolic extract

of *Solanum nigrum*. The leaf extract and fractions of *S. nigrum* inhibited the growth of pathogenic microorganisms. The extract and fractions showed varying degree of inhibitory effects. Results showed that the most susceptible bacterial strain was *B. subtilis* and *S. aureus* to extract and fractions of *Solanum nigrum* leaves. While the most susceptible fungal strain was *Rhizopus solani*. The plant extract and fractions demonstrated no antifungal activity against *Aspergillus niger*. Overall *S. nigrum* leaves extract and fractions exhibited mild antibacterial activity while antifungal activity was not significant. In the continuation of global effort for the isolation of new and potent bioactive principles from plants, the present study was focused on the determination of minimum inhibitory concentration (MIC) of leaf extract of *Solanum nigrum,* which may play an important role in the modern drug discovery program. However, further studies are needed, including toxicity evaluation and purification of active antibacterial constituents from *Solanum nigrum* extracts looking towards a pharmaceutical use.

Conclusion

This study demonstrated that methanolic extract and different fractions of *S. nigrum* leaves have different degree of antimicrobial activity. It is concluded that leaf extract and fractions of *S. nigrum* were mildly potent as antibacterial agent. While Antifungal activity of *S. nigrum* leaves extract/fractions was poor. Therefore we would like to state that constituents of leaves extract/fractions of *S. nigrum* may serve as a potential source of industrial drugs useful in chemotherapy of some bacterial infections.

Table:1 Antimicrobial activity in terms of inhibition zones and minimum inhibitory concentration of methanolic extract and different fractions of S. nigrum leaves against selected bacterial and fungal strains[a]

Tested Microorganisms	Methanolic extract and different fractions					Rifampcin	Fluconazole
	100% Methanol	Ethylacetate	Chloroform	n-butanol	n-hexane		
Diameter of inhibition zone[b]							
Bacillus subtilis	5.10±0.16[a]	25.5±0.21[f]	7.33±0.19[b]	23.5±0.21[e]	8.18±0.17[c]	26.5±1.73[d]	------
Pasturella. multocida	5.11±0.18[a]	22.5±0.31[d]	13.5±0.21[b]	15.2±0.36[c]	5.35±0.19[a]	27.5±1.65[e]	------
Staphylococcus aureus	24.5±0.22[c]	24.3±0.25[c]	16.8±0.38[b]	3.16±0.07[a]	17.3±0.41[b]	30.4±0.86[c]	------
Escherichia coli	13.0±0.35[b]	16.5±0.21[c]	16.5±0.21[c]	17.2± 0.45[c]	7.25±0.15[a]	25.5±0.21[d]	------
Aspergillus niger	1.10±0.26[a]	1.22±0.20[a]	2.0±0.35[b]	1.75±0.25[ab]	1.32±0.30[a]	------	14.3±0.32[c]
Aspergillus flavus	13.5±0.25[e]	3.10±0.30[b]	2.7±0.3[a]	5.3±0.26[c]	10.8±0.44[d]	------	24.8±0.76[f]
Rhizopus solani	3.02±0.35[a]	11.2±0.42[b]	11.3±0.25[b]	3.5±0.25[a]	12.8±0.32[c]	------	18.87±0.98[a]
Minimum inhibitory concentration (MIC)[c]							
Bacillus subtilis	300±0.36	30.2± 0.36	260± 0.38	38.4±0.08	255 ± 0.47	------	------
Pasturella. multocida	300±0.19	42.4± 0.36	170± 0.47	115±0.31	299 ± 0.38	23.2± 0.40	------
Staphylococcus aureus	34.3±0.40	34.8±0.08	86.4± 0.36	400±0.35	80.4± 0.82	20.2± 0.36	------
Escherichia coli	180±0.45	98.2±0.05	98.2± 0.40	80.4±0.31	258±0.35	5.25± 0.40	------
Aspergillus niger	500±0.45	484±0.35	450±0.45	462±0.82	472±0.36	30.2±0. 45	130±0.40
Aspergillus flavus	170±0.40	400± .024	440±.034	290±0.352	230± 0.82	------	32.1±0.36
Rhizopus solani	400±0.35	220± 0.35	220± 0.35	350±0.29	180± 0.40	------	62.1±0.36

[a]Values are mean ± SD of three separate experiments.
[b]Diameter of inhibition zone (mm) including disc diameter of 6 mm.
[c]Minimum inhibitory concentration, MIC (mg/mL).
Letters in superscript show the significance of the results against single strain.

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