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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2020 17(3): 2057-2065.

OPEN ACCESS

Antimicrobial Activity, Phytochemical screening, Antioxidant Potential and Total phenol of Medicinal Plant of *Solanum nigrum* L.

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The whole plant of *Solanum nigrum* L. was examined for phytochemical assessment antibacterial and antifungal activity. The methanol, n-hexane, Distilled Water and Ethanol solvent extract was utilized to determine the antimicrobial impact of extract on *Staphylococcus aureus*. The phytochemical evaluation indicated the existence of saponins, terpenoid, flavonoids, phenol, tannin, steroid and glycoside. Data exhibited that *Staph aureus* was inhibited by methanol (8mm), Ethanol (8mm), n-hexane (1mm) Distilled Water (2mm) The inhibition zones against *Venturia inaequilis* fungi were ranged from 1.8mm to 3.1 mm. Largest zone was seen for the ethanol 3.1 mm (53%) accompanied by n-hexane 2.9mm (50%) and distilled water 2.1 mm (36%) while minimum zone of inhibition were observed Methanol extract 1.8 mm (31%). The anti-oxidant varying at various concentration at 60µg/ml (61.45±0.11) at 80µg/ml (65.23±0.10) as well as 100µg/ml (71.78±0.12) correspondingly. Total phenolic content of aqueous soluble fraction associated with crude extract was (1.40%) of dried extract.

Keywords: Medicinal plants; Antimicrobial activity, Phytochemical screening, Total phenol, antioxidant activity.

INTRODUCTION

Pathogenic microorganisms usually shown severe threats towards the health of humans as well as and animals. Infective diseases are the 2nd major reason death all over the world (WHO, 2002). Human being are now being infected by contagious microbes from times immemorable until this time. bacteria such as *Klebsiella pneumoniae*, and *Escherichia coli* create skin infection, septicemia, respiratory disorder. *E. coli* normally cause the gynecological infections. *Staphylococcus aureus* creates intra-abdominal infections, bone and joint infections, lower respiratory infections as well as skin problem.

(Karch, 2014). *Bacillus subtilis* acts as an opportunistic pathogen, generating poisonous substance that may commonly cause to food poisoning (Eley, 1992). *Aspergillus fumigatus* induces allergic broncho-pulmonary aspergillosis and chronic pulmonary infections (Segal, 2009). While as 1% of most vaginal yeast-based infections exist caused by *Saccharomyces cerevisiae* (McCullough *et al.*, 1998). Medicinal plants are the initial source which utilizing by people to take care of pathogenic infections It's predictable that generally there are approximately 2.5-5.0 lakh species of plants in the world (Borris, 1996). Included in this just a small percentage has

been investigated for phytochemicals and medicinal attributes. In reality just less than 1% of a number of 250,000 higher plants have already been investigated with regards to their phytochemistry (Petlevski et al. 2001). Medicinal plants are high sources of antimicrobial phytochemicals significantly alkaloids, flavonoids, phenols, saponins, terpenes tannins, and terpenoids (Tiwari and Singh, 2004; Lewis and Ausubel 2006). Previous study confirmed the antimicrobial potential of herbal extracts against various pathogenic microbes (Sharvani et al., 2015; Hussain et al., 2015). It is valuable to point out that antimicrobial Phytoconstituents would be the growing options for the control over pathogenic microorganisms. The phytochemicals are two kinds such as primary phytochemical that might have never such a powerful action in medicine as the secondary phytochemical are all over the world utilized for numerous disease, probably the most essential secondary phytochemical are Alkaloids, Terpenoids (Heinrich et al., 1998). Secondary phytochemicals are substances that reveal no purpose to be included in primary metabolic process, they function as supplement role. Instead of medicinal utilizes such phytochemicals are utilized because of the plant due to their protection as well (Heinrich et al., 1998). Terpenoids perform an important role in many pharmacological study and activities, medicinally it perform an important role in anticancer, anti-malarial and anti-fever likewise (Briskin, 2000). Alkaloids are additionally essential secondary chemical that show an important use for the anesthetic agent.. Alkaloids always produce fluctuation in physiology (Islam et al., 2008). Phenol and its particular compound are distributed in several plants, phenolic compound are utilized for the treatment of numerous disorder and also because of its poisonous action stop the growing of various pathogens (Kiran and Bargali 2009). Flavonoids, sterol, and saponins are crucial secondary chemical contained in above 85 families, because of their many function and particular chemistry these are typically utilized to treat numerous condition (Marks and Clay 1996).

Often called black nightshade which is the member of the Solanaceae family. Locally, it is recognized as "Kamacho". It is a small lived perennial shrub that always grows in damp habitats. *Solanum nigrum* L is really a indigenous utilized significant medicinal plant with numerous therapeutic characteristics that as anti-proliferative, anti-seizure, anti-inflammatory, antipoisson, antioxidant and antipyretic properties

(Ramya et al., 2011). Some indigenous utilizes regarding the plant through the treatment of pain, inflammation and fever (Acharya and Pokhrel 2006; Zakaria et al., 2006). Its roots are decoction mixed with some sugar to boost fertility in females. Root juice normally utilized to take care of asthma and whooping cough. Leaf paste can be used to take care of rabies as well as for wound healing. In Algeria, lentire plant decoction is commonly utilized to treat burns off and dermal infections (Ramya et al.2011). The current study was carried out to find out the antimicrobial potential of the *Solanum nigrum* L plant against *Staphylococcus aureus* bacteria and *Venturia inaequalis* fungai strains.

MATERIALS AND METHODS

Plants collection and Extraction

Selected plants were shade dried and ground into a fine powder. From each plant 50g of powder were soaked in 200ml of different solvents (methanol, ethanol, distilled water and nhexane) and powder of the selected plant was stored at room temperature for two weeks and was vigorously shaken each day. The impregnated plant material was first filtered through plain filter and whattmann filter paper # 41. By rotary evaporator under vacuum, solvents were completely vaporized to obtain the crude extracts. The filtrates acquired were kept in a refrigerator (Choudhary et al., 2011).

Antibacterial activity

The antibacterial potential of selected plants was determined by a modified agar well diffusion assay reported by Khan et al., (2011).

Requirements/ Materials

Plant samples, nutrient agar and broth, bacterial strain cultures, saline solution bacterial slants, sterile cork borer, disc (for diffusion), micropipette, Petri plates, organic solvent (DMSO), incubator, spirit lamp, Filter paper.

Preparation of samples

Samples were prepared from three plants i.e, *Solanum nigrum* L using methanol, ethanol, n-hexane and distilled water.

Preparation of media for bacteria

1. Media was prepared by adding 0.13g of nutrient broth to 10ml of dist. water, adjusting the pH to 7.0 & autoclaved.

2. Nutrient agar (NA) medium was prepared by dissolving 23gm/10ml nutrient agar in dist. water, adjusting the pH to 7.0 and autoclaved.

Bacterial strains used

A gram positive strain called *Staphylococcus aureus* was used in the study. The bacteria were preserved at 4°C on nutrient agar medium.

Antifungal assay

The antifungal activity of selected plants was determined by agar well diffusion assay reported by Khan *et al.*, (2011) with some modifications.

Microorganism used:

Venturia inaequalis, an ascomycetes fungi was used in the study

Assay procedure

Media was prepared by taking 6.5 g Sabouraud agar and dissolve in 100 ml of dist. water. The pH was adjusted to 5.6. Methanol is used as a negative control. 15 ml of each medium was poured into a P. dish (9cm), allowed to solidify. Subsequently, a 7-day-old well-tested fungal culture was obtained with a pre-sterilized cork drilling machine and placed upside down in the center of the plate. Petri dishes were place for 7 days in the dark at 25 ± 2 ° C in incubator. Extension diameter (mm) was measured at 24-hour intervals for seven days. The assay was performed in triplicate.

The rate of fungal inhibition was calculated by the following formula:

$$\% \text{ inhibition of fungi growth} = \frac{\text{Linear growth in test sample} \times 100}{\text{Linear growth in control}}$$

Phytochemical assay

Phytochemical process is carried out after plant extract preparation by using the detailed protocol is as follows:

Test for saponins

A total of 3ml from extract was added to 5ml of dist. water in a test tube and shaken vigorously thus will result in a stable foam, a +v sings for saponins.

Test for steroid

To 5 ml of extract, 2 ml of chloroform and conc. H₂SO₄ was added. Red color production in chloroform lower layer indicates steroids presence.

Test for Glycosides

3ml of extract was mixed with 2ml of chloroform, then 2ml of conc. H₂SO₄ was added carefully and gently shaken. A reddish brown colour was obtained which shows the presence of steroidal ring.

Test for Terpenoids

A total of 5 ml extract was mixed in 2ml of chloroform and dissolve till dryness. Then 2ml of conc. H₂SO₄ was included and heat up for approximately two minutes. A grayish colour appeared which indicate the existence of terpenoids.

Test for Flavonoids

5 ml of extract had been included to 2ml of 2% NaOH solution which resulted in yellow colour. Then a small number of drops of dilute acid were put into make the solution colourless which is a positive notice for the existence of flavonoids.

Test for Phenols and Tannins

From extract a total of 0.5 ml was combined 2ml of water and 2 to 3 drops of FeCl₃ were added. The appearance of blackish color indicate the existence of tannins and phenols.

Total phenolic assay

The magnitude of total phenol was identified through the use of Folin Ciocalteu assay reported by (Oliveira *et al.*, 2008). Plant sample extract 0.1g were added with 60 to 80ml of distilled water. Now it is heated and boiled for 30 minutes in a sand bath and filtered. Final volume was up to 100ml with distilled water. From the above extract 0.1ml added to test tube (screw cap) plus 3.9ml dist water is added and agitated. Add 1ml of Folin ciocalteu reagent and then added 5ml of 20% of sodium carbonate and shake vigorously. Keep it at room temperature for 20 minutes. After that O.D of sample will be observed at 720nm wave length. Experiments were performed in triplicate.

Antioxidant assay

Antioxidant assay was performed by scavenging effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Oliveira *et al.*, 2008). A total 0.25 ml DPPH 0.8 mm in MeOH was included with test tubes to accurately weigh the weighted amounts of the extracts break up in 3.75 ml MeOH, accordingly to an extraction concentration ranging from 0.01 and 0.2 mg / ml. ml. Subsequently preparing, the samples were kept at room temperature for half an hour in the dark. Absorbance at 517 nm was calculated utilizing a

UV / Vis V-530 spectrophotometer and examined having a non-extracted control. A blank for every sample was ready utilizing methanol rather than of the DPPH solution. Ascorbic acid had been utilized as the reference compound. Antioxidant activity was shown as a percentage of DPPH radical inhibition and was measured by comparison:

$$\% \text{ scavenging DPPH free radical} = \frac{100 \times (1 - AS)}{AD}$$

These values are defined as the inhibitory concentration of the extract required to reduce the first DPPH radical concentration by 50% and shown in mg/ml.

STATISTICAL ANALYSIS

Standard deviation was applied on the data and recorded in mean values then converted into percent. The Formula of calculation in percent is as follows:

Percent inhibition =

$$\frac{\text{Zone of inhibition of extract (mm)}}{\text{Zone of inhibition of standard (mm)}} \times 100$$

RESULTS

Antibacterial activity of selected plants extracts

The current study performed to realize the antimicrobial activity of crude extracts (Distilled Water, ethanol, methanol, and n-hexane) of *Solanum nigrum* L. against *Staphylococcus aureus* bacteria strains. The *Solanum nigrum* L whole plant were assessed utilizing the agar disc diffusion method by measuring the diameter of growth inhibition zones with 50 µl of distilled water, ethanol, methanol and n-hexane leaf extract. Antibacterial activity was carried out against the bacterial gram positive, *Staphylococcus aureus*

In *Solanum nigrum* L various zone of inhibition were observed. Zones of inhibition were varied from 1mm to 8mm in *Solanum nigrum* L. plant extracts. Recorded zone of inhibition about multiple extract Methanol extract 8mm, accompanied by ethanol 8mm, Distilled water 2mm and n-hexane 1mm, the greatest zone of inhibition seen in methanol and ethanol 8mm even though the smallest zone of inhibition against *Staphylococcus aureus* seen in n-hexane 1mm (Table. 1). Highest zone of inhibition percentage (60%) was found at Methanol and ethanol extract while the lowest zone of inhibition percentage

(7%) observed in n-hexane extract (Table 2) Evaluation regarding the data reported that methanol, ethanol and Distilled Water extracted samples possessed a strong inhibitory impact against *Staphylococcus aureus* in comparison with the n-hexane extracted samples. Methanol and ethanol extracts were more efficient against *Staphylococcus aureus* at both the concentrations in comparison to the n-hexane extracts. (Fig 1) Furthermore the data reveal that n-hexane extract was not valuable working when compared extracts such as Methanol ethanol

Anti-fungal activity:

The inhibitory impact of multiple extract (viz distilled water, methanol, ethanol and n-hexane) of *Solanum nigrum* L, were studied against fungal strains noticed in *Solanum nigrum* L many zone of inhibition were found. The inhibition zones were varied from 1.8mm to 3.1 mm in *Solanum nigrum* L plant extracts. (Table 3). Maximum zone of inhibition was seen for ethanol 3,1mm (53%) accompanied by n-hexane 2.9mm (50%) after which distilled water 2.1mm (36%) while lower limit zone of inhibition was with Methanol 1.8mm (31%) against *Venturia inaequilis* fungi (Table 4). Highest zone of inhibition percentage (47%) was found at n-hexane extract while the lowest zone of inhibition percentage (26%) observed in distal water extract (Fig 2)

Phytochemical study of *Solanum nigrum* L.

The phytochemical screening reflect that various phytochemicals were found in methanolic extract of *Solanum nigrum* L. (whole plant). Such as saponins, terpenoid, flavonoids, phenol and tannin steroid and glycoside. Methanolic extracts indicated the existence of greater amounts of flavonoids compounds than ethanolic one. (Table 5).

Total Phenol Contents of *Solanum nigrum* L

The total phenolic content regarding the aqueous extracts was finf out by using Folin-Ciocalteu reagent. Within the assay, gallic acid was applied as a standard compound and total amount of phenolic contents were indicated as mg/g gallic acid equivalent utilizing the standard curve equation: $y = 0.0012x - 0.0018$, the word y represent the absorbance at 720 nm and x is total phenolic content in *Solanum nigrum* L extracts indicated in mg/gm. The finding revealed that total phenol contents of dried powder of *Solanum nigrum* L. as well as its extracts are revealed in (Table 6). *Solanum nigrum* L. whole plant have

(1.40%) of total phenolics.

Table1: Zone of inhibition of *Solanum nigrum* L plants extracts against *Staphylococcus aureus* bacteria.

Name of the Organism	PLANTS EXTRACT	Zone of Inhibition in (mm)	Mean± Standard Deviation	Chi -Square
Staphylococcus Aureus	Distilled Water	2	2.0±0.17	0.5
	Ethanol	8.4	8.4±0.16	
	Methanol	8.4	8.4±0.21	
	n-hexane	1	1±0.17	
	Control Erytheromycin	14	14±0.15	

Table: 2. Percentage inhibition of *Solanum nigrum* L plants extracts against *Staphylococcus aureus* bacteria.

Name of the Organism	PLANTS EXTRACT	Percentage (%)
Staphylococcus aureus	Distilled Water	14%
	Ethanol	60%
	Methanol	60%
	n-hexane	7%
	Control Erytheromycin	100%

Table3:Zone of inhibition in (mm) against fungal strain *Venturia inaequalis*

Name of the Organism	Plant Extract	Zone of Inhibition in (mm)	Mean± Standard Deviation	Chi –Square
Venturia inaequalis	Distilled Water	1.8	1.8±0.208	0.5
	Ethanol	3.1	3.1±0.115	
	Methanol	2.9	2.9±0.115	
	n-hexane	3.2	3.2±0.208	
	Control (methanol)	6.8	6.8±0.152	

Table: 4 Percentage inhibition of selected plants extracts against fungal strain *Venturia inaequalis*.

Name of the Organism	PLANT EXTRACTS	Percentage (%)
Venturia inaequalis	Distilled Water	26%
	Ethanol	46%
	Methanol	43%
	n-hexane	47%
	Methanol (-ve contr)	100%

Table 5: Phytochemical screening of *Solanum nigrum* L.

S.No	PHYTOCHEMICAL COMPOUND	Methanol extract
1	Saponin	+
2	Steroid	+
3	Glycoside	+
4	Terpenoid	+
5	Flavonoid	++
6	Phenol and Tannin	+

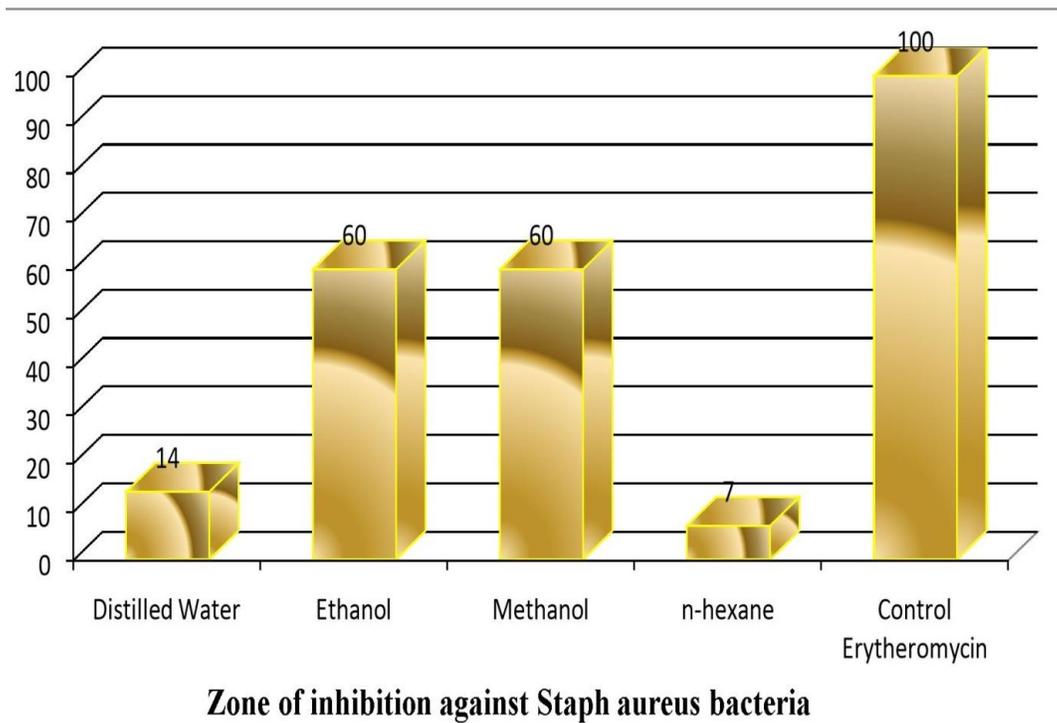


Figure 1: Inhibition zone of *Solanumnigrum* L extracts against *Staphylococcus aureus* bacteria.

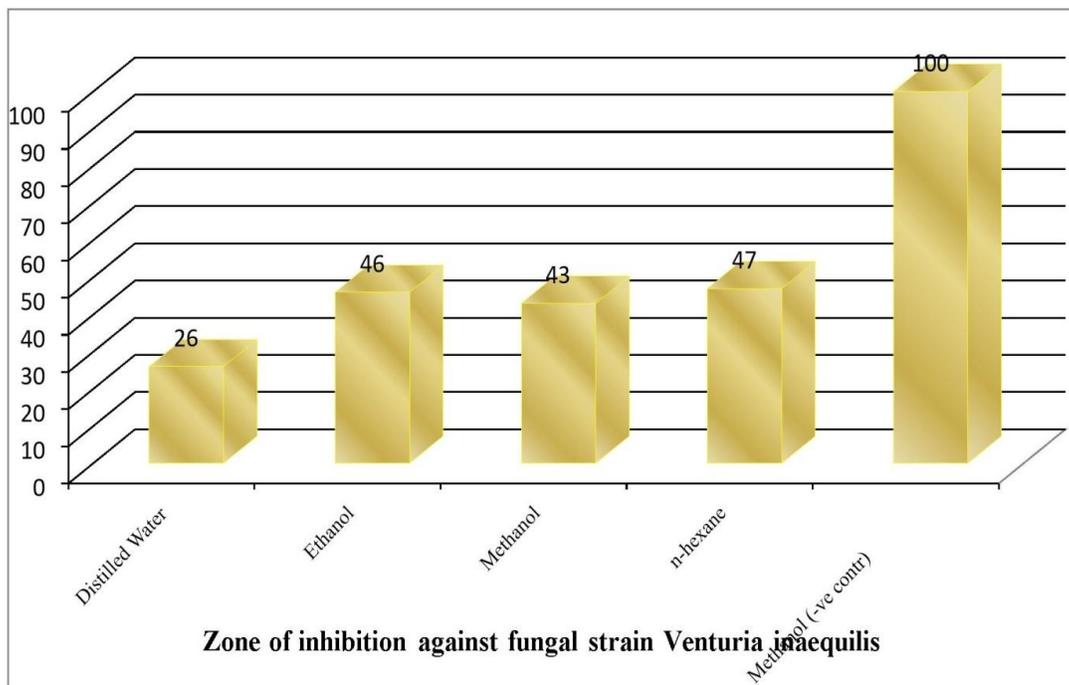


Figure 2: Zone of inhibition of *Solanum nigrum* L extracts against fungal strain *Venturia inaequilis*.

Table 6: Total phenolic analysis of *Solanum nigrum* L plants extracts by Folin-Ciocalteu assay

Treatment	Sample weight (g)	O.D	Blank	O.D Blank	$X = y + \frac{0.0018}{0.0012}$	Total phenol (ppm)	%age
<i>Solanum nigrum</i> L. 1	0.13	0.15	0.113	0.039	34	14961.1	1.51±1.17
<i>Solanum nigrum</i> L. 2	0.13	0.15	0.113	0.037	32.33	14166.7	1.40±1.15

Table 7: Antioxidant potential of aqueous plant extracts of *Solanum nigrum* L plant.

Concentration	Anti-oxidant activity (%)	
	Mean	Mean± Standard Deviation
60 µg	38	38.17±0.05
80 µg	43	43.32±0.07
100 µg	50	49.67±0.41

Antioxidant activity:

The anti-oxidant potential of *Solanum nigrum* L was find out utilizing aqueous extracts by scavenging effect assay. Multiple concentration (60, 80 and 100 µg/ml) regarding the extracts were utilized in the assay as shown in (Table 7). At 60µg/ml the aqueous fraction of *Solanum nigrum* L was recorded (38.17±0.05). At 80 /ml, *Solanum nigrum* L showed the antioxidant activity (43.32±0.07) but at 100µg/ml express (49.67±0.41).

DISCUSSION

Phytochemicals are bioactive chemicals associated with the plant origin. These are typically the secondary metabolites because their need is less them. The amount and quality of phytochemicals contained in various part of the plant fluctuate (Lahlou, 2004). Effective finding of biologically active ingredients from plant content is essentially influenced by the kind of solvents utilized within the extraction method (Tiwari *et al.*, 2011). In the current examination methanol, n-hexane, Distilled Water, Ethanol extract of *Solinum nigrum* L revealed presence of Saponin, Steroid, Glycoside, Terpenoid, Flavonoid, Phenol and Tannin. Similar results was reported by Qadir *et al.*, (2014) aqueous and alcoholic extract of *Solinum nigrum* L exhibited the existence of six compounds alkaloids, saponins, proteins and free amino acids, lignin and polysterol and lack of some other five compounds oils and fats, tannins and phenolic compounds, gums and mucilage, flavonoids and glycosides. Phytochemical and antibacterial activities of *Solinum nigrum* L leaf extract by Qadir *et al.*, (2014) showed the

existence of most phytochemicals as that of with the exception of terpenoids and phenol in both the extract of aqueous and methanol. Likewise the whole plant of *Solanum nigrum* L revealed nonexistence of terpenoids and volatile oils whereas great amount of alkaloids, saponins, glycosides and flavonoids; medium amount of tannins and coumarins (Ashrafudoulla *et al.*, 2016) . Phytochemical investigation of *Solanum nigrum* L utilizing hexane and benzene extracts indicated the existence of saponins, phytosterols, tannins and fixed oils and fats. The ethanolic and the aqueous extract indicated carbohydrates, flavonoids, coumarins and phytosterol (Ashrafudoulla *et al.*, 2016). *Solinum nigrum* L Plants have been playing a important role in human medicine. The susceptibility regarding the crude extracts on the basis of zones of growth inhibition various regarding solvent extract. Zones of inhibitions had been seen in multiple extracts that as distilled water, ethanol, methanol and n-hexane. Methanolic and Ethanolic extract of *Solanum nigrum* L show maximum zone of inhibition, distilled water was noticed with medium zone of inhibition, while n-hexane was seen with minimal inhibitory concentration. The Same study was carried out by (Sridhar *et al* 2011). Who stated that ethanolic extract of the dried fruits of *Solanum nigrum* L were examined for antimicrobial activity? The current results indicated that *Solanum nigrum* L. have low amount of phenolic contents (1.40%). The similarly studies had been performed on *Solinum nigrum* L by Oliveira *et al.*, (2008); Saddiqe *et al.*, (2013) who stated that such plants are a prospective source of phenolic compounds and hence explaining our results. The potent anti-

oxidant property of plant extracts is because of the property of having hydroxyl groups existing within the phenolic compounds (Cai *et al* 2004). Various reports of the scientific study indicate that antioxidant activity 26 of phenolic compounds contained in plants have redox properties, that enable it to perform as reducing agents and hydrogen donors (Mustafa *et al.*, 2010). The outcomes are in respect towards the results exhibited by Oliveira *et al.*, (2008); Saddiqe *et al.*, (2013 when a concentration dependent antioxidant activity were documented for *Solanum nigrum* L being probably the most effective one. The finding obtained in the assay indicated that these plants are extremely vital from medicinal point of view, and it needs.

CONCLUSION

Current study have validated the reported utilization of *Solanum nigrum* L plant in the indigenously therapeutic systems regarding cure of different contagion. Through the results of this investigation, it is determined that the crude extracts of the *Solanum nigrum* L plant might have vital antimicrobial properties, particularly against fungal strains utilized into the study. The plant may contain potent antimicrobial substance, excellent for the cure of different fungal and bacterial infections. But, more examination is required towards the natural ingredient isolation,poisonous effect and healthcare provider to be able to utilize the magnificent compound(s) that efficient antimicrobial agents.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

Funding sources. This research work was sponsored by Prime minister Reimbursement scheme, Pakistan.

ACKNOWLEDGEMENT

This research work was sponsored by Prime minister Reimbursement scheme, Pakistan which is highly acknowledged. The authors extend their high appreciation and acknowledgment towards the NIFA staff by providing moral support to the authors.

AUTHOR CONTRIBUTIONS

All author contributed in the paper here. Sher Hayat designed and carry out the experiments AHK wrote the manuscript. Ahmad Hasan, Saira Naz, Qaisar ali, Ateeq ur Rahman, Zubir shah performed Data analysis, Ashfaq Ahmad and

Khaleeq Ahmad helped in plant collection identification dehydration and powder preparation. Imran Khan and Ali Hazrat provide technical suggestions on the draft and identified the language and grammatical mistakes. IK supervised all the stages. All authors read and approved the final version.

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