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Phytochemistry and Antibacterial Assay of Fruit, Leaf and Stem Extracts of *Solanum nigrum* L. in Different Solvent

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Abstract

Solanum nigrum L. (black nightshade) is a wild vegetable with numerous ethnomedicinal potentials. In this study phytochemical analysis and antibacterial activity of fruit, leaf and stem extracts of *Solanum nigrum* L. prepared in four solvents i.e., ethanol, chloroform, petroleum ether and distilled water were inspected. Phytochemical examination showed the presence of significant constituents like, saponins, tannins, steroids, terpenoids, alkaloids and flavonoids. Antibacterial activity was determined by zone of Inhibition using agar well diffusion method in contradiction of five bacterial strains viz. *Basillus subtilis, Salmonella paratyphi, Escherichia coli, Proteus vulgaris* and *Vibrio cholera*. Zone of inhibition ranged from 8.5 mm to 33.0 mm where stem extracts in chloroform showed no detectable zone of inhibition against *B. subtilis*. Leaf and stem extracts also showed no detectable zone of inhibition against *S. paratyphi*. The MIC value measured using serial dilution method against bacterial strains ranged from 250µg/ml to 1000µg/ml. Fruit extracts in ethanol and chloroform both, showed greater zone of inhibition against *V. cholera* as compared to tetracycline. This investigation sanctions that the ethanol and chloroform extracts from fruit of *Solanum nigrum* L. possess potential antibacterial action.

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Introduction

Solanum is one of the largest and extensively diverse genera of the family Solanaceae. In Pakistan *Solanum* is characterized by 15 species, of which 11 species are important from medicinal point of view. Taxonomically, this is a multifaceted genus, because of the occurrence of various hybrid and controversial taxonomic status (Zubaida *et al.* 2010). It can grow in a variety of soil types like, stony, dry, shallow or deep soils (Kiran *et al.,* 2009). Numerous chemical constituents are found in *S. nigrum* including glycoalkaloids such as tannins, solanine, solasodine, solamargine, solanigrine, steroidal genin (gitogenin) and polyphenolic compounds (Potawele *et al.* 2008).

A very crucial chemical constituent of *Solanum* species is Solasodine. It is a steroidal alkaloid which is not soluble in water and it is involved in the manufacture of many steroid drugs especially corticosteroids (Alvarez *et al.* 1994). It was found that *S. nigrum* contains many compounds like essential oils which might be found in different parts of the particular plant (Rao *et al.* 2012; Rotimi *et al.* 2012).

It is medicinally used since antiquity and traditional folklore designates that it has been used for fever, inflammation and wounds treatment (Ong, 2003). Leaves and fruits of *S. nigrum* are chewed and swallowed to cure ulcer of mouth (Kingston *et al.* 2007; Mohana *et al.* 2008) and also used as diuretic, tonic, antidiarrhoea, antimalaria, and in the treatment of eye, heart and skin diseases (Karmakar *et al.* 2010).

It encompasses phytochemicals which have antimicrobial activity against a wide range of gram-positive bacteria (Baohung, 2002). Bacteria and viruses have developed resistance against available chemotherapeutics in market therefore; it is strappingly required to substitute these chemotherapeutics with naturally obtained phytochemicals which can be used as antimicrobial medicines (Iwu *et al.* 1999).

In pharmaceutical industries, natural raw material especially plant parts play a vital role in manufacture of medicines and other drug development programs (Baker *et al.* 1995). In this contest, World health Organization (WHO) is playing its role to make strategies, guidelines and standards for the manufacture of medicines from natural plant materials and also emphasizes on the importance of traditional medicines (WHO, 2002).

Under developed countries 80% population of the world rely on traditional medicine obtained from plants for primary health care. In the recent decades, ethno-medicine has gained significant reputation, because it is safe and have no side effects (Prusti *et al.* 2008). Although conventionally available synthetic antibacterial drugs are associated with undesirable side effects and resistance problem, therefore this investigation was carried out with the aim of exploring the phytochemistry and antibacterial activity of extracts from *Solanum nigrum* L against important pathogenic bacteria.

Materials and methods

Collection of plants

Fresh plants were collected from different sites nearby Mirpur University of Science and Technology (Bhimber Campus) Pakistan. Collected plants were shade dried at room temperature for 20 days and then leaves, fruits and stems were ground separately into fine powder using mortar and pestle.

Preparation of extracts

Four solvents viz. ethanol (C_2H_5OH; polarity 5.1), chloroform ($CHCL_3$; 4.1), petroleum ether ($C_2H_5OC_2H_5$; 0.1) and distilled water (H_2O; 10.2) were used as extraction solvents. The ground plant parts were weighed and 250g of each part was soaked in 500 ml of each solvent for 10 days. The crude extracts from each part were obtained by maceration method. The filtrates were concentrated by evaporation. These concentrated extracts of each part in different solvents were kept in refrigerator for further use.

Phytochemical analysis

The extracts of leaves, fruits and stems in different solvents were subjected to phytochemical screening qualitatively following the method described by Harborne (1973), Kokate (1994) and Sofowara (1993). For each component zone of inhibition and minimum inhibitory concentration were carried out. Following standard procedures were carried out to check the presence of phytochemicals in the extracts.

Test for Saponins

Mixture of filtrate 10ml and distilled water 5ml was obtained. This mixture was vigorously shaken. Appearance of persistent forth was formed. To the froth 3-4 drops of sulphuric acid (H_2SO_4) were added and shaken vigorously. Production of foam and its persistence for 10 minutes was considered the indicator for the presence of saponins (Sofowara, 1993).

Test for Tannins

Ferric chloride test was accomplished for the revealing of tannins**.** Few drops of 1% neutral ferric chloride solution was combined with each extract, development of blackish blue color was considered as indicator for the presence of tannins (Kokate, 1994).

Test for Flavonoids

Small amount of extract was added in 2ml of Methanol in a test tube. Few magnesium ribbon and conc. HCl were added slowly from the sides of the test tube. Appearance of pink, red colour were the indicative for the presence of flavonoids (Harborne, 1973).

Test for Steroids

2 ml of acetic anhydride was added to 0.5g of extracts of each sample. Then 2 ml sulphuric acid (H_2SO_4) was added. The colour changed from violet to blue or green indicated the presence of steroids.

Test for Terpenoids

Little amount of extract was added to 2ml of chloroform and 3ml of con. H2SO4 to form a monolayer of reddish brown color of the interface was considered as an indicative for terpenoids (Harborne, 1993).

Test for Anthraquinones

Small concentration of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones (Evans, 2002).

Test for alkaloids

Small amount of extracts were kept disjointedly with few drops of diluted hydrochloric acid (HCl) and then filtered. The filtrate was experienced with numerous alkaloidal agents like, Wagner's reagent, Mayer's reagent and Dragendorffs reagent. The orange precipitate, the creamish precipitate and brown precipitate, were the indicators for the presence of alkaloids (Salehi-Surmaghi, 1992).

Antibacterial activity

Determination of Inhibition Zone

For the determination of zone of inhibition all extracts were analyzed for antibacterial activity by using agar well diffusion method (Perez *et al.* 1990; Jorgenson *et al.* 2007). After well formation the PDA slants were inoculated with the test bacterial strains viz. *Bacillus subtilis, Salmonella paratyphi, Vibrio cholera, Proteus vulgaris and Escherisichia coli*; while the wells (5mm) were carefully inoculated with the extracts of *S. nigrum*.

For inducing bacterial strains inoculums, petri plates were inoculated with the bacterial strains by streaking and incubated at 35±2 ^{0}C for 24 hours. After 1 day (24hrs) zone of inhibition was determined by calculating the diameter of cleared area i.e., zone of inhibition, including the 5mm well.

Negative and positive controls

Potato dextrose agar (PDA) slant without any bacterial inoculation for checking aseptic conditions of the lab was used as negative control while one PDA slant was used for comparison of antibacterial activity of extracts with standard antibiotic drug tetracycline (100µg/ml) and served as positive control.

Minimum inhibitory concentration (MIC)

Serial dilution method was used to measure MIC of crude extracts and their fractions as described by Manisha *et al.* (2009). Two fold (10^{-2}) dilutions of each extract were prepared using Muller Hinton Broth. A series of 7 dilutions were prepared. Final concentrations of 1000–15.62 µg/ml were prepared.

Procedure for checking MIC

MIC of each extract was checked by the method described by Indumathi and Mohandas (2014). Briefly, the test tubes were autoclaved for 15 minutes and then sterile tubes were labeled from 1 to 9. 8th tube was taken as a control for checking sterile conditions of solution whereas, 9th test tube was used to check viability of bacterial strains. Griseofulvin 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. Bacterial culture 0.01 ml was inoculated in all the test tubes. 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 MIC for that test organism.

Statistical analysis

SPSS program (SPSS Inc. Chicago IL Version 12.0) was used to get accuracy in measurement. Every reading was noted thrice. Confidence interval for mean was 95%. Level of significance was ($P<0.05$).

Results

Phytochemical analysis of extracts of *S. nigrum* leaves in ethanol showed presence of tannin, steroid, flavonoid, saponin, terpenoid and alkaloids.

Table 1. Phytochemicals found in extracts of *S. nigrum*.

Phytochemicals	Leaves				Fruit				Stem			
	Eth	Chl	Pet	Dis	Eth	Chl	Pet	Dis	Eth	Chl	Pet	Dis
Tannins	+	+	-	+	-	-	-	-	-	-	-	-
Steroids	+	-	-	+	+	-	-	+	+	-	-	+
Flavonoids	+	-	-	+	+	-	-	+	+	-	-	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	-	-	+	+	-	-	+	+	-	-	+
Tests for Alkaloids												
Mayer's test	+	+	-	+	+	+	-	+	+	-	-	+
Dragendroff's	+	+	-	+	+	-	-	+	+	-	-	+
Wagner's test	+	+	+	+	+	+	-	+	+	-	+	+

Key: + = present, - = absent

Eth = Ethanol; Chl = Chloroform; Pet = Petroleum ether; Dis = Distilled wate.

In chloroform extract, presence of tannin, saponin and alkaloids were noticed. In petroleum ether saponin and alkaloids were present by Wagner's test. While tannin, steroid, flavonoid, saponin, terpenoid and alkaloids were present in aqueous extracts as shown in table 1. Steroid, flavonoid, saponin, terpenoid and alkaloids were found in ethanolic extracts of fruits while saponin and alkaloids were present in fruit extracts in chloroform. Only saponins were found in fruit extracts in petroleum ether whereas, aqueous fruit extracts showed presence of steroid, flavonoid, saponin, terpenoid and alkaloids.

Table 2 shows that MIC of fruits is much lower than the stem and ranged from 250µg/ml against the *V. cholera* to 1000µg/ml against *S. paratyphi*. MIC of ethanolic extracts is lower than the extracts in other solvents viz. chloroform, petroleum ether and distilled water. *B. subtilis* and *P. vulgaris* have lower MIC as compared to other bacterial strains.

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

Bacterial strains	MIC (µg/ml)											
	Ethanol			Chloroform			Petroleum ether			Distilled water		
	L	F	S	L	F	S	L	F	S	L	F	S
B. subtilis	250	500	250	500	250	500	250	500	1000	500	500	500
S. paratyphi	250	500	500	500	500	1000	500	250	500	1000	1000	1000
E. coli	500	250	500	500	500	500	500	500	500	500	500	1000
P. vulgaris	250	500	500	250	500	500	250	250	1000	500	500	500
V. cholera	500	500	500	250	500	250	500	250	500	500	1000	1000

L= Leaves; F= Fruit; S= stem.

Table 3 illustrates that fruit extracts of *S. nigrum* in ethanol and petroleum ether showed higher zones of inhibition than tetracycline (standard antibiotic) against *E. coli* and *V. cholera* as shown in figure 1. Fruit extracts in chloroform also showed higher zone of inhibition than tetracycline against *V. cholera*. Zone of inhibition exhibited by extracts of fruits in ethanol, chloroform and petroleum ether against *V. cholera* were measured as 31.83±0.72, 31.33±0.66 and 32.07±0.16 respectively while, fruit extracts in ethanol and petroleum ether against *E. coli* was measured to be 25.66±0.33 and 25.56±0.72.

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

		Zone of inhibition (mm)				
Solvent		*B. subtilis*	*S. paratyphi*	*E. coli*	*P. vulgaris*	*V. cholera*
Ethanol	Leaves	20.50±0.28	19.33±0.72	20.33±0.33	23.50±0.28	24.33±0.16
	Fruits	23.16±0.16	25.83±0.44	25.66±0.33*	30.16±0.44	31.83±0.72*
	Stems	8.16±0.44	14.16±0.44	17.33±0.33	18.00±0.28	19.33±0.16
	Tetracycline	28.66±0.26	30.16±0.33	25.26±0.66	30.33±0.16	30.66±0.44
Chloroform	Leaves	18.33±0.33	20.66±0.33	19.42±0.54	24.00±0.33	22.72±0.56
	Fruits	26.56±0.44	24.33±0.66	24.56±0.46	27.52±0.16	31.33±0.66 *
	Stems	ND	13.72±0.27	17.24±0.16	19.66±0.44	18.24±0.86
	Tetracycline	28.66±0.26	30.16±0.33	25.26±0.66	30.33±0.16	30.66±0.44
Petroleum ether	Leaves	23.66±0.44	20.33±0.33	23.66±0.16	17.33±0.33	22.56±0.44
	Fruits	27.56±0.42	25.66±0.16	25.56±0.72*	22.16±0.16	32.07±0.16 *
	Stems	11.16±0.66	9.42±0.33	17.54±0.28	12.72±0.66	18.33±0.33
	Tetracycline	28.66±0.26	30.16±0.33	25.26±0.66	30.33±0.16	30.66±0.44
Distilled water	Leaves	12.24±0.44	ND	17.33±0.28	ND	7.56±0.16
	Fruits	21.56±0.23	8.66±0.44	17.56±0.33	16.33±0.66	14.72±0.33
	Stems	8.33±0.33	ND	9.72±0.16	ND	ND
	Tetracycline	28.66±0.26	30.16±0.33	25.26±0.66	30.33±0.16	30.66±0.44

Confidence interval for mean was 95%. Result = Mean ± SEM ($P<0.05$); n = 3

*Values are significantly different from control ($P<0.05$). ND = not detected.

Stem and leaf extracts of *S. nigrum* in distilled water showed no detectable zone of inhibition against *S. paratyphi* and *P. vulgaris*. Leaf extracts in distilled water showed minimum zone of inhibition (7.56±0.16) against *V. cholera* whereas the highest zone of inhibition (32.07±0.16) was shown by fruit extracts in petroleum ether.

Discussion

Plants from the solanum genus have extraordinary pharmacological potential. Morpholine is a carcinogenic compound and it is isolated as a white crystalline substance from *Solanum nigrum* (Mary and Okiemen, 2004) and also a comprehensive review on phytochemicals and pharmacological activity of *Solanum nigrum* is documented (Melina and Giuseppina, 2012).

F = fruit extracts; T = twigs/stems extracts; L = Leaf extracts.

Fig. 1. Picture showing zones of inhibition of ethanolic extracts of *S. nigrum* against *V. cholera*.

Phytochemical studies of *S. nigrum* showed the presence of different compounds like tannins, saponins, terpenoids, steroids, flavonoids with alkaloids and *Solanum nigrum* possess significant antioxidant and antibacterial activities (Gbadamosi and Afolayan, 2016). Sridhar *et al.* (2011) studied phytochemicals and found matching results with present study. In another study the ethanol and methanol extract of *Solanum nigrum* were found to have strong activity against different types of bacteria (Hussain *et al.* 2013).

Investigation of minimum inhibitory concentration (MIC) of *S. nigrum* in the present study ranged from 250 µg/ml to 1000 µg/ml. MIC of ethanolic fruits extracts was lowest (250 µg/ml) against *E.coli* whereas, stem extracts in distilled water was highest (1000 µg/ml) against *V.cholera, E. coli* and *S. paratyphi*. It was found that fruits and leaf extracts of *S. nigrum* have more antibacterial potency as; they have lowest MIC values (250µg/ml) for *E. coli, S. paratyphi, B. subtilis, P. vulgaris* and *V. cholera*. Ethanolic stem extracts showed lower MIC whereas, higher MIC values were seen by stem extracts in other solvents. Present study is in accordance with the study of Indhumathi and Mohandass, (2014) revealed that ethanolic extracts of *S. nigrum* have MIC value of 500, 500 and 250 µg/ml against *V. cholera, S. paratyphi* and *B. subtilis* respectively. Present study also found same results for ethanolic fruit extracts against *V. cholera* and *S. paratyphi,* but the MIC value of ethanolic fruit extracts recorded against *B. subtilis* was 500µg/ml.

On the other hand our results are not in agreement with the results obtained by Sridhar *et al.* (2011). Present study qualifies fruit extracts to have lower MIC values whereas study of Sridhar *et al.* (2011) found stem extracts to have lower MIC values.

This investigation finds significant antibacterial activity against *V. cholera* and *E. coli* by fruit extracts of *S. nigrum* in ethanol, chloroform and petroleum ether. The zone of inhibition measured against *V. cholaera* by fruit extracts of ethanol, chloroform and petroleum ether was 31.83±0.72, 31.33±0.66 and 32.07±0.16 respectively. These zones of inhibition were higher than the zone of inhibition measured against *V. cholera* by tetracycline. Fruit extracts in ethanol and petroleum ether also showed greater zone of inhibition than tetracycline against *E. coli.*

Indhumathi and Mohandass, (2014) found that ethanolic fruit extracts have higher zone of inhibition than the ciprofloxacin against *Staphylococcus aureus*, in our study fruit extracts in ethanol also showed higher zone of inhibition than the tetracycline against *V. cholera* and *E. coli.* Our results are also in accordance with Almazini *et al.* (2009) as, fruits extracts in both studies showed greater antibacterial activity than leaf and stem extracts.

Conclusively, the fruit extracts of *Solanum nigram* contains important phytochemicals that bear persuasive antibacterial assets. Additional exploration on the isolation and identification of antibacterial constituents may provide a clue to effective chemical entities for clinical use. Identification and separation of antibacterial compounds from *Solanum nigram* fruit extracts will go a long way in developing new drugs in competent and concerned Pharmacological Centres.

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