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Effect of Metal Ions on Antimicrobial Activity of *S. Nigrum* Against Various Pathogens

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Subject: Pharmaceutics

Abstract

The present study is aimed to focus on antimicrobial activity with the effect of metal ion of plant sample *Solanum nigrum* which was collected from the Lucknow region. The plant extracts were prepared in five solvents (methanol, acetone, ethyl acetate, hot water and normal water) to check the anti-bacterial and anti-fungal activity against bacterial pathogens (*P. aeruginosa, S. aureus and E. coli*) and fungal pathogens (*M. canis, C. albicans, T. rubrum and A. niger*) by agar well diffusion method. The two metal ions (Zinc and Lead) were used at concentration of 0.1%. The leaf, stem and fruit parts of *S. nigrum* were used for antimicrobial analysis, out of which the fruit extract showed the best activity against *S. aureus* and *E. coli* with maximum zone of inhibition ranging from 12 mm to 16.5 mm. The activity of all parts of *S. nigrum* was enhanced in the presence of metal ion. The lowest concentration (highest dilution) of the extract was regarded as MIC. Among the different types of tested *S. nigrum* parts extract, the fruit extract with different solvents showed the least MIC value at concentration ranging from 2.7µg/ml to 57µg/ml. A lowest MIC value of fruit (0.14 OD at conc. of 57µg/ml) was obtained against *S. aureus*. The acetonic extract of leaves also showed the lowest MIC value (0.12 OD at conc. of 96µg/ml) against *S. aureus.* The phytochemical analysis of *S. nigrum* parts revealed the presence of secondary metabolites which were terpenoids, sterols, flavonoids, tannins, phenols in leaves and fruits and terpenoids, sterols, saponin and flavonoids in stem.

Key words: *Metal ion, Antibacterial, Antifungal, Plant extract, Secondary metabolites.*

Introduction:

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan's. Antimicrobial drugs either kill microbes (micro-biocidal) or prevent the growth of microbes (micro-biostatic). Various parts of medicinal plants like the leaves, flowers, fruits, roots and bark extract, infusion, decorations and powders have proven useful in curing a wide range of health related issues[1]. This plant synthesize a vast array of secondary metabolites, that are important for modern medicine (e.g., Taxol, an anticancer drug was first obtained from the bark of Pacific Yew tree-Taxusbrevifolia). Clinical efficacy of many synthetic antibiotics is questioned now-a-days with the emergence of multidrug pathogens. The increasing failures of chemotherapeutics and antibiotics exhibited by pathogenic microbial infection have led to screening of several medicinal plants for potential antimicrobial activity [2]. Medicinal plants possess potent medicinal value that is due to the presence of variety of phytochemical constituents in the plants tissue which cast a definite physiological action on the human body. Very few of these chemicals are toxic also [3]. Novel molecules obtained in many cases are highly active against microbes. *Solanum nigrum* is commonly known as Black Nightshade, Makoy and Deadly Nightshade. The plant has been extensively used in traditional medicine of India and other parts of the world to cure liver disorders, chronic skin ailments (Psoriasis and Ringworm),

inflammatory conditions, fever, painful periods, eye disorders etc. The plant *S. nigrum* belongs to family Solanacea (genus Solanum). The generic name of *S. nigrum* is considered to be derived from the lation "Solamen" to refer to the quieting or sedative effect associated with many species (Edmond.JM, *et al*). This family consists of 90 genera and 2000-3000 species. In this family the *S. nigrum* constitutes the largest and the most complex genus consists of more than 1500 species. *Solanum nigrum* grows as a weed all over the dry part of India. *S. nigrum* generally elaborated a wide spectrum of medicinal plants such as antiseptic, antidysentric, gastro antiulceric, anti-diabetic, anti-cancer, antioxidant, neuroprotective and antimicrobial property [4]. The present study is carried out by evaluation of antimicrobial activity of *S.nigrum* and also to check the effect of metal ion on antimicrobial activity of *S. nigrum* and their phytochemical analysis.

Methodology:

Preparation of plant extract:
Samples (*Solanum nigrum* plant) was collected from surroundings. The various parts of plant (*leaves, stem, fruits and roots*) were separated and were washed properly. After drying under sunlight, the sample was air dried in hot air oven at 40-50 ^{0}C for 2-3 days. The air dried samples were further grinded separately in mortar-pestle and filtered by the help of muslin cloth to get their fine powder. The sample's powder was individually mixed with different solvents in 1:10 ratio. After mixing, the solution of solvent and sample powder was incubated in complete dark for 1-2- days. After the completion of incubation period each solutions were filtered by the help of filter paper (Whattman paper) in a washed and air dried Petri plate. The obtained filtrate was then placed in hot air oven and incubated until they get solidified. The weight of Petri plate having the solid filtrate was then measured in order to calculate the difference. Further the DMSO (Dimethyl Sulphoxide) was added to the obtained filtrate.

Tested microorganisms:

Bacterial cultures were obtained from IMTECH, Chandigarh. Subcultures were maintained by MRD LifeSciences, Lucknow. One gram positive culture- *Staphylococcus aureus* (MTCC 2940) and two gram negative cultures- *Pseudomonas aeruginosa* (MTCC 2453) and *E. coli* (MTCC739) were used and the four fungus cultures were used: *Microsporum canis*,

Sample collection

Solanum nigrum was used as a plant for antimicrobial analysis, In this process the herbs and shrubs were searched, where wild weeds grown naturally. Finally the healthy plants were identified and collected from the backyard area of MRD LifeSciences laboratory, Vibhuti Khand, Gomti Nagar, Lucknow, Uttar Pradesh.

Solvent used

Organic solvents were used for the preparation of plant extracts. Secondary metabolites were needed from plants which were organic in nature and as "like dissolve like" organic solvents were used to dissolve secondary metabolites. During extraction solvents diffuse into the solid plant material and solubilize compounds with similar polarity [5].The following five solvents were used for plant extract preparation out of which the first three are organic solvents: Acetone, Methanol, Ethyl Acetate, Hot Water, and Normal Water.

Candida albicans , *Trichophyton rubrum* and *Aspergillus niger*.

Agar well diffusion & antibiotic optimization test:

Agar Well Diffusion is one of the methods to analyze the antimicrobial or antibiotic activity against various microorganisms. The activity is determined by measuring the diameter of zone of inhibition in mm. The culture media (Nutrient Agar, NA for bacterial & Potato Dextrose Agar, PDA for fungal culture) were prepared. The Petri plates and prepared culture media were then autoclaved. In LAF, the media were carefully poured in the Petri plates and allowed to get solidified. After the media solidified, wells were made in the plates with the help of sterile borer (5mm). The sample or extract compound were then loaded into the wells and plates were incubated at standard temperature required by culture to grow 37^{0}C for bacteria and 28^{0}C for fungus). The growth or activity were observed after the incubation and was determined by measuring the diameter of zone of inhibition in mm [6,7].

Antibiogram analysis for bacteria and fungi:

Stage 1- Agar Plate Preparation & Spreading of Cultures

The culture media (*i.e.*, Nutrient Agar for bacterial & Potato Dextrose Agar for fungal strain) were

prepared. The Petri plates and prepared culture media were then autoclaved. In LAF, the media were carefully poured in the Petri plates and allowed to get solidified. After the media solidified, 20 µl of pathogenic culture (bacterial and fungal strain) was poured over the media with help of micropipette. The poured cultures were then spread all over the media surface uniformly with the help of sterile test tube. The bottom of test tube was first dipped in ethanol and then heated under flame. Each plate was marked properly with the culture name respectively.

Stage 2- Well Preparation & Sample Loading

After spreading of cultures, wells were made in the plates with the help of sterile borer (5mm). Three wells were made in the triangle manner.

In Absence of Metal Ion-1st well was loaded with 50µl of Plant Extract (PE). 2nd well was loaded with 50µl of antibiotic (Ofloxacin). 3rd well was loaded with 50µl of distilled water.

In Presence of Metal Ion-1st well was loaded with 50µl of Plant Extract (PE). 2nd well was loaded with 45µl of PE and 5µl of metal ion (either Zn or Pb). 3rd well was loaded with 50µl of metal ion only.

Stage 3- Incubation & Determination of Zone Of Inhibition (ZOI)- After loading of sample, the plates were incubated in straight position for overnight at required temperature. For bacterial culture- the plates were incubated at 37^0 C in hot air oven. For fungal culture- the plates were incubated at 28^0 C. After the incubation period the plates were observed to determine the activity by measuring the diameter of zone of inhibition. The diameter of wells was measured horizontally and vertically and average value was noted down.

Minimum Inhibitory Concentration (MIC)

MIC is an important diagnostic laboratory test which is generally performed in microbiology. It is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. As much as the value of MIC will be low it will show that antimicrobial agent have better potential against microorganisms. It is the most basic laboratory measurement of the activity of an antimicrobial agent against microorganisms (or organisms)[8].For performing MIC of single extract, twelve washed test tubes were taken. Each test tube was filled with 3ml of culture media (*i.e.*, nutrient broth (NB) for bacteria and potato dextrose broth (PDB) for fungus culture).The test tubes were then autoclaved properly. After autoclaving, the test tubes were taken to the LAF where they were allowed to cool for few minutes. Out of the twelve test tubes, six were treated as control (*i.e.*, Blank- without any culture) and remaining six was treated with culture (pathogens) to obtain the MIC value. For each culture test tube there was a control test tube. In the 1st test tube of both the control and cultured test tube, 500µl of extract was added and mixed properly. The test tubes were then serially diluted till the 6th test tube and finally 500µl was discarded from the last one (*i.e.*, 6th test tube).After the serial dilution, except the six control test tubes the other six were inoculated with 20µl of pathogenic culture .Finally the test tubes with culture were incubated in shaker incubator for overnight and the controls were stored in refrigerator. After the incubation period, the MIC values were determined by taking the Optical Density (OD) of the samples serially (*i.e.*, from 1st to 6th test tube) at 600nm in spectrophotometer.

Phytochemical Analysis:

Phytochemical analysis is a type of chemical assay which is used to identify the presence of various phytochemicals in the plant extracts such as in leaf, stem, fruit, root, seed etc. The most of the phytochemicals are classified as secondary compounds (metabolites) of the plant, often their function is completely not known. However the most of the secondary metabolites have the defense mechanism against plant pathogens [9].

Test for Alkaloids: About 0.2 gm of plant extracts was taken in separate test tube and warmed with 2% Sulphuric acid (H_2SO_4) for 2 minutes. And it was filtered in separate test tube and few drops of Dragendrofs reagent were added and observed for the presence of orange red precipitates for the presence of alkaloids.

Test for Terpenoids and Sterols: About 0.5g of plant extract was taken in separate test tubes with 2 ml of chloroform and concentrated Sulphuric acid (H_2SO_4) was added carefully to form a layer. Observed for presence of reddish brown color interface which show positive results for the presence of terpenoids and sterols.

Test for reducing sugars: A test tube was taken and 2 ml of crude plant extract was added along with 5 ml of D/W and filtered. The filtrate was boiled with 3-4 drops of Fehling's solution A and B for 2 minutes. Observed for orange red precipitate which indicates the presence of reducing sugars.

Test for Saponins: About 0.2 gm of plant extract was taken in the test tube and 5 ml of distilled water

was added and then heat to boil. Observed for frothing (appearance of creamy mass of small bubbles), this shows the presence of Saponins.

Test for Tannins and Phenols: To small quantity of plant extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. Dark green solutions were observed which indicates the presence of tannins.

Test for Flavonoids : About 0.2 gm plant extract was taken in separate test tubes and diluted with Sodium hydroxide (NaOH) and then diluted Hydrochloride (HCl) was dissolved and observe for yellow solutions that turns colorless indicate the presence of flavonoids.

Test for Phlobatannins : About 0.5 gm of plant extract was taken in a test tube and dissolved with distill water and filtered. The filtrate was boiled with 2% Hydrochloric acid (HCl) solution. Observe for red precipitate shows the presence of Phlobatannins.

Table 1: Common properties of organic solvents used for sample extraction

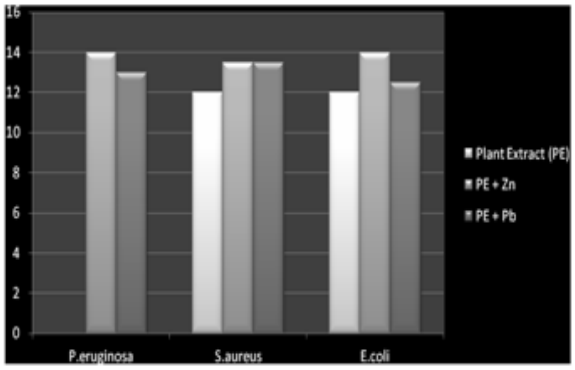
S.No.	Solvents	Formula	MW	BP(^{0}C)	MP(^{0}C)	D(g/ml)	Solubility in H_2O (g/100g)	Dielectric Constant
1.	Acetone	C_3H_6O	58.08	56.20	-94.3	0.786	Miscible	20.7(25)
2.	Methanol	C_4H_8O	88.11	77	-83.6	0.895	8.7	6 (25)
3.	Ethyl Acetate	CH_4O	32.04	64.6	-98	0.791	Miscible	32.6(25)

Results: Antibacterial Activity of Fruit Extract

Antibacterial activity of Fruit + Ethyl Acetate Extract

Table 2- Tabular representation of antibacterial activity of Ethyl acetate extract.

Sr.No	Pathogens	ZOI (in mm) of PE	ZOI (in mm) of PE+ Zn	ZOI (in mm) of PE+Pb
1.	*P. aeruginosa*	0.0	14.0	13.0
2.	*S. aureus*	12.0	13.5	13.5
3.	*E. coli*	12.0	14.0	12.5



Graph 1: Graphical representation of antibacterial activity of Ethyl acetate extract

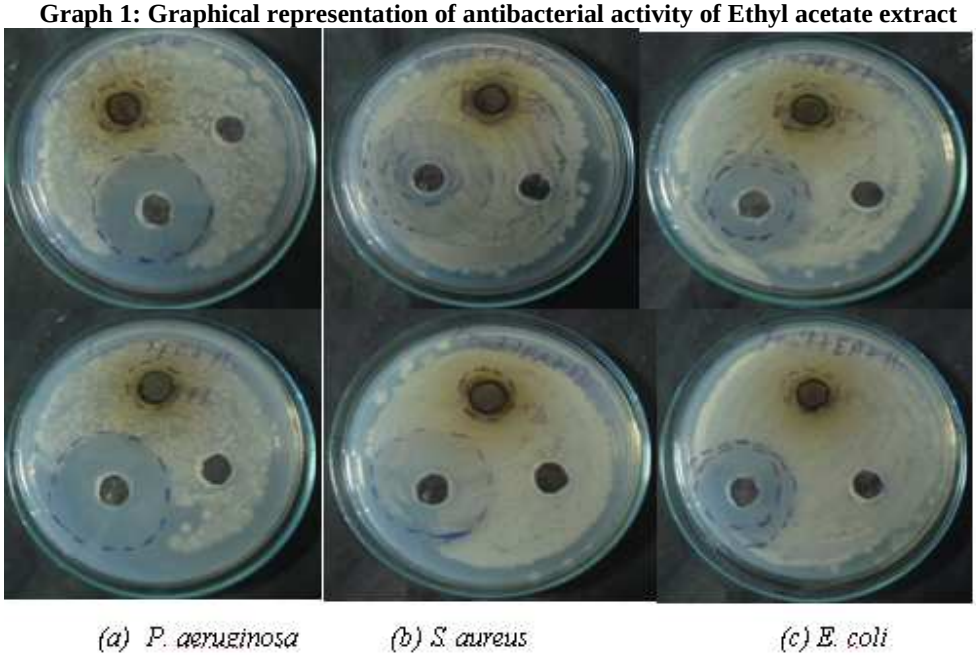
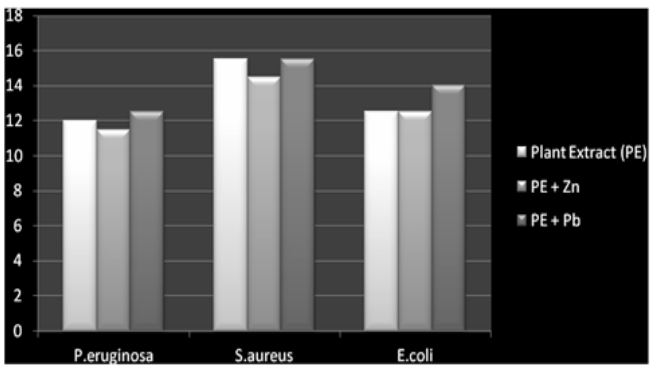


Figure 1: *Petri plate's showing the zone of inhibition (ZOI) of fruit-ethyl acetate extract against-(a) P. aeruginosa, (b) S. aureus (c) E. coli. 1st row- with zinc (Zn) & 2nd row-with lead (Pb).*

Antibacterial activity of Fruit + Methanolic Extract

Table 3: Tabular representation of antibacterial activity of methanol extract

Pathogens	ZOI (in mm) of PE	ZOI (in mm) of PE+ Zn	ZOI (in mm) of PE+Pb
P. aeruginosa	12.0	11.5	12.5
S. aureus	15.5	14.5	15.5
E. coli	12.5	12.5	14.0



Graph 2: Graphical representation of antibacterial activity of methanol extract.

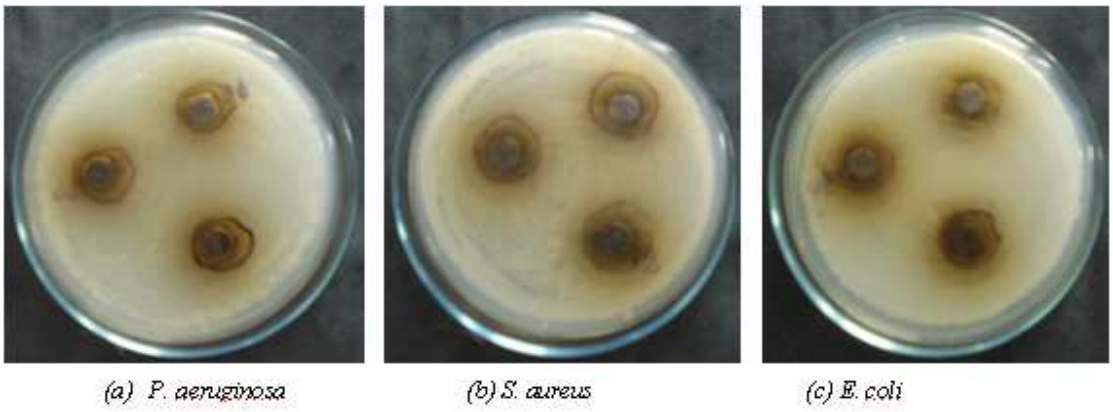
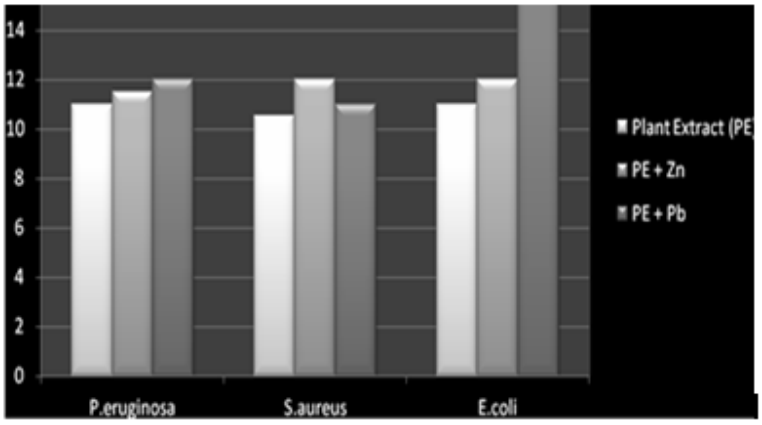


Figure 2: *Petri plate's showing the zone of inhibition (ZOI) of fruit-methanol extract against-(a) P. aeruginosa, (b) S. aureus (c) E. coli.*

Antibacterial activity of Fruit + Hot water Extract

Table 4: Tabular representation of antibacterial activity of hot water extract

Pathogens	ZOI (in mm) of PE	ZOI (in mm) of PE+ Zn	ZOI (in mm) of PE+Pb
P. aeruginosa	11.0	11.5	12.0
S. aureus	10.5	12.0	11.0
E. coli	11.0	12.0	16.5



Graph 3: Graphical representation of antibacterial activity of hot water extract

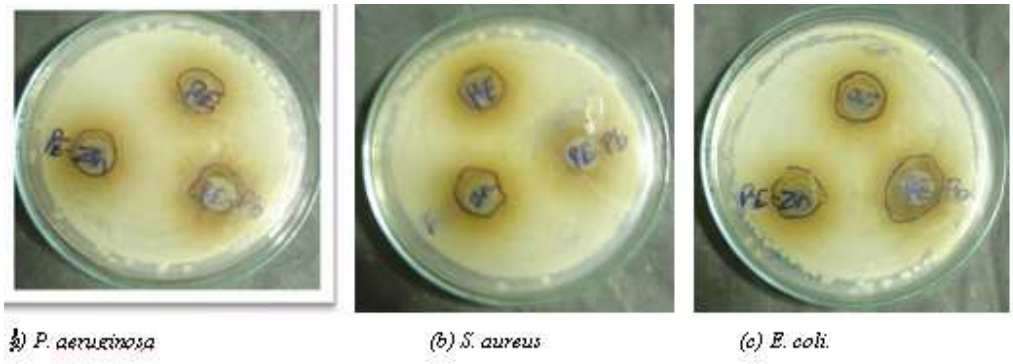
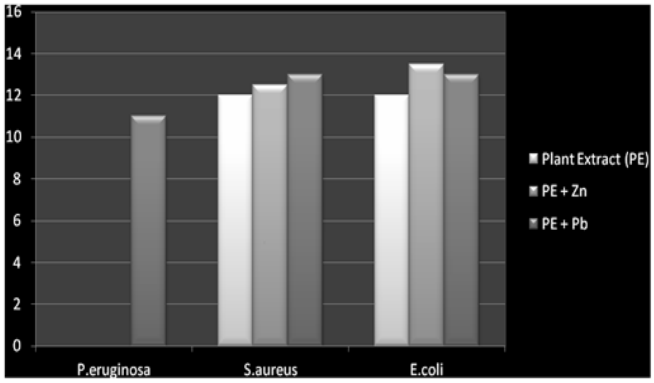


Figure 3:-*Petri plate's showing the zone of inhibition (ZOI) of fruit-hot water extract against-(a) P. aeruginosa, (b) S. aureus (c) E. coli*

Antibacterial activity of Fruit + Acetone Extract

Table 5: Tabular representation of antibacterial activity of acetone extract

Pathogens	ZOI (in mm) of PE	ZOI (in mm) of PE+ Zn	ZOI (in mm) of PE+Pb
P. aeruginosa	0.0	0.0	11.0
S. aureus	12.0	12.5	13.0
E. coli	12.0	13.5	13.0



Graph 4: Graphical representation of antibacterial activity of acetone extract.

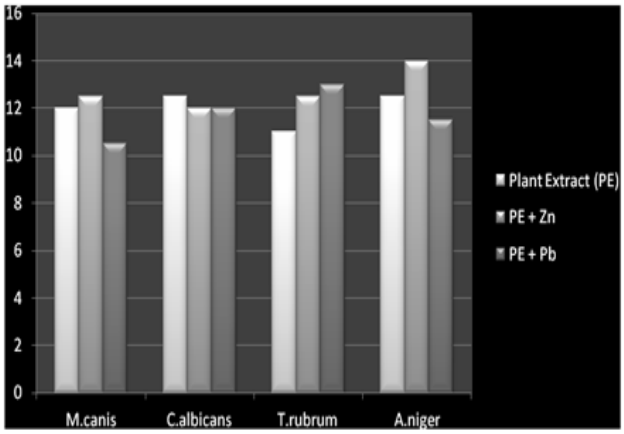
There was no antibacterial activity of stem acetone extract against any bacterial pathogens either in absence or presence of metal ion. The zone of inhibition was zero.

Antibiogram Results of *S. nigrum* Parts with Effect of Metal Ion against Fungal Pathogens:

Antifungal activity of fruits + Methanolic Extract.

Table 6: Tabular representation of antifungal activity of methanol extract

Pathogens	ZOI (in mm) of PE	ZOI (in mm) of PE+ Zn	ZOI (in mm) of PE+Pb
M. canis	12.0	12.5	10.5
C. albicans	12.5	12.0	12.0
T. rubrum	11.0	12.5	13.0
A. niger	12.5	14.0	11.5



Graph 5: Graphical representation of antifungal activity of methanol extract.

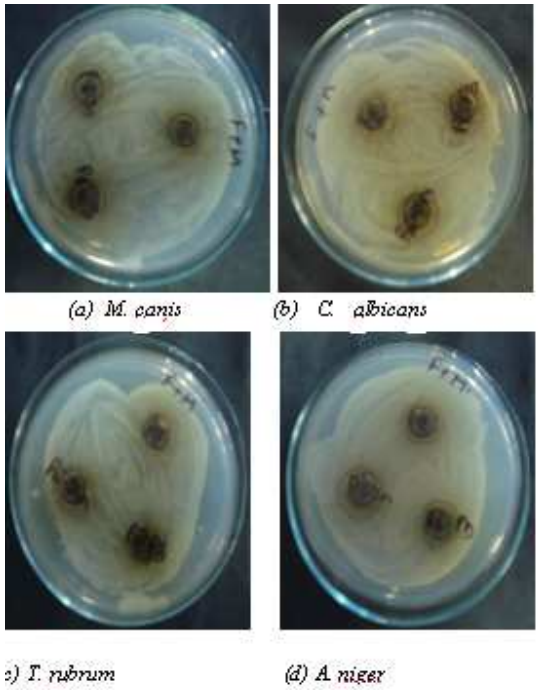


Figure 4: ***Petri plate's showing the zone of inhibition (ZOI) of fruit methanol extract against-(a) M. canis (b) C. albicans (c) T. rubrum (d) A. niger.***

Table 7: The overall results of *S. nigrum* parts extract against both bacterial and fungal strain are as follows:-

S.No	Solvents Used	Against Bacteria			Against Fungus		
		Leaf	Stem	Fruit	Leaf	Stem	Fruit
1.	Methanol	Positive	Positive	Positive	Negative	-	Positive
2.	Acetone	Positive	Positive	Positive	Negative	-	-
3.	Ethyl Acetate	Positive	Negative	Positive	Negative	-	-
4.	Hot Water	Negative	Negative	Positive	Positive	-	-
5.	Normal Water	Negative	Negative	-	Positive	-	-

Statistical Evaluation of Antimicrobial Activity

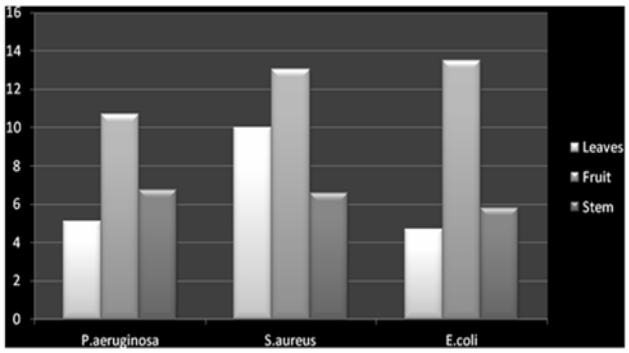
By the method of mean, the overall results for each *S. nigrum* parts (Leaf, Stem and Fruit) extracts and their comparison is done against each bacterial strain. The mean value of each part extract has been calculated against each strain which is as follows:-

S.No	Pathogens	Total Value of ZOI (in mm)	Mean (in mm)

1.	*P. aeruginosa*	14.5+13+11.5+12.5+11.5+12+0+11	85.5/8=10.69
2.	*S. aureus*	13.5+13.5+14+15+12+11+12.5+13	104.5/8=13.06
3.	*E. coli*	14+12.5+12.5+14+12+16.5+13+13.5	18/8=13.5

Graphical Representation of Overall Results of *S. nigrum* Parts:

By plotting a graph on the above obtained mean against each strain, the comparison between each part of *S. nigrum* will be clear that which has the highest antibacterial activity against the pathogenic culture.



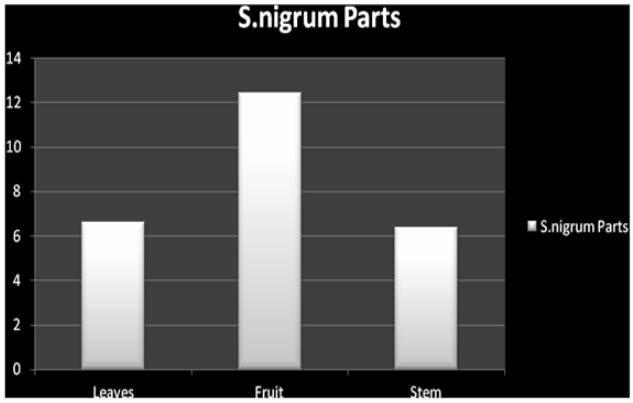
Graph 4.12:- Graph for *S. nigrum* parts against bacterial strains.

Table 4.17:- Table showing the average value (Mean) of zone of inhibition (ZOI) of each part (Leaf, Fruit and Stem) of *S. nigrum* against bacterial strain.

S.No	*S. nigrum* Parts	Average Value (Mean) of ZOI	Mean
1.	*Leaves*	5.1+10.0+4.7	19.8/3= 6.60
2.	*Fruits*	10.69+13.06+13.5	37.24/3 =12.41
3.	*Stems*	6.75+6.56+5.81	19.12/3 = 6.37

NOTE:- Average value (Mean) of Zone of Inhibition (ZOI) of each parts (Leaf, fruit and stem) to compare the activity between each other.

Graph 4.13: Graph showing the average value (Mean) of zone of inhibition (ZOI) of each part (Leaf, Fruit and Stem) of *S. nigrum* against bacterial strain.



4.1. Minimum Inhibitory Concentration (MIC)

NOTE: - The initial concentration of each plant extract was 250mg/ml and 0.5ml of plant extract was added for serial dilution.

MIC results of fruit extracts against *S. aureus*

Table 4.25:- MIC results of fruit extracts against *S. aureus*

T.T.No	Conc. (mg/ml)	Methanolic Extract OD at 600nm	Acetone Extract OD At 600nm	Ethyl Acetate Extract OD at 600nm	Hot water Extract OD at 600nm
1.	20.83	0.0	0.07	0.35	0.0
2.	3.47	0.25	0.13	0.39	0.07
3.	0.57	0.27	**0.17**	0.50	**0.14**
4.	0.96	0.33	0.34	0.43	0.69
5.	0.016	0.54	0.49	**0.30**	0.68
6.	0.0027	**0.25**	0.43	0.45	0.63

The value showing the MIC of fruit extract at different concentration (conc.). The value 0.25 of methanolic extract at conc. of 0.0027mg/ml, 0.17of acetonic extract and 0.14 of hot water extract at conc. of 0.57mg/ml and 0.30 of ethyl acetate extract at conc. of 0.016mg/ml showing the least conc. of extract at which the growth of culture was inhibited and called as MIC value.

4.1.1. Phytochemical Analysis of Fruit

Table 4.28:- Phytochemical Analysis of Fruits

Sr.No.	Phytochemicals	Colour Indication	Result (+ve or - ve)
1.	Terpenoids& Sterols	Red Brown ring	Positive
2.	Phlobatannins	-	Negative
3.	Saponins	-	Negative
4.	Flavonoids	Yellow to Colorless	Positive
5.	Tannins	Green color	Positive
6.	Phenols	Green color	Positive
7.	Reducing Sugar	-	Negative
8.	Quinones	-	Negative

DISCUSSION

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan's. Antimicrobial agents either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Various parts of plants like the leaves, flowers, fruits, roots, stems and the bark have proven useful in curing a wide range of health related issues[10]. The present study was carried out to analyze the antibacterial and antifungal activity of *Solanum nigrum* and Mustard seeds (both yellow and black) with effect of metal ion and to identify the presence of secondary compounds by phytochemical analysis. The overall results showed that the *S. nigrum* fuits showed the best activity alone as well as with metal ion[11]. The used part of sample (*S. nigrum*) were leaves, fruits and stems with five solvents i.e., methanol, acetone, ethyl acetate, hot water and normal water out of which the first three

were organic solvents. The plant extracts with organic solvents showed better results as compared to water (either hot or normal) extracts. The phytochemical analysis of *S. nigrum* parts revealed the presence of secondary metabolites which were terpenoids, sterols, flavonoids, tannins, phenols in leaves and fruits and terpenoids, sterols, saponin and flavonoids in stem.

The maximum zone of inhibition was recorded in the case of fruit extracts against *S. aureus* and *E. coli* ranging from 12mm to 16.5 mm of zone of inhibition. The activity of *S. nigrum* parts against fungal strain was mild both alone and in presence of metal ion. So, finally it was found out that *S. nigrum* parts have significantly good antibacterial activity as compared to antifungal activity. The lowest concentration (highest dilution) of the extract was regarded as MIC [22]. Among the different types of tested extract, the fruit extract with different solvents showed the least MIC value at concentration ranging from 57µg/ml to 2.7µg/ml. A lowest MIC value of fruit (0.14 at conc. of 57 µg/ml) was obtained against *S. aureus*. The acetonic extract of leaves also showed the lowest MIC value (0.12 at conc. of 96µg/ml) against *S. aureus.*

CONCLUSION

After doing all the work it was concluded that *S. nigrum* have good anti-bacterial and mild anti-fungal properties. Fruits of *S. nigrum* was shown to have maximum anti-bacterial activity both in presence and absence of metal ion. However the antimicrobial activity was found to be enhanced due the effect of metal ions. Preliminary phytochemicals analysis revealed the presence of active secondary metabolites which needs to be analyzed for further research purpose. Molecular characterization of the genes responsible for various antimicrobial activity and by screening, identifying and purifying various active molecules the desired drug can be designed after various process of research and evaluation. One big advantage of using *S. nigrum* as medicinal plant is that it is widely available and thus possible future drug agent will be cheap. Being a herbal plants, drugs obtained will have almost no side effect. Further research is needed to identify the exact novel molecules and its mode of action.

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References:

1. Nostro.A., Germano.M.P.,Angelo V., Marino A and Canatelli M.N. Extraction Methods and Bioautography of evaluation of Medicinal Plants and Antimicrobial Activity. *Letters in Applied Microbiology*.2000,30,379-384.

2. Iwu, M.W., Duncan, A.R and Okunji, C.O. New Antimicrobials of Plant origin. In JanickJ.ed. Perspectives on New Crops and new Uses. Alexandria, VA: ASHS Press.1999,457-462.

3. Prakash.S and Jain.A.K. Antifungal Activity and Preliminary Phytochemical Studies of Leaf extract of *Solanum nigrum* Linn.*International Journal of Pharmacy and Pharmaceutical Sciences*. 2011, 3(4).

4. Jainu M, Devi CSS. Antiulcerogenic and ulcer healing effect of *Solanum nigrum* (L.) on experimental ulcer models: Possible mechanism for the Inhibition of acid formation, *Journal of laboratory and Clinical Medicine*.2005, 98,417-427.

5. Ncube NS., Afoayan AJ., Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*. 2008,7(12),1797-1806.

6. Andrews.J.M.Determination of minimum inhibitory concentration, J. Antimicrob.Chemother.2001,48 (5),16.

7. Thongson.C., Davidson.P.M., Mahakarrchanakul.W and Weiss.J. Antimicrobial activity of ultrasound-associated solvent extracted species. *Lett. Appl. Microbiol*, 2004,39: 401-406.

8. Bauer.AW., Kirby .WM., Sheris JC., Turck M. Antibiotic susceptibility testing by a standardized single disc method,*Am J ClinPathol*, 1966,45,149-158.

9. Thenmozhi.M., Sivaraj R., R.H.Y. A comparative Phytochemical analysis of *Alstoniascholaris,lawsonia inermis,Ervatamiadivaricata* and *Asparagus racemosus*.IJPRD. 2010,2(9):86-91.

10. Kaushik. D., Jogpal.V., Kaushik.P, Lal.S., Saneja., Sharma.C and Aneja.K.R. Evaluation of activities of *Solanum nigrum* Fruit Extract. *Archives of Applied Science Research* 2009, 1(1),43-50.

11. Zubair.M., Rizwan.K., Rasool.N., Afshan.N.,, Shahid.M and Ahmed uddin.V. Antimicrobial potential of various extract and fraction of leaves of *Solanum nigrum. International Journal of Phytomedicine*.2011,3,63-67.