Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2016, 6(4):57-68

ISSN : 2249-7412
CODEN (USA): AJPSKY

Studies on phytochemical constituents and antimicrobial activities of leaves, fruits and stems of *Solanum nigrum* L.

Pankaj Kumar[1]*, Jitendra Kumar[2], Raghuveer Kumar[1] and R. C. Dubey[3]

[1]*Department of Microbiology, Dolphin (PG) College of Science & Agriculture, Chunnikalan, Fatehgarh Sahib, Punjab, India*
[2]*Department of Biotechnology, Dolphin (PG) College of Science & Agriculture, Chunnikalan, Fatehgarh Sahib, Punjab, India*
[3]*Department of Botany and Microbiology, Gurukul Kangri Vishwavidyalaya, Haridwar-249404, Uttarakhand, India*

ABSTRACT

Medicinal plants are being used as traditional medicines to cure several infectious and non-infectious diseases because of their products are safe in contrast to the synthetic medicines that are considered as unsafe to human including other animals and environment. We selected Solanum nigrum L. (Makoi) which belongs to family Solanaceae to evaluate antimicrobial activities against six pathogenic bacteria Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeroginosa and Enterobacter aerogens. Phytochemical analysis of the plant (crude extract of leaves, fruits and stems) showed the presence saponins, tannins, alkaloids, terpenoides, flavonoides and steroids etc. The extract of leaves, fruits and stemsprepared using five different solvents (hexane, chloroform, acetone, ethanol and water) showed antimicrobial activates against all selected pathogenic bacteria. But water extract of leaves, acetone extract of fruits and hexane extract of stems showed maximum zone of inhibition such as21.30$\pm$1.50mm, 16.67$\pm$0.58mm and 14.30$\pm$1.52mm respectively against Pseudomonas aeroginosa. Thus, we can say that S. nigrum possess broad spectrum antimicrobial activities against several pathogenic bacteria and can be used clinically to find novel antibacterial compounds for various diseases.

Key words: *Solanum nigrum,* Antimicrobial activities, Pathogenic bacteria

---

INTRODUCTION

According to World health organization approximately 80% world population use traditional medicines for the treatment of several diseases, because the herbal products safe in contrast to the synthetics, that are regarded as unsafe to human and environment. There are many plant/herbs having different medicinal value used against various disease since early time. Among them *Solanum nigrum* L.(black nightshade "Makoi") is one of plant having great importance in Ayurvedic medication. Itis a member of family *Solaneace*. The member of this family known for the presence of natural products of medical significance mainly steroidal lactones, glycosides, alkaloids and flavonoids. This herb is antiseptic, antidysentric and diuretic in nature and used in the treatment of cardiac, skin disease, psoriasis, herpi virus and inflammation of kidney. The medicinal plant have antimicrobial agent, that kills or inhibit the growth of microorganisms such as *Echerichia coli, Bacillus subtillus, K. pneumonia, V. cholera, Micrococcus luteus, Salmonella typhiurium, Aspergillus niger, A.fumigatus, Candida albicans*, etc.Indians are using traditional medicine to treat several diseases since ancient time. The traditional medicine still plays an important role in the primary health care. Diosgenin is a naturally-occurring steroidal saponin present in *S. nigrum*. Diosgenin serves as

important starting material for the production of corticosteroids, sexual hormones, oral contraceptives as well as other steroidal drugs. It is also used in the treatment of diseases such asdiabetes, leukemia, hypercholesterolemia, climacteric syndrome and colon cancer [5].

Several worker reported antimicrobial activities of extract of various plant against different microorganisms, Gram positive bacteria; (*Micrococcus varians*, *M.luteus*, *Staphylococcus aureus*) and Gram negative bacteria; (*Salmonella typhi*, *Pasteurella maltocida*, *E. coli*, *K.pneumoniae*, *V. cholera*and some species of fungi; (*Aspergillus niger*, *A.flavus*, *A.fumigates*)[11,1 and 14]. Yogananth *et al.*[18] reported antimicrobial activity of extracted stem and leaf of *S. nigrum*using three different solvents like Ethanol, Hexane and Chloroform against eight bacterial cultures (*Enterococcus faecalis*, *E. coli*, *K.pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella typhi*, *Staphylococcus aureus* and *V.cholerae*). India and other parts of the world are using *S. nigrum* to cure liver disorders, chronic skin ailments (Psoriasis and ringworm) fevers, diarrhoea, eye diseases, hydrophobia etc. It possesses properties like antimicrobial, antioxidant, cytotoxic properties, antiulcerogenic, and hepatoprotective activity. They are also useful in otopathy, ophthalmopathy, rhinopathy and hepatitis [18].

In Punjab, several research works is going on regarding antimicrobial activity of plants against various microorganisms, but very less or no focus on antimicrobial activity of *S. nigrum* to treat disease. Hence based on above fact and medicinal value of this plant we have selected this plant to study various extract of this plant against pathogenic microorganisms.

MATERIALS AND METHODS

Collection and processing of plant sample:
The fresh and disease free upper parts (leaves, fruits, and stems) of plant *S. nigrum* were collected from the different field areas of Village-Chunni Kalan, Fatehgarh Sahib, Punjab (India) in the month of January, 2015. After proper cleaning collected parts were rinsed with autoclaved water and shade dried at room temperature for overnight followed by incubator at 34℃ (±2℃) for 10-11 days of leaves, stems and 15 days of fruits. After drying, the microbes free plant samples were grounded to coarse powder. The samples were weighed before loading in the Soxhlet apparatus for extraction (Table -01).

Table:-1. Loss of moisture in samples after drying

Name of samples	Fresh weight	Dry weight	Weight loss	Loss (%)
Leaves	600g	90g	510g	85.00%
Fruits	550g	94g	456g	82.91%
Stems	600g	130g	470g	78.34%

Collection and processing of upper parts of *S. nigrum*.
Fresh upper parts were collected and processed. Around 90g of leaves, 94g of fruits, 130g of stems of dried powder was obtained of which 50g of each leaves, fruits and stems were taken for extraction of crude extracts.

Extraction of active principles:
To extract active principle from different plant materials were prepared by using solvents such as: hexane, chloroform, acetone, ethanol and double distilled water with the help of Soxhlet apparatus. The solvent was evaporated at their respective temperature with the help of distillation apparatus and the extract obtained was cooled and dried under vacuum and preserved at 4℃ for further use.

Soxhlet extraction [16]
The soluble active principles from solid material are extracted by repeated washing with either an organic solvent or distilled water under reflux in special glassware. Earlier prepared sample were placed in an extraction chamber, which is suspended above a flask containing the solvent and below a condenser. Approximately 400ml of solvent is used to perform each extraction in 1000ml RBF (Round Bottom Flask) for 70-72 hours. The flask was heated at the bottom. The solvent evaporates and move up from the flask into the water cooled containing the sample. The solvent is redistilled from the solution in the flask and condenses in the chamber, repeating the extraction with fresh solvent. The process can be repeated as many times as necessary. The soxhlet extraction is usually completed when the solution in the soxhlet chamber is of the same colors as the pure solvent. This means that nothing more is being

extracted from the plant part used by the solvent. At the end of the extraction process, the flask is evaporated and the mass of the remaining material is separated.

Solvents used for extraction:
Initial screening of plants parts for possible antimicrobial activities was done by using crude aqueous or alcohol extraction followed by various organic extraction methods. Extraction of active ingredient from selected plant's parts were carried out using various solvents (Hexane, Chloroform, Acetone, Ethanol (60%), Distilled water) in sequential manner of increasing order of polarity. After extraction, the extracted material was evaporated to separate the active ingredients from the solvent. The concentrate was stored in airtight bottles for further use (Table- 02).

Table:-2. Different solvent and its boiling points, polarity and solubility

Solvent used	Boiling point	Polarity	Solubility in water (%)
Hexane	65-70℃	0.0	0.001
Chloroform, Acetone, Ethanol (60%), Distilled water	65-70℃	4.1	0.815
Acetone, Ethanol (60%), Distilled water	56-60℃	5.1	100
Ethanol (60%), Distilled water	70-80℃	5.2	100
Distilled water	95-105℃	9.0	100

Distillation:
The extract along with the active components obtained through the soxhlet extract was taken in the RBF and boiled. The vapours were passed through the T-tube of the condenser, where they condensed and fell back in the conical flask through the L-tube for about 3-5 hrs, the solvent got separated from the active principle, which gets collected in the RBF. Further, using hot water bath, slowly heat up the crude extracts at its respective temperatures. The prepared extracts were kept at 4℃ in the refrigerator for at least 24 hrs before the subsequent tests.

Physical Examination and Percentage Yield of Extract
Physical analysis:
Physical parameters like colour, odor and solubility (in organic solvent) of extract was analysed to check nature and properties of extract.

Calculation of percentage yield:
Percentage yield of extract was calculated by following method.

$$\text{Percentage yield (v/w)} = \frac{\text{Weight of extract recovered}}{\text{Weight of plant sample loaded}} \times 100$$

PHYTOCHEMICAL STUDIES:
Chemical tests of the extracts were carried out qualitatively following standard procedures to identify the phytochemical constituents present in extract.

Test for Saponins (Foam test):
Approximately 50mg of crude extract was dissolved in 2ml distilled water in a test tube. Tubes were vigorously shaken to produce the foam. The presence of foam indicated the Saponins[17].

Test for Tannins (Nitric acid test):
Approximately 50mg of plant extract were transferred in 2ml of 69% nitric acid in a test tube. The formation of raddish to yellow colour indicated the presence of tannins [6].

Test for Alkaloids (Mayer's test):
500-600mg dried powdered sample and 8ml (1% HCl) were mixed. After 10-12 minutes of incubation at room temperature the mixture were vortexed for 2 minutes and filtrated.

1ml of Mayer's reagent were added in 2ml of filtrate. Appearance of turbidity or precipitate indicated the presence of alkaloids [8].

Test for Terpenoides (Salkowski test):
Approximately 50mg extract was added to 1ml of Chloroform. 1.5ml concentrated sulfuric acid was carefully added along the wall of test tube. Raddish brown coloration of the interface indicates the presence of Terpenoides[15].

Test for Flavonoides:
50mg of plant 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 indicated the presence of flavonoids [7 and 8].

Test for Glycosides (Keller-Kiliani test):
Approximately 50mg of plant extract added in0.5ml of Feric chloride (1M). Concentrated acetic acid and conc. sulfuric acid were added carefully along the wall of test tube. Raddish brown coloration at the junction of both layers and bluesh green colour at the upper layer indicated the presence of Glycosides [7].

Test for Protein:
50mg of plant extract or filtrate and 2ml of Millons reagent mix thoroughly and heated. White precipitate turns brick red after boiling indicated the presence of protein [13].

Test for Steroids (Salkow Skiss test):
Approximate 50mg of extract was dissolved in 1ml acetone. 1ml conc. sulfuric acid was added. Formation of two phase with red or yellow colour indicating the presence of Steroids and methylated steroids [7].

ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS:
Microbial cultures used for antibacterial assay:
The pure cultures (lyphilysed) of microbes were procured from MTCC, IMTECH (Institute of microbial technology) sector-39 A, Chandigarh, India. A total of six bacterial cultures were used in the present study. All the pure cultures were maintained on slants of Nutrient agar media (NAM) and were stored at 4°C. Mueller Hinton Agar (MHA) was used for testing of antimicrobial efficiency. The microorganisms which were selected for the determination of antibacterial activity of extract are as follows (Table - 03):

Table: -3. Selected bacterial culture

Microorganisms	MTCC No.
Staphylococcus aureus	3160
Bacillus cereus	8733
Escherichia coli	443
Salmonella typhimurium	3231
Pseudomonas aeroginosa	3542
Enterobacteraerogens	111

Solvent used for dissolving extracts:
The dilution of the extracts was prepared by dissolving in Dimethyl sulfoxide{DMSO -$(CH_3)_2SO$} (Fig.-01)for evaluating the antibacterial activity against selected pathogenic microorganism. DMSO being a very good solvent for experimental purposes and hence selected for dissolving the extracts to be used for testing the antibacterial efficacy. It easily diffuses into the agar medium and is miscible in organic solvents as well as in water. Normally it does not exhibit antimicrobial activity and hence also serve as a control. It is a colourless hydroscopic liquid with mild garlic odor having a boiling point of 189℃.

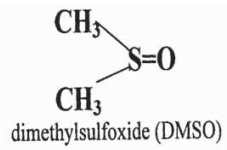

Fig:-1. Structure of DMSO

Diffusibility of solvent (DMSO):
Wells were prepared in the agar plates using cork borer. The diameter of the wells prepared was approximately 9mm. 100µl of 7.8% DMSO in water and ethanol extract and pure (100%) DMSO was added in acetone, chloroform

and hexane extract in wells and kept overnight in an incubator to test the diffusibility. It was found that the solvent diffused well in the medium and hence, selected for antibacterial study.

Preparation of various dilutions of extracts:
Various dilutions from extracts obtained from upper parts of *S. nigrum* were prepared for testing their antibacterial efficacy against selected microorganisms. 7.8% DMSO was used in water, ethanol and pure DMSO used in acetone, chloroform and hexane extract as a control. The concentrations of extract were used:- 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml.

Agar well assays method (Agar diffusion method):
100µl cultures of test organism were spreaded uniformly on MHA plates. After inoculation, wells (9mm diameter) were prepared using sterile cork borer. 100µl of the extracts of different concentrations were transferred in well. One well having 100µl (7.8% DMSO) served as control. The plates were then allowed to stabilize for about 1 hour and then incubated at 37°C for 24 hours. The experiment was carried out in triplicate.

Measurements of zone of inhibition:
The diameters of zones of inhibition were measured in millimeter (mm). It was determined as the diameter of inhibition zone around the wells as an average of four measurements per well at 4 dimension's that is 0°, 45°, 90°, 135°.

RESULTS

The extract (leaves, fruits and stems) *S.nigrum* were obtained by soxhletion. These extracts were addressed for their bioactive potential against the standard microbial culture. *S.nigrum* extract possess broad range of activity against the selected bacterial culture.

Nature of extracts:
The upper parts (leaves, fruits, stems) extract of *S.nigrum* was extracted with the solvents such as hot water, ethanol (60%), acetone, chloroform and hexane. After extraction the colour of the extract was noted. The colour of various solvent extracts are as follows (Table- 04).

Table:- 4. Colour of different parts (leaves, fruits and stems) of *S. nigrum* indifferent solvents

S.no.	Extracts	Different colours of *S. nigrum* extract		
		Leaves	Fruits	Stems
1.	Hexane	Yellow	Greenes yellow	Greenes yellow
2.	Chloroform	Green	Light yellow (turbidity)	Raddish yellow(brick colour)
3.	Acetone	Yellow	Light yellow (turbidity)	Light yellow (turbidity)
4.	Ethanol	Yellow	Yellow	Light yellow (turbidity)
5.	Water	Raddish yellow (brick colour)	Brown	Raddish yellow(brick colour)

PHYTOCHEMICAL ANALYSIS:-
Saponins test (Foam test):
The phytochemical compound of saponins are present in large quantity in ethanol extract, medium quantity in water and acetone extract, small quantity in chloroform extract, where as absent in hexane extract of leaves. The saponins are present in medium quantity in water extract, small quantity in ethanol extract, but absent in acetone, chloroform and hexane extract of fruits. The saponins are present in large quantity in water extract, medium quantity in ethanol extract, small quantity in acetone extract, but absent in chloroform and hexane extract of stems. All the results are as follows (Table- 05).

Table:- 5. The Phytochemical compound in leaves, fruits and stems extract of *S. nigrum* in different solvents

		Saponins			Tannins			Alkaloids			Terpenoids		
S.No.	Extract	Leaves	Fruits	Stems	Leaves	Fruits	Stems	Leaves	Fruits	Stems	Leaves	Fruits	Stems
1.	Water	++	++	+++	++	+++	+++	+	+	++	+	+	+
2.	Ethanol	+++	+	++	++	+++	++	++	+++	+++	++	++	++
3.	Acetone	++	-	+	+++	++	+	+++	++	+	+++	+++	+++
4.	Chloroform	+	-	-	+++	+	+++	-	-	-	-	+++	-
5.	Hexane	-	-	-	-	-	+++	-	-	-	-	-	-
6.	Control	-	-	-	-	-	-	-	-	-	-	-	-

Bearing in mind that: (-)= Indicates to the absence of an active substance. (+)= Indicates the presence of phytochemicals small quantity. (++)= Indicates the presence of phytochemicals medium quantity . (+++)= Indicats the presence of phytochemicals large quantity.

Table:- 6. The Phytochemical compound in leaves, fruits and stems extract of *S. nigrum* in different solvents

S.No.	Extract	flavonoides			glycosides			proteins			steroids		
		Leaves	Fruits	Stems	Leaves	Fruits	Stems	Leaves	Fruits	Stems	Leaves	Fruits	Stems
1.	Water	++	++	++	+	+	++	++	+++	++	++	+	+
2.	Ethanol	+++	+++	+++	++	++	+++	+++	+++	++	++	++	++
3.	Acetone	+	++	+	++	++	+	+++	++	++	++	+	++
4.	Chloroform	-	-	-	+++	++	++	+++	-	-	+	+	+
5.	Hexane	-	-	-	+++	+++	+	+	-	-	+	-	-
6.	Control	-	-	-	-	-	-	-	-	-	-	-	-

Bearing in mind that: (-)= Indicates to the absence of an active substance. (+)= Indicates the presence of phytochemicals small quantity. (++)= Indicates the presence of phytochemicals medium quantity . (+++)= Indicats the presence of phytochemicals large quantity.

Tannins test (Nitric acid test):

The phytochemical compounds of tannins are present in large quantity in acetone and chloroform extract, medium quantity in water and ethanol extract, where as absent in hexane extract of leaves. The tannins are present in large quantity in water and ethanol extract, medium quantity in acetone extract, small quantity in chloroform extract, but absent in hexane extract of fruits. The tannins are present in large quantity in water, chloroform and hexane extract, medium quantity in ethanol extract, small quantity in acetone extract of stems. All the results are as follows (Table-05).

Alkaloids test (Mayer's test):

The phytochemical compounds of alkaloids are present in large quantity in acetone extract, medium quantity in ethanol extract, small quantity in water extract, where as absent in chloroform and hexane extract of leaves. The alkaloids are present in large quantity in ethanol extract, medium quantity in acetone extract, small quantity in water extract, but absent in chloroform and hexane extract of fruits. The alkaloids are present in large quantity in ethanol extract, medium quantity in water extract, small quantity in acetone extract, but absent in chloroform and hexane extract of stems. All the results are as follows(Table- 05).

Terpenoids test (Salkowski test):

The phytochemical compounds of terpenoids are present in large quantity in acetone extract, medium quantity in ethanol extract, small quantity in water extract, where as absent in chloroform and hexane extract of leaves. The terpenoids are present in large quantity in acetone and chloroform extract, medium quantity in ethanol extract, small quantity in water extract, but absent in hexane extract of fruits. The terpenoides are present in large quantity in acetone extract, medium quantity in ethanol extract, small quantity in water extract, but absent in chloroform and hexane extract of stems. All the results are as follows (Table- 05).

Flavonoids test:

The phytochemical compounds of flavonoids are present in large quantity in ethanol extract, medium quantity in water extract, small quantity in acetone extract, where as absent in chloroform and hexane extract of leaves. The flavonoids are present in large quantity in ethanol extract, medium quantity in water and acetone extract, but absent in chloroform and hexane extract of fruits. The flavonoids are present in large quantity in ethanol extract, medium quantity in water extract, small quantity in acetone extract, but absent in chloroform and hexane extract of stems. All the results are as follows(Table- 06).

Glycosides test (Keller Kiliani test):
The phytochemical compounds of glycosides are present in large quantity in chloroform and hexane extract, medium quantity in ethanol and acetone extract, small quantity in water extract of leaves. The glycosides are present in large quantity in hexane extract, medium quantity in ethanol, acetone and chloroform extract, small quantity in water extract of fruits. The glycosides are present in large quantity in ethanol extract, medium quantity in water and chloroform extract, small quantity in acetone and hexane extract of stems. All the results are as follows (Table- 06).

Protein test:
The phytochemical compounds of protein are present in large quantity in ethanol, acetone and chloroform extract, medium quantity in water extract, small quantity in hexane extract of leaves. The protein are present in large quantity in water and ethanol extract, medium quantity in acetone extract, but absent in chloroform and hexane extract of fruits. The protein are present in medium quantity in acetone, ethanol and water extract, but absent in chloroform and hexane extract of stems. All the results are as follows (Table- 06).

Steroids test (Salkow Skiss test):
The phytochemical compounds of steroids are present in medium quantity in water, ethanol and acetone extract, small quantity in chloroform and hexane extract of leaves. The steroids are present in medium quantity in ethanol extract, small quantity in water, acetone and chloroform extract, but absent in hexane extract of fruits. The steroids are present in medium quantity in ethanol and acetone extract, small quantity in water and chloroform extract, but absent in hexane extract of stems. All the results are as follows (Table- 06).

ANTIBACTERIAL ACTIVITY:-
Antimicrobial potential of extracts of *S. nigrum* leaves:
Among the different types of extracts of *S. nigrum* leaves, water extract at 50 mg/ml concentration showed maximum (**21.30±1.50mm)** zone of inhibition against *P. aeruginosa*. There is no zone of inhibition observed in negative control (DMSO havivg no extract) in all cases. The activities of two antibiotics(Streptomycine and Ciprofloxacin) were also observed against *E. coli*, *S. typhimurium* and *P. aeruginosa*(Fig. – 02a and Table- 07).

Fig - 02: Effect of (a) water extract of leaves (b) acetone extract of fruits and (c) hexane extract of stems of *Solanum nigrum* L. on *Pseudomonas aeruginosa*

Table:- 7. Screening of antibacterial activities of different extract of *S. nigrum* leaves

TO	Diameter of zone of inhibition (mm)*.																						
	Hexane extract				Chloroform extract				Acetone extract				Ethanol extract				Water extract				DMSO and Antibiotics		
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	E	F	G
SA	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	**4.00 ±1.00**	**5.67 ±0.58**	**7.30 ±0.58**	**8.00 ±1.00**	3.30 ±0.58	4.67 ±0.58	7.00 ±1.00	9.30 ±2.08	7.67 ±1.50	9.67 ±0.58	12.00 ±1.00	16.30 ±1.50	9.67 ±0.58	13.00 ±1.00	14.67 ±1.15	16.67 ±0.58	0.0 ± 0.0	10.30 ±0.58	24.30 ±0.58
BC	3.67 ±0.58	4.00 ±1.00	5.30 ±0.58	6.00 ±1.00	**7.67 ±0.58**	8.30 ±0.58	10.00 ±1.00	12.00 ±1.00	4.00 ±1.00	5.67 ±0.58	7.00 ±1.00	7.30 ±0.58	4.00 ±1.00	**5.67 ±0.58**	**7.00 ±1.00**	**8.00 ±1.00**	12.00 ±1.00	13.67 ±0.58	16.00 ±1.00	17.67 ±0.58	0.0 ±0.0	14.00 ±1.00	19.67 ±1.50
EC	**0.00 ±0.00**	4.30 ±0.58	5.67 ±0.58	7.30 ±0.58	6.00 ±1.00	8.00 ±1.00	9.30 ±0.58	10.30 ±0.58	**0.00 ±0.00**	**2.00 ±0.58**	**3.67 ±0.58**	**5.67 ±0.58**	**11.67 ±0.58**	**13.00 ±1.00**	14.67 ±0.58	**18.30 ±0.58**	10.30 ±0.58	11.67 ±1.50	13.67 ±0.58	16.00 ±1.00	0.0 ±0.0	20.30 ±0.58	26.67 ±1.50
ST	3.00 ±1.00	3.67 ±0.58	4.67 ±0.58	6.00 ±1.00	6.67 ±0.58	**8.67 ±0.58**	9.67 ±0.58	11.30 ±0.58	4.30 ±0.58	5.67 ±0.58	8.00 ±1.00	9.67 ±0.58	4.30 ±0.58	6.00 ±1.00	8.00 ±1.00	9.67 ±0.58	7.67 ±0.58	10.00 ±1.00	12.00 ±1.00	13.30 ±0.58	0.0 ±0.0	18.30 ±0.58	25.30 ±0.58
PA	**8.00 ±1.00**	**10.30 ±0.58**	**10.67 ±0.58**	**12.30 ±0.58**	7.30 ±0.58	**8.67 ±0.58**	**11.00 ±1.00**	**12.67 ±0.58**	**8.00 ±1.00**	**9.30 ±0.58**	**10.67 ±1.15**	**13.30 ±1.50**	10.67 ±0.58	12.30 ±0.58	**15.30 ±1.50**	**16.67 ±0.58**	**13.67 ±0.58**	**15.67 ±1.50**	**19.00 ±2.00**	**21.30 ±1.50**	0.0 ±0.0	29.00 ±1.00	36.00 ±1.00
EA	4.00 ±1.00	5.30 ±0.58	6.30 ±0.58	8.00 ±1.00	6.67 ±0.58	8.30 ±0.58	10.30 ±0.58	11.00 ±1.00	6.00 ±1.00	7.67 ±0.58	10.00 ±1.00	11.00 ±1.00	**3.67 ±1.50**	6.00 ±1.00	7.68 ±0.58	10.00 ±1.00	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	0.0 ±0.0	16.30 ±0.58	18.30 ±0.58

*Abbreviation: TO- Test Organisms; SA - **S. aureus; BC - B. cereus; EC - E. coli; ST - S. typhimurium; PA - P. aeruginosa; EA - E. aerogens;** A= 6.25mg/ml, B= 12.5mg/ml, C= 25mg/ml, D= 50mg/ml, E= control (7.8% DMSO), F= Streptomycine (20µg/ml), G= Ciprofloxacine (20µg/ml), (0) = Denotes no ZOI. * = Value are mean of three replication ± Standard deviatin (SD).*

Table:- 8. Screening of antibacterial activities of different extract of *S. nigrum* Fruits

TO	Diameter of zone of inhibition (mm)*.																						
	Hexane extract				Chloroform extract				Acetone extract				Ethanol extract				Water extract				DMSO and Antibiotics		
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	E	F	G
SA	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	**3.67 ±0.58**	**5.00 ±1.00**	**6.00 ±1.00**	**6.67 ±0.58**	**3.67 ±0.58**	5.67 ±0.58	8.00 ±1.00	9.00 ±1.00	3.67 ±0.58	6.00 ±1.00	8.67 ±0.58	11.00 ±1.00	6.00 ±1.00	9.67 ±0.58	12.00 ±2.00	13.67 ±0.58	0.00 ±0.00	10.30 ±0.58	24.30 ±0.58
BC	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	5.00 ±1.00	6.30 ±0.58	7.67 ±0.58	9.00 ±1.00	7.30 ±1.50	9.67 ±0.58	12.30 ±1.15	14.00 ±1.00	4.30 ±0.58	5.00 ±1.00	6.30 ±0.58	8.00 ±1.00	7.76 ±0.58	9.00 ±1.00	10.30 ±0.58	11.67 ±0.58	0.00 ±0.00	14.00 ±1.00	19.67 ±1.50
EC	3.67 ±0.58	5.00 ±1.00	6.00 ±1.00	7.30 ±0.58	**8.00 ±1.00**	8.30 ±0.58	9.00 ±1.00	10.00 ±1.00	6.30 ±0.58	8.00 ±1.00	10.30 ±0.58	11.30 ±0.58	**2.30 ±0.58**	**3.67 ±0.58**	**5.67 ±0.58**	**7.68 ±0.58**	**8.30 ±0.58**	**11.00 ±1.00**	**12.30 ±0.58**	**14.00 ±1.00**	0.00 ±0.00	20.30 ±0.58	26.67 ±1.50
ST	2.30 ±0.58	4.00 ±1.00	5.00 ±1.00	6.67 ±1.5	6.00 ±1.00	8.67 ±0.58	9.30 ±1.50	10.00 ±1.00	6.00 ±1.00	8.30 ±0.58	9.67 ±0.58	13.67 ±0.58	**8.00 ±1.00**	**10.00 ±1.00**	**11.67 ±0.58**	**14.30 ±0.58**	**2.30 ±0.58**	**3.67 ±0.58**	6.67 ±0.58	8.67 ±0.58	0.00 ±0.00	18.30 ±0.58	25.30 ±0.58
PA	**5.67 ±0.58**	**6.67 ±0.58**	**8.00 ±1.00**	**10.00 ±1.00**	7.67 ±0.58	**9.00 ±1.00**	**10.67 ±0.58**	**12.30 ±0.58**	**10.00 ±1.00**	**13.00 ±1.00**	**15.30 ±0.58**	**16.67 ±0.58**	**2.30 ±0.58**	5.30 ±0.58	8.00 ±1.00	10.30 ±0.58	**2.30 ±0.58**	4.00 ±1,00	**5.30 ±0.58**	**6.30 ±0.58**	0.00 ±0.00	29.00 ±1.00	36.00 ±1.00
EA	2.30 ±0.58	4.67 ±0.58	5.67 ±0.58	6.00 ±1.00	6.67 ±0.58	8.00 ±1.70	10.30 ±0.58	10.67 ±0.58	4.30 ±0.58	**5.30 ±0.58**	**7.00 ±1.00**	**8.30 ±0.58**	6.00 ±1.00	7.67 ±0.58	9.30 ±0.58	10.00 ±1.00	3.67 ±0.58	7.68 ±0.58	10.00 ±1.00	12.00 ±1.00	0.00 ±0.00	16.30 ±0.58	18.30 ±0.58

*Abbreviation: TO- Test Organisms; SA - **S. aureus; BC - B. cereus; EC - E. coli; ST - S. typhimurium; PA - P. aeruginosa; EA - E. aerogens;** A= 6.25mg/ml, B= 12.5mg/ml, C= 25mg/ml, D= 50mg/ml, E= control (7.8% DMSO), F= Streptomycine (20µg/ml), G= Ciprofloxacine (20µg/ml), (0) = Denotes no ZOI. * = Value are mean of three replication ± Standard deviatin (SD).*

Table:- 9. Screening of antibacterial activities of different extract of *S. nigrum* Stems

TO	Diameter of zone of inhibition (mm)*.																						
	Hexane extract				Chloroform extract				Acetone extract				Ethanol extract				Water extract				DMSO and Antibiotics		
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	E	F	G
SA	3.67 ±0.58	6.00 ±1.00	7.67 ±0.58	9.30 ±0.58	**0.00 ±0.00**	4.00 ±1.00	**4.67 ±0.58**	6.00 ±1.00	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	**0.00 ±0.00**	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	10.30 ±0.58	24.30 ±0.58
BC	4.30 ±0.58	5.67 ±0.58	8.00 ±1.00	12.00 ±1.00	3.67 ±0.58	6.30 ±0.58	7.67 ±0.58	**10.30 ±0.58**	2.67 ±0.58	4.30 ±0.58	5.00 ±1.00	6.30 ±0.58	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	14.00 ±1.00	19.67 ±1.50
EC	**2.67 ±0.58**	5.00 ±1.00	7.30 ±0.58	9.67 ±0.58	3.30 ±0.58	4.30 ±0.58	6.30 ±0.58	7.00 ±1.00	2.30 ±0.58	4.00 ±1.00	6.30 ±0.58	8.00 ±1.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	20.30 ±0.58	26.67 ±1.50
ST	**2.67 ±0.58**	**3.67 ±0.58**	**6.00 ±1.00**	**7.67 ±0.58**	5.00 ±1.00	6.30 ±0.58	8.30 ±0.58	**10.30 ±0.58**	3.67 ±0.58	5.67 ±0.58	**8.00 ±1.00**	**10.30 ±0.58**	**4.00 ±1.00**	**5.67 ±0.58**	**8.00 ±1.00**	**10.00 ±1.00**	**3.67 ±0.58**	**5.67 ±0.58**	**7.30 ±0.58**	**7.67 ±0.58**	0.00 ±0.00	18.30 ±0.58	25.30 ±0.58
PA	**7.67 ±0.58**	**10.00 ±1.00**	**11.67 ±0.58**	**14.30 ±1.52**	**7.00 ±1.00**	**8.00 ±1.00**	**9.00 ±1.00**	9.30 ±0.58	**6.00 ±1.00**	**7.30 ±0.58**	7.76 ±0.58	9.00 ±1.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	2.30 ±0.58	4.00 ±0.58	6.00 ±1.00	0.00 ±0.00	29.00 ±1.00	36.00 ±1.00
EA	4.00 ±1.00	6.00 ±1.00	8.30 ±0.58	9.67 ±0.58	3.30 ±0.58	**3.67 ±0.58**	5.30 ±0.58	**5.67 ±0.58**	4.30 ±0.58	5.67 ±0.58	7.67 ±0.58	9.30 ±0.58	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	16.30 ±0.58	18.30 ±0.58

*Abbreviation: TO- Test Organisms; SA - **S. aureus; BC - B. cereus; EC - E. coli; ST - S. typhimurium; PA - P. aeruginosa; EA - E. aerogens;** A= 6.25mg/ml, B= 12.5mg/ml, C= 25mg/ml, D= 50mg/ml, E= control (7.8% DMSO), F= Streptomycine (20µg/ml), G= Ciprofloxacine (20µg/ml), (**0**) = Denotes no ZOI. * = Value are mean of three replication ± Standard deviatin (SD).*

Antimicrobial potential of extracts of *S. nigrum* fruits:
Among the different types of extracts of *S. nigrum* fruits, acetone extract at 50 mg/ml concentration showed maximum (**16.67±0.58mm**) zone of inhibition against *P. aeruginosa*. There is no zone of inhibition observed in negative control (DMSO having no extract) in all cases. The activities of two antibiotics(Streptomycine and Ciprofloxacin) were also observed against *E. coli*, *S. typhimurium* and *P. aeruginosa*(Fig. – 02b and Table- 08).

Antimicrobial potential of extracts of *S. nigrum* stems:
Among the different types of extracts of *S. nigrum* stems, acetone hexane at 50 mg/ml concentration showed maximum (**14.30±1.52mm**) zone of inhibition against *P. aeruginosa*. There is no zone of inhibition observed in negative control (DMSO having no extract) in all cases. The activities of two antibiotics(Streptomycine and Ciprofloxacin) were also observed against *E. coli*, *S. typhimurium* and *P. aeruginosa*(Fig. – 02c and Table- 09).

Statistical Analysis:
The mean zone of inhibition caused by the solvent extract of *S. nigrum* leaf, fruit and stem and standard dug (Streptomycine and Ciprofloxacine) were calculated and reliability of the samples was assessed by calculating standard deviation (SD).

DISCUSSION

The Plant belonging to the genus *Solanum* has been reported to have remarkable pharmacological activity. The leaf, fruit and stem extract of *S. nigrum* have various biologically active compounds, which could serve as potential source of drugs in herbal medicine [4]. Our results of the qualitative phytochemical test of all the extract (water, ethanol, acetone, chloroform and hexane) of leaf, fruit and stem of *S. nigrum* confirmed the presence of Tannins, Alkaloids, Teroenoids, Steroids, Flavonoides Steroids and Glycosides, etc. which is similar to the finding of Indhumathi and Mohandav[9], Jasim*et al.*,[10]. Modilal *et al.*,[12]also reported the similar results.

The antibacterial activities of *S. nigrum* leaves, fruits and stems extract have studies against six pathogenic bacteria. The result obtained from our experiments indicated that various extract (water, ethanol, acetone, chloroform and hexane) of *S. nigrum* leaf, fruit and stems significantly inhibited the growth of all six selected pathogenic bacteria in different way. Among different extract of *S. nigrum* leaves water extract showed best inhibition zone (21.30±1.50mm, at 50mg/ml) against *P. aeruginosa* followed by ethanolic extract against same bacteria. While ethanolic extract of leaves showed good zone of inhibition (18.30±0.58mm at 50mg/ml) against *E. coli* followed by water extract against same bacteria. Our finding are very similar to Alo*et al.*,[2].

The acetone extract of *S. nigrum* fruits inhibited the growth of *P. aeruginosa* with the inhibition zone diameter of 16.67±0.58mm, at 50mg/ml, which is best among all extract of fruits against different bacteria followed by water extract against *E. coli* (14.00±1.00mm, at 50mg/ml) and ethanolic extract against *S. typhimurium* (14.30±0.58mm, 50mg/ml). Similarly Kaushik *et al.,* [11] reported the results of ethanolic extract of *S. nigrum* and was found to inhibit the Gram positive bacteria at all the tested concentrations (100mg, 75mg, 50mg and 25mg) whereas Gram negative bacteria did not showed any activity at a concentration of 25mg/ml. The extract showed good activity against the tested microorganisms except for *P. aeruginosa*, which showed low activity as compare to other tested microorganisms. The extract was found to be most effective against *S. aureus* showing the maximum zone of inhibition (25.6mm) followed by *B. sublitis* (24.3mm) whereas incase of Gram negative bacteria, ethanolic extract was found to be most effective against *E. coli* showing the maximum zone of inhibition (18.6mm) followed by *P. aeruginosa* (13.6mm) [11].

The hexane extract of *S. nigrum* stem showed better growth inhibition (14.30±1.52mm at 50mg/ml) against *P. aeruginosa* and *B. cereus* (12.00±1.00mm at 50mg/ml) followed by acetone extract of *S. nigrum* stem against *S. typhimurium* (10.30±0.58mm at 50mg/ml). Similarly, Yogananth*et al.*,[18] reported the antibacterial activity of hexane extract of stem and leaf, which inhibit the growth of *Staphylococcus flexneri, S. aureus, K.pneumoniae* and *P. aeruginosa.* Among these the stem extracts showed the maximum antibacterial activity against *E.coli* (5.36 mm), followed by *P. mirabilis*(3.46 mm) than leaf extract.

The ethanolic extract of *S.nigrum* stem failed to inhibit all the tested bacteria accept *S. typhimurium*, while water extract inhibited only *S. typhimurium* and *P.aeruginosa*. Hence, the difference in antimicrobial properties of a plant extract might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature,

light water), contamination by field microbes, adulteration and substitution of plants, incorrect preparation and dosage. In the same vein reported that inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials. Subsequently, Chukwuka *et al.*[3] also noted that medicinal plants with no antibacterial activity may be due to astringent properties possessed by extract of the plants. Alo, *et al.*[2] also reported the antimicrobial activity of the crude extract of leaves, fruits and stems of *S. nigrum* against different bacterial strains.

The clear zone of the growth inhibition was noted around the well due to diffusion of various compounds. The diameter of zone denotes the relative susceptibility of the test microorganisms to a particular antimicrobial compound. The results obtained of the crude extract were compared with the standard antibiotic (Streptomycin and Ciprofloxacin). All the tested organisms are sensitive to different plant extract and standard antibiotics also. Since the extracts are crude, the active principle present in these extract, may be would be in low concentration, hence, we can say that active compound purified from crude extract would exhibit better result than standard antibiotics.

The present investigation ensure that crude extracts of selected plants contain antimicrobial properties, which are used in different parts of country and other country as valuable traditional meditational medicine. However further studies are needed, including toxicity evaluation and purification of active antimicrobial compounds from *S. nigrum* extracts.

CONCLUSION

Many medicinal plants have been found effective to cure the several bacterial diseases. Due to increasing resistance to antibiotic by microorganisms and side effects of synthetic antibiotics, medicinal plants are now gaining popularity in the treatment of bacterial infections. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics. Extensive research in the area of isolation and characterization of the active principles of these plants is required so that better, safer and cost effective drugs for treating bacterial infections can be developed.

REFERENCES

[1] Abbas K, Niaz U, Hussain T, Saeed M A, Javaid Z, Idress A,Rasool S. *J.Acta.Poloniae. Pharm. Drug Res.***2014**, 71 (3), 415-421.
[2]AlO M, Hassan W H B., Ahmod MS, Al-R A J.*Sandi Pharm. J.***2012,** 20, 371-379.
[3]Chukwuka KS, Ikheloa JO, Okonko IO, Moody J.O., Mankinde, T.A.*Adv. Appl. Sci. Res.***2011,**2(4), 37-48.
[4]Chukwuma E R, Obioma N, Christopher O L.*Pak. J. Nutrition***2010**,9 (7), 709-715.
[5]Desai S, Tatke P,Gabhe S Y.*Int. J.Pharm. Sci. Res.***2015**,6 (2), 676-681.
[6]EvansWC, MajorVA, ThanMP.*PlantaMedica***1965,**13, 353-358.
[7]Harborne J B.Londan. *Champan Hall Ltd.*, 2nd edition. **1973,**49-188.
[8]Harborne J B,*Champan Hall, Londan. New York,* 2nd edition**1984,** 24-25.
[9]Indhumathi T,Mohandass S.*Int.J.Curr.Microbiol.App.Sci.***2014,** 3(6), 939-949.
[10] Jasim H, Hussein A O, Hameed I H.*J.PharmacognosyPhytother.***2015,**7(4), 56-72.
[11] Kaushik D, Jogpal V, Kaushik P.*J.Archives Appl. Sci. Res.***2009,**1(1), 43-50.
[12] Modilal M R D.*Int. J. Pure Appl. Zool.***2015,**3 (2), 210-215.
[13] OguyemiAO,Sofowora A.*Ife-Ife University Ife***1979,**20-22.
[14] Prasad C, Kumar V, Srivastava SK, *Int. J.Curr.Trends Pharma. Res.***2015,**3(3), 878–884.
[15] Sofowora A.*J. Willey Sons Ltd Ibadan***1982,**2, 8-14.
[16] Soxhlet F. *Dingler'sPolytechnisches J* (in German) **1879,**232, 461–465.
[17] Tona L, Kambu K, Ngimbi N. *J. Ethnopharmacol,***1998,**61, 57-65.
[18] Yogananth N, Buvaneswari S,Muthezhilan R,*Global J.Biotech.Biochem.***2012**,7 (3), 86-89.