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Antifungal assay of *Solanum nigrum* Linn. fruit, leaves and stems extracts in different solvents

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Abstract

Antifungal activity of fruit, leaf and stem extract of *Solanum nigrum* L. prepared in four solvents i.e., ethanol, chloroform, petroleum ether and distilled water were investigated. Agar well diffusion method was utilized to study antifungal activity against five fungal strains viz. *Aspergillus's Niger, A. flavors, Saccharomyces cervisae, Alternaria alternate* and *Fusarium oxysprum*. Zone of inhibition was measured ranging from 9.3±0.66mm of stem extracts in distilled water against *A. flavus* and 32.42±0.66mm of fruit extracts in chloroform against *A. niger*. However the stem extracts in distilled water showed no detectable zone of inhibition against *A. niger* and *A. alternate*. Minimum inhibitory concentration (MIC) of extracts of S. nigrum was measured by serial dilution method. MIC was found ranging from 250µg/ml of ethanolic leaf extracts against *A. Niger* to 1000µg/ml of leaf extracts in chloroform against *F. oxysporum*. Fruit extracts in chloroform and leaf extracts in ethanol showed greater zones of inhibition as compared to standard antifungal Grisofulvin. Fruit extracts in ethanol showed 32.42±0.66mm and fruit extracts in ethanol showed 28.16±0.72mm zone of inhibition against *A. Niger* and *F. oxysporum* respectively.

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Introduction

S. nigrum L. contains constituents that are beneficial for treatment of diseases as well as they are known for their effective role in diverse activities. Some of major chemical constituents of this plant are glycoalkaloids, glycoproteins and polysaccharides. Polyphenolic compounds like gallic acid, catechin, protocatechoic acid (PCA), caffeic acid, epicatechin, rutin and naringenin (Sikdar and Dutta, 2008). Steroids, alkaloids of steroids, steroidal glycosides, solamargine and solasonine are abundantly found in *S. nigrum* L. extracts. These are found much effective in the activities against the liver diseases like cirrhoses and fibrosis which are induced by carbon tetrachloride (Raju *et al.,* 2003).

S. nigrum L. is used as an ailment in various diseases such as pain, inflammation and fever (Zubaida *et al.,* 2010). This species is used as a medicine against a number of diseases; it is frequently used as an antioxidant, anti-inflammatory, hepato protective and antipyretic agent. Photochemical studies of that particular plant have revealed that several active and effective constituents are present in it which can cure chronic diseases (Zakriya *et al.,* 2006). *S. nigrum* L. has been used for treatment of cardalgia and gripe because it has antidysentric and antiseptic characteristics. In the conditions of abdominals upsets an enema like infusion of *S. nigrum* L. is used. Extracts of it are beneficial to treat cirrhoses of liver and other liver inflammations. Fresh extracts of it are also useful in food poisoning especially it is used as antidote to opium poisoning (Akansha *et al*., 2008).

Microbes have developed resistance against chemotherapeutics; there is a need to replace these chemotherapeutics by naturally obtained photochemical which might be used as antimicrobial medicines (Iwu *et al*., 1999). Few extracts of *S. nigrum* were more antibacterial as compared to standard antibiotics available in market (Mazher *et al.,* 2016). In this sense *S. nigrum* may be used to prepare antibiotics which will have greater antibiotic activity. In this study we will study the antifungal potential of *S. nigrum*.

Materials and methods

Collection of Plants

Fresh plants were collected in the months of November and December, 2014 from different sites nearby Mirpur University of Science and Technology, Bhimber Campus. Collected plants were shade dried and then grinded to fine powder. Leaves, fruits and twigs were grinded separately. These grinded parts were weighed and 250g of each part was soaked in ethanol, chloroform, petroleum ether and distilled water. Extracts were obtained by maceration method. After filtration, extracts were concentrated by evaporation. These concentrated extracts of each part in different solvents were kept in refrigerator for use in further tests.

Solvents Used

- Ethanol C_2H_5OH having polarity of 5.1
- Chloroform $CHCL_3$ having polarity of 4.1
- Petroleum ether $C_2H_5OC_2H_5$ having polarity of 0.1
- Distilled water H_2O having polarity of 10.2

Standard Antibiotic

For checking antifungal activity of *Solanum nigrum* L. was signified by comparing Griseofulvin. Griseofulvin was grinded to powder and 5µg/ml was used as a standard drug for fungal strains.

Drying and Grinding of Plant Parts

Whole plant was cut from just above the ground. Leaves, fruits and twigs were separated. These parts were dried in shade at room temperature for 20 days. Dried parts were grinded to fine powder using mortar and pestle.

Extraction of Crude Extracts

Maceration method was used to obtain crude extracts of plant parts. Powdered plant parts were soaked in respective solvents. Electrical balance was used to measure the weight of dried plant parts; 250g of each dried plant part was soaked in 500ml of respective solvent. Each plant part was soaked in respective solvent for 10 days. After 10 days the solution was filtered using Whatman filter paper. Filtrate was dried and weighed using electrical balance.

Assessment of Percentage Yield of Extracts

Well dried plant extracts were used to calculate percentage yield. For calculating percentage yield following formula was used; % Extraction yield = (Weight of well dried plant extract / Weight of powdered plant material used) × 100.

Antifungal Activity

Preparation of Nutrient Medium

For antifungal activity Potato Dextrose Agar (PDA) was prepared by dissolving 39 grams of PDA in 900ml distilled water in a beaker. Then the solution was heated on spirit lamp so that all the solute may dissolve efficiently. After that the volume of solution was raised to 1000ml (1litre). The solution was poured in funnel and neck of the funnel was closed by cotton plug and sealed tightly by cling tape.

Sterilization of Nutrient Medium

PDA solution was kept in funnel and sealed tightly. Then all the instruments along with funnel containing the PDA solution were covered with newspaper separately and placed in autoclave. After autoclaving for 15 minutes all the instruments (cork borer, spatula, Petri dishes and funnel containing the solution) were kept in incubator. PH of culture medium was adjusted to 5.6±0.2 by adding few drops of 0.01M NaOH and 0.01M HCl. PDA medium was poured in Petri dishes in laminar flow. The solution was left for 2 hours for solidification.

Determination of Antimicrobial Activity (Inhibition Zone)

For the determination of zone of inhibition published method of Jorgenson *et al.* (2007) was followed. Fruit, leaves and stems extracts were analyzed for antifungal activity by using agar well diffusion method. After solidification, cork borer was used to make wells in PDA slants. Each well measured 5mm. After well formation the PDA slants were inoculated by the test fungal strain. In the wells extracts of *Solanum nigrum* L. were inoculated carefully. Fungal strains were inoculated under aseptic conditions in laminar flow. For inducing fungal strains inoculums needle was firstly made red hot in the flame of spirit lamp. Red hot needle was dipped in ethanol solution. Very small amount of strains was streaked by needle into the perspective Petri dishes. These Petri dished were incubated at 35±2°C for 24 hours. After 24 hours zone of inhibition was determined by measuring the diameter of clear area i.e., zone of inhibition, including the 5mm well. SPSS the statistical program was used to get accuracy in measurement. Every reading was noted thrice. Confidence interval for mean was 95%. Level of significance was ($P<0.05$).

Negative and Positive Controls

Two PDA slants were left without any fungal inoculation for checking aseptic conditions of the lab. These slants were used as negative control. Two PDA slants were used for comparison of antifungal activity of extracts with standard antibiotic drug Grisofulvin was used at 100µg/ml concentrations. These PDA slants were termed as Positive control.

Minimum Inhibitory Concentration (MIC)

Serial dilution method was used to measure MIC (Minimum Inhibitory Concentration) of crude extracts and fractions of these crude extracts.

Drug preparations for test

Two fold (10^{-2}) dilutions of each extract were prepared by serial dilution method. Muller Hinton Broth was used to prepare dilutions. A series of 7 dilutions were prepared. Final concentrations of 1000–15.62 µg/ml were prepared.

Procedure for checking MIC

For checking MIC (Minimum Inhibitory Concentration) of each extract following procedure was followed (Indumathi and Mohandas, 2014).

Test tubes were autoclaved for 15 minutes and then sterile tubes were labeled from 1 to 9, 8^{th} tube was taken as a control for checking sterile conditions of solution whereas, 9^{th} test tube was used to check viability of fungal strains. Grisofulvin was used as control. One ml of diluted Muller Hinton Broth was transferred to the test tubes from 1 to 9.

One ml of solution of extract was transferred to 1st test tube and shaken well. From this homogenous mixture present in 1st test tube one ml was transferred to 2nd test tube and shaken well. One ml of solution in 2nd test tube was transferred to 3rd and from 3rd to 4th and from 4th to 5th and from 5th to 6th and from 6th to 7th test tube. Fungal culture 0.01ml was inoculated in all the test tubes.

Results

After this, all the test tubes were incubated for 24 hours at 35±2°C. After incubation of 24 hours turbidity or optical density (OD) value was observed by spectrophotometer method. The least test tube in which growth failed to occur was the Minimum Inhibitory Concentration (MIC) for that test organism.

Table 1. Percentage yield of extracts of different parts of *Solanum nigrum* L. in different solvents.

Plant Part	Solvent used	Weight of Powder (g)	Weight of extracts (g)	Percentage extraction yield (%)
LEAVES	Ethanol	250	16.00	6.45
	Chloroform	250	10.92	4.36
	Petroleum ether	250	03.21	1.28
	Distilled water	250	24.00	9.60
FRUITS	Ethanol	250	05.80	2.32
	Chloroform	250	10.60	4.24
	Petroleum ether	250	05.75	2.35
	Distilled water	250	10.50	4.25
STEMS	Ethanol	250	07.00	3.65
	Chloroform	250	10.71	5.35
	Petroleum ether	250	02.59	1.30
	Distilled water	250	09.39	4.69

% Extraction yield = Weight of well dried plant extract / Weight of powdered plant material used × 100.

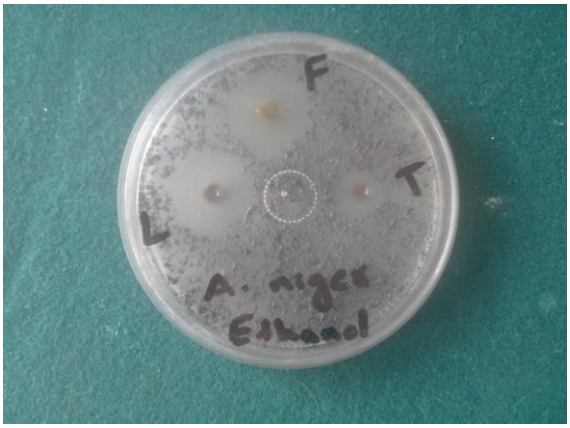
Table 2. The MIC of extracts of *S. nigum*.

Fungal strains	MIC (µg/ml)											
	Ethanol			Chloroform			Petroleum ether			Distilled water		
	L	F	S	L	F	S	L	F	S	L	F	S
A. niger	500	500	1000	1000	500	1000	1000	500	1000	1000	1000	1000
A. flavus	250	250	500	500	250	1000	500	500	500	500	500	1000
S. cervisae	250	500	1000	500	250	1000	500	500	1000	1000	500	500
A. alternate	250	500	1000	500	500	500	500	250	500	1000	500	1000
F. oxysporum	250	250	1000	250	250	1000	500	500	1000	500	500	1000

Table 3. Zone of Inhibition of bacterial strains against extracts of *S. nigrum*.

		Zone of inhibition (mm)				
Solvent		*A. niger*	*A. flavus*	*S. cervisae*	*A. alternata*	*F. oxysporum*
Ethanol	Leaves	21.66±0.16	15.26±0.16	22.26±0.16	21.33±0.26	17.33±0.66
	Fruits	32.42±0.66*	19.66±0.33	26.28±0.66	23.66±0.66	28.16±0.72*
	Stems	18.33±0.33	15.66±0.44	14.44±0.66	17.33±0.33	9.44±0.44
	Grisofulvin	30.33±0.33	32.16±0.66	27.72±0.33	32.66±0.44	27.33±0.72
Chloroform	Leaves	18.33±0.28	17.66±0.33	24.28±0.66	19.66±0.16	14.72±0.66
	Fruits	25.28±0.66	22.16±0.44	22.33±0.72	26.54±0.44	23.16±0.44
	Stems	17.16±0.66	13.72±0.16	14.16±0.44	15.28±0.72	8.33±0.33
	Grisofulvin	30.33±0.33	32.16±0.66	29.72±0.33	32.66±0.44	27.33±0.72
Petroleum ether	Leaves	19.72±0.16	16.16±0.44	20.16±0.72	18.66±0.66	17.33±0.44
	Fruits	21.33±0.33	21.33±0.66	22.44±0.33	26.33±0.72	19.44±0.16
	Stems	14.44±0.16	16.28±0.66	19.16±0.16	13.16±0.44	14.33±0.33
	Grisofulvin	30.33±0.33	32.16±0.66	29.72±0.33	32.66±0.44	27.33±0.72
Distilled water	Leaves	10.16±0.33	15.16±0.44	18.33±0.16	19.72±0.26	20.16±0.66
	Fruits	14.72±0.16	17.72±0.44	16.16±0.33	19.16±0.66	20.33±0.33
	Stems	ND	9.33±0.66	10.16±0.44	ND	10.72±0.16
	Grisofulvin	30.33±0.33	32.16±0.66	29.72±0.33	32.66±0.44	27.33±0.72

Confidence interval for mean was 95%. Result = Mean ± S.E. ($P<0.05$).



Discussion

In this study maceration method was implied to get the extracts from different parts of *S. nigrum*. Different extracts with differing polarities were used to get extracts. Highest percentage of extraction 9.6% was noticed by leaves extract in distilled water whereas lowest percentage extraction was 1.28% of leaves extract in petroleum ether. Percentage extraction was measured by dividing the well dried extract by total plant part powder used and by multiplying with 100.

Minimum inhibitory concentration (MIC) of extracts of *S. nigrum* against test fungal strains was determined by serial dilution method. Having different concentration of extracts 7 dilutions were made ranging from 1000µg/ml to 15.62µg/ml. MIC was found to be ranging from 250µg/ml of fruit extracts in ethanol against *A. flavus* to 1000µg/ml of stems extract in distilled water against *F. oxusporum*. Every test was revised thrice and weighted average was considered as the result.

The results of present research were different from the results of Abad *et al.,* 2007. Difference may be due to different mediums used or different test organisms used. It may also be due to difference of procedures followed. Almazini *et al.* (2009), Atanu *et al.* (2011) investigated the minimum antifungal concentration by using percentage mortality as measuring meter whereas for present study serial dilution method was used to investigate MIC. Present research also found matching results as found by previous studies of Indumathi and Mohandas, 2014.

Extracts of *S. nigrum* L. in different solvents showed significant antifungal activity. Antifungal activity of extracts was measured by agar well diffusion method. Wells of 5mm were prepared by cork borer. Results of zone of inhibition showed that fruit extracts are more potent antifungal whereas, stems showed little antifungal activity. Contrary to it ethanolic extracts showed significant antifungal activity as compared to other solvents´ extracts. Some extracts of stems in distilled water showed no detectable zones of inhibition. Minimum zone of inhibition was 9.5mm that of stems extract in distilled water against *Aspergillus flavus* and the highest antifungal zone was 27.5mm that of fruit extracts in ethanol against *Alternaria alternata*. Present study is also in accordance with Gogoi *et al.,* 2012. Just the measuring methods are different. Due to difference in measuring method results vary a little bit.

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