IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2017 Vol.:11, Issue:1

Phytochemical Analysis and Assessment of Antimicrobial Activity of *Solanum nigrum*

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ISSN 2349-7203
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Submission: 20 November 2017
Accepted: 30 November 2017
Published: 30 December 2017

Keywords: Medicinal plants, phytochemicals, *Solanum* and antimicrobial activity.

ABSTRACT

The present study deals with the qualitative screening of phytochemicals and evaluation of the antimicrobial activity of *Solanum nigrum* leaf extracts. Aqueous, chloroform and butanol extracts of dried and ground plant materials were prepared using Soxhlet apparatus. Qualitative phytochemical screening of the extracts showed positive for carbohydrates, chloride, tannins, alkaloids, flavonoids, saponins phenolic compounds and steroids. Antimicrobial activities of the extracts were evaluated by agar well diffusion method. The extracts exhibited significant antimicrobial effects against all the tested bacterial and fungal pathogens. The aqueous extracts of leaf showed highest activity on the Gram positive bacteria *S. aureus* and fungus *A. fumigatus.* The analysis of bioactive compounds present in the plant extracts involving the applications of common phytochemical screening assay as well as HPLC is discussed.

INTRODUCTION

Plants have been used for medicinal purposes long before prehistoric period. Recently, WHO estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare needs. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. Medicinal plants are extensively used to cure various infectious diseases in human beings[1]. Phytonutrients phytochemicals are numerous and are highly beneficial to health, protecting our body against various diseases[2]. Medicinal plants are being used as traditional medicines to several infectious diseases because of their products are safe in contrast to the synthetic medicines including other animals and environment[3].

Solanum nigrum Linn is a medicinal branched herb belongs to the family Solanaceae and has beneficial effects on cancer, ulcer and microbial infections[4]. The phytochemical investigation of *Solanum nigrum Linn* shows the presence of saponins, tannins, and alkaloids. A dietary intake of *Solanum nigrum* supplies our body with nutrients that offer protection against numerous diseases[5]. All parts of this plant are used in the traditional medicine as a remedy for treating various diseases like cough, cold, asthma, skin diseases and liver problem.

MATERIALS AND METHODS

Plant sample

The plant *Solanum nigrum* was collected from Marthandam, Kanyakumari District, Tamil Nadu, India. The leaf of the plant used for this study was rinsed severally with clean tap water and shade dried in a dark place at room temperature for few days. The dried plant parts were cut into small pieces ground in electric chopper to get fine powder for further use.

Preparation of extracts

The powdered plant materials were subjected to Soxhlet extraction using aqueous, acetone, dimethyl sulfoxide, chloroform and ethanol. Each 5 g of powder of plant material was filled separately in the thimble and extracted successively with 60 ml of solvents using a Soxhlet extractor for 3 h. After solvent evaporation, each of these solvent extracts was weighed and preserved in room temperature until further use.

Qualitative analysis phytochemical constituents

All the extracts were subjected to systematic phytochemical screening for testing the presence of various phytochemical constituents by the method followed by standard protocols[6]. Phytochemical test includes carbohydrates, amino acids, proteins, vitamin C, chloride, tannin, alkaloids, flavonoids, phlobatannins, steroids, phenols and saponins.

Antimicrobial activity of plant extracts

Antimicrobial activities of the plant extracts were determined by well diffusion method[7]. Four bacterial pathogens such as *Escherichia coli, Klebsiella pneumonia, Bacillus subtilis,* and *Staphylococcus aureus* and three fungal pathogens such as *Aspergillus niger, Aspergillus fumigatus* and *Penicillium* sp. were used for this investigation. The test bacterial strains were inoculated into Nutrient broth and incubated at 37°C for 24 h. After the incubation period, the culture tubes were compared with the turbidity standard. Fungal inoculums were prepared by suspending the spores of fungus (as previously cultured) in saline water mixed thoroughly, made turbidity standard and used.

Fresh bacterial culture of 0.1 ml having 108 CFU was spread on Muller Hinton agar plates using sterile cotton swabs. The fungal strains were spread on Potato dextrose agar. Wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50 µl of plant extracts using micropipette in aseptic condition. The plates were kept in a refrigerator to allow pre-diffusion of extract for 30 min and then incubated at 37°C for 24 h and 28-30–37°C for 3-4 days for bacterial and fungal cultures respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

Reverse phase HPLC purification

Isolation of the molecule was performed using a reverse phase HPLC system (Cyberlab, USA). C-18 column was applied using acetonitrile: water (65:35) as mobile phase. The solvent sand samples applied in this system were sonicated for 0.5 h prior to HPLC. 10 µL of the TLC purified sample was injected in HPLC column and eluted with an isocratic elution continued for 20 min (flow rate of 1 mL/min at 215 nm).

RESULTS

Qualitative analysis of phytochemical constituents

Phytochemical screening of the plant extracts was performed for the analysis of phytochemical constituents. In the leaf material, aqueous extract showed positive results for amino acid, chloride, tannins, alkaloids, flavonoids, Phlobatannins, steroids, phenolic compounds and saponins; Butanol extract showed positive results only for chloride, tannins and phenolic compounds; chloroform extract showed positive results for carbohydrate, tannins, phenolic compounds and saponins (Table 1).

Table 1: Qualitative analysis of phytochemical constituents

Sr. No.	Chemical constituents	Leaf extracts		
		Aqueous	Chloroform	Butanol
1	Carbohydrates	-	+	-
2	Protein	-	-	-
3	Amino acid	+	-	-
4	Vitamin C	-	-	-
5	Chloride	+	-	+
6	Tannins	+	+	+
7	Alkaloids	+	-	-
8	Flavonoids	+		-
9	Phlobatannins	+	-	-
10	Steroids	+	-	-
11	Phenolic compounds	+	+	+
12	Saponins	+	+	-

'+' presence of compound; '-' absence of compound

Antimicrobial activity of plant extracts

Antimicrobial activity of the plant extracts was determined by well diffusion method against various bacterial and fungal pathogens. The leaf of butanol extract showed inhibition activity only on *K. pneumonia* (10 mm) and *A. fumigatus* (12 mm); chloroform extract showed activity on *E. coli* (10 mm), *S. aureus* (12 mm), *B. cereus* (10 mm), *A. fumigatus* (12 mm)

and *A. niger* (10 mm); and aqueous extract showed activity on *E. coli* (10 mm), *S. aureus* (14 mm), *B.cereus* (12 mm), *A.niger* (12 mm) and *Penicillium sp* (10 mm) (Table 2).

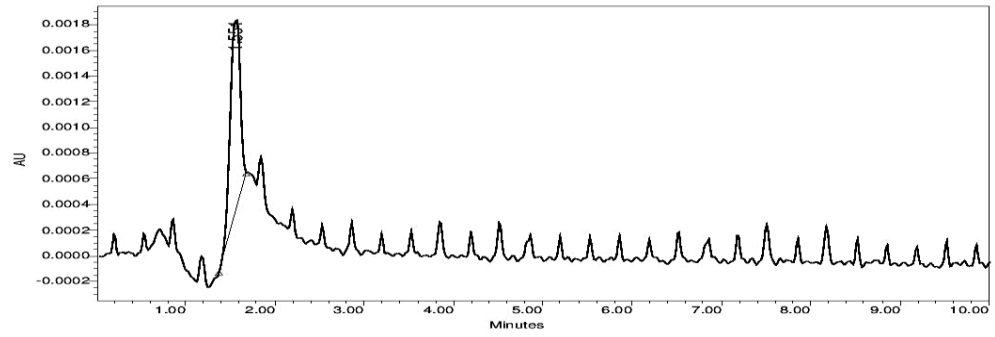
Table 2: Antimicrobial activity of *Solanum nigrum* leaf extracts

Sr. No.	Test organisms	Leaf		
		Aqueous	Chloroform	Butanol
1.	*E.coli*	10mm	10mm	-
2.	*K. pneumoniae*	-	-	10
3.	*S. aureus*	14mm	12mm	-
4.	*B. cereus*	12mm	10mm	-
5.	*A. fumigatus*	-	12mm	12
6.	*A. niger*	12mm	10mm	-
7.	*P. chrysogenum*	10mm	-	

Zone of inhibition in 'mm'

Identification and characterization

High performance liquid chromatography (HPLC) is a versatile, robust and widely used technique for the isolation of natural products[8]. In the present study, the TLC purified sample was loaded on an analytical HPLC and results were observed in Figure 1.



	RT (min)	Peak Type	Area (V*sec)	% Area	Height (V)	% Height	Integration Type	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)
1	1.551	Unknown	12070	100.00	1535	100.00	BB	19	1.367	1.683	1.367

	Baseline End (min)	Slope (V/sec)	Offset (V)
1	1.683	2.437742e-003	-3.448579e-003

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Figure 1: High performance liquid chromatography (HPLC) analysis of compound from *Solanum nigrum*

DISCUSSION

The result of the phytochemical screening indicates the presence of carbohydrates, amino acids, chloride, tannins, alkaloids, flavonoids, phenolic compounds and steroids. The pharmaceutical activity of the plant extracts might be due to the presence of secondary metabolites called phytochemicals. Qualitative analysis for phytochemicals was carried out by phytochemical screening revealed that the leaves of *Solanum nigrum* contain carbohydrates, flavonoids, saponins, tannins, alkaloids, phenols and steroids[9]. The phytochemical constituents of the plant products serve as a defense mechanism[10]. *Solanum nigrum* contains many active components are glycoalkaloids, glycoproteins and polysaccharides, polyphenolic compounds such as gallic acid, catechin, protocatechuic acid, caffeic acid, epicatechin, rutin and naringenin[11]. The scientific investigation from many research findings shows that this plant has various bioactive ingredients such as alkaloids, solanins, saponins, flavonoids, tannins, steroidal glycoalkaloids, steroidal and vitamins[12].

In the present study, the plant extracts of *Solanum nigrum* inhibited the growth of entire tested pathogenic strains. The aqueous extracts of leaf showed highest activity. The antimicrobial of activity may due to the presence of phytochemical constituents like alkaloids, flavonoids, tannins and steroids.

CONCLUSION

The phytochemical analysis showed that antimicrobial activity of *Solanum nigrum* was due to the presence of phytochemical compounds such as Alkaloids, Saponins, Steroids and Proteins. The result indicated that studies carried out on medicinal plant having traditional claims of effectiveness. These plants could serve as useful sources for new antimicrobial agent.

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