

Research Article

Effect of Metal Ions on Antibacterial Activity of *Aloe Barbadensis Mill.* & *Coriandrum Sativum* Against Various Pathogens

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Abstract: The present study is carried out by evaluation of antibacterial activity of Aloe vera and Coriander plant samples against 3 bacterial pathogens (*E.coli*, *P.aeruginosa* and *S.aureus*). In this work the activity was checked in presence and absence of metal. The Zn metal was used for this work after optimization of some metals. The five solvents were used for plant extraction - Methanol, Ethyl Acetate, Chloroform, Ethanol and Hot Water. According to result basis the best results were obtained in the case of Aloe vera and Coriander for methanolic extract in the presence of Zn which was done by agar well diffusion method. MIC was done by broth dilution method. The MIC value obtained 0.06 mg/ml for methanolic aloe vera against *S.aureus* and 0.02mg/ml for coriander methanolic extract against *S.aureus*. Also the phytochemical analysis showed presence of secondary metabolites in both samples which were responsible for antibacterial activity.

Keywords: agar well diffusion method, broth dilution method and secondary metabolites

INTRODUCTION

An antimicrobial is a substance that kills or inhibit the growth of microorganism such as bacteria, fungi, protozoan etc. Antimicrobial drugs either kill microbes (micro-biocidal) or prevent the growth of microbes (micro-biostatic). Various parts of this plant were useful in curing a wide range of health related issue. This plant synthesizes a vast array of secondary metabolites that are important for medicines. Clinical efficacy of many synthetic antibiotics is questioned now days with the emergence of multidrug resistance pathogens. The increasing failures of chemotherapeutics and antibiotics exhibited by pathogenic microbial infection have led to the screening of several medicinal plant for potent microbial activity. Aloe vera is one of the medicinal plant looking like a cactus with green, dagger shaped leaves that are fleshy, tapering, spiny, margined & filled with a clear viscous gel [1]. The species in Aloe vera L. have been used as ethnic medicine in many different countries for centuries possessing functions such as, anti-cancer, anti-inflammatory, anti-virus, antibacterial, antihelminthic, antifungal, aphirodisiac, purgative, antiseptic & cosmetic evacuating protecting liver & increasing immunity [2]. The Aloe leaf contain over 75 nutrients, 200 active compounds including 20 minerals, 18 amino acid & 12 vitamins, controls the ageing process of skin. The washing of eyes with aloe vera protects eyes from UV rays coming from sun. Now days it is commonly used in medicines, in juices, drinks & cosmetics products.

The leaves of Coriander were also used in antimicrobial analysis. Food preservative is an old topic & people have been using high salted, high molasses system acid, alcohol, smoking, under water,

underground storage & so on to extend food shelf life. With the industrial development chemical preservative are widely used in the food processing industry. However, with the development of food industry as well as great attention of peoples to food safety & have higher demand to food preservative method & try to food more secure & efficient preservatives. Coriander has been widely studied & reported about its fruit its fruit in domestic & foreign. There essential oil is mainly extracted from its fruits & there are relatively few research report about physiological functions of stems & leaves including antiseptic efficiency. Coriander can be used as a condiment at the same time it has preservative function. It has a good prospect of application as a natural food preservative [3,4].

The main objective of this study was to check the antimicrobial activity of *Aloe barbadensis mill.* & *Coriandrum sativum*. The further work was proceeding with antibiogram analysis of aloe vera & coriander with effect of metal ion and also perform Minimum inhibitory concentration to know the least concentration and phytochemical analysis to check the presence of secondary metabolites.

MATERIALS AND METHODS

Sample Collection

The sample was collected from the Gomtinagar, Lucknow, where the healthy plant of aloe vera was grown. From there the healthy leaves of aloe vera were collected. The second sample coriander was collected from the vegetable shop.

Solvents Used

Organic solvents were used for the preparation of plant extracts. Organic solvents have a common

structure (at least 1 carbon & 1 hydrogen atom), low molecular weight, lipophilicity and volatility and they exist in liquid form at room temperature.

Secondary metabolites were needed from plants which are organic in nature and organic solvents were

used to dissolve secondary metabolites. During extraction solvents diffuse in to the solid plant material and solubilise compound with similar polarity. The following five solvents were used for plant extraction out of which first three are organic solvents-Methanol, Ethyl Acetate, Chloroform, Ethanol and Hot Water [5].

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

Sl. No.	Solvents	Formula	MW	BP(°C)	MP(°C)
1.	Chloroform	CHCl ₃	119.38	61.15	-63.55
2.	Ethyl acetate	CH ₄ O	32.04	64.6	-98
3.	Methanol	C ₄ H ₈ O	88.71	77	-83.6

Preparation of Plant Extract

- Samples (aloevera & coriander leaves) were collected from surroundings.
- The leaves of plants were separated and washed properly.
- The samples were placed in sunlight.
- After complete dry the sample was grinded in mortar-pestle to get their powder form.
- The sample's powder was individually mixed with different solvents in 1:10 ratio.
Note-Ratio will be 1:10.
- After mixing, the solution was place in dark for 48hrs.
- After completion of 48hrs the sample was filtered by the help of filter paper in a washed and air dried petri plate.

Note-The weight of blank petri plate was taken in order to calculate the difference after collecting the filtrate.

- The obtained filtrate was then placed in to hot air oven and incubated for 24hrs for complete dry.
- The weight of petri plate having the solid filtrate was then measured in order to calculate the difference.

After that the DMSO (Dimethyl sulphoxide) was added to the obtained filtrate. Note-Adding double the amount of DMSO to filtrate will give the conc .of 500mg/ml and so on. Finally the solution obtained was the plant extract [6].

Pathogens Used

The antibiogram analysis of aloevera and coriander were performed against three bacterial pathogens& four fungal pathogens which are listed below-

- Pseudomonas aeruginosa*
- Staphylococcus aureus*
- Escherichia coli*

Agar Well Diffusion & Antibiotic Optimization Test

Agar well diffusion is one of the method to analyze the antimicrobial or antibiotic activity against various microorganisms. The activity is determined by measuring the diameter of zone of inhibition in mm [7].

Procedure

- The culture media (i.e Nutrient Agar For bacteria & potato dextrose broth for fungus) were prepared.
- The petri plates and culture media were then autoclaved.
- After autoclaving, they were place in LAF.
- In LAF, the media were carefully poured in to petriplates and allowed to get solidified.
- After the media solidified, wells are made in the petri plates with the help of well puncture machine.
- The sample or extract were then loaded in to the wells and the plates were incubated for overnight at 37°C Temp.
- The growths were then observed next day and the diameter of the zone of inhibition was measured in mm.

Antibiotic optimization test-As its name suggest, it is the optimization of different antibiotic against pathogens by agar well diffusion method to obtain the best antibiotic among the tested antibiotics.

Procedure

- Culture media were prepared and autoclaved.
- The culture media were then poured in petriplates in LAF.
- After the media get solidified, the pathogenic culture was spreaded (20µl) over the media and plates were marked with culture name.
- After spreading wells are made in triangle manner.
- After making wells the antibiotics (Ampilox, ofloxacin & novamox) were loaded (50µl) in to the wells.
- The petri plates were then incubated for overnight in incubator.
- The zone of inhibition were observed and measured to determine the maximum activity of antibiotic against pathogens.
- The antibiotic with maximum activity against all tested pathogen was considered as good antibiotic and was opted for further work.

Effect of metal ions on antibiogram analysis

Stage 1: Agar plate preparation & spreading of cultures

- The culture media (Nutrient agar) were prepared.
- The petri plates and prepared culture media were then autoclaved.
- After autoclaving they were placed in LAF (laminar air flow).
- In LAF, the media were carefully poured in the petri plate and allowed to get solidified.
- After the media solidified, 20µl of pathogenic culture was poured over the media with the help of micropipette.
- The poured cultures were then spread all over the media surface uniformly with the help of sterile test tube bottom.
- 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 well puncture machine. Three wells were made in the triangle manner.

Note: The sample loading was done in two way-one the absence of metal ion and second in the presence of metal ion.

In Absence of metal ion

- 1st well loaded with 50µl of plant extract (PE).
- 2nd well loaded with 50µl of antibiotic (ofloxacin).
- 3rd well loaded with 50µl of distilled water(D/W).

In presence of metal ion

- 1st well loaded with 50µl of plant extract(PE).
- 2nd well loaded with 25µl of plant extract and 25µl of metal ion (zinc).
- 3rd well 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 37°C for bacteria & 25°C for fungus.
- After incubating for overnight the plates are observed for measuring the zone of inhibition.
- The diameter was measuring vertically & horizontally and the average value was calculated.

Minimum Inhibitory Concentration (MIC)

MIC means the minimum inhibitory concentration. It is the lowest concentration of an antibiotic that will inhibit the growth of microorganism after overnight incubation. As much the value of MIC will low it will

show that antimicrobial agent have better potential against microorganism [8, 15, 16].

Procedure

- For performing MIC of single extract, twelve test tubes were taken.
- Each test tube was filled with 3ml of culture media (nutrient broth).
- The test tubes were then autoclaved.
- After autoclaving the test tube were placed in LAF for cooling.
- Out of the twelve test tubes, six were blank i.e without culture & other six containing the culture & pathogen.
- In the 1st test tube of both control & culture the 500µl of extract was added & mixed properly.
- The test tubes were then serially diluted till the 6th test tube & finally 500µl was discarded from the last one.
- 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.
- After the incubation period the MIC values was determined by taking the optical density of the sample serially 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 extract. The most of the phytochemicals are classified as secondary metabolites of the plant. The secondary metabolites are responsible for the antimicrobial activity of the plant [9, 17].

Test for deoxy sugars

A volume of the 5ml of the plant extract was treated with 2ml of glacial acetic acid containing a drop of ferric chloride solution. Then it was underplayed with 1ml conc. sulphuric acid. A brown ring of the interface indicates the presence of deoxy sugar characteristics of cardio glycosides.

Test for reducing sugars

The extract were treated with 5ml of fehling solution & kept in boiling water bath. The formation of yellow or red colour precipitate indicate the presence of reducing sugars.

Test for saponin

2gm of powdered sample was boiled in 20ml distilled water bath & filtered. The 10ml of filtrate was mixed with 5ml distilled water & shaken vigorously, a suitable persistent froth formed. The frothing was mixed with 3 drops of olive oil & shake it. The formation of emulsion was then observed.

Test for oil & fats

Press a small quantity of powder between 2 filter papers. Oil stain on the filter indicate the presence of fixed oil.

Test for terpenoids

A volume of 5ml of plant extract was mixed in 2ml chloroform & conc. Sulphuric acid was added to form a layer. A reddish brown colour of the interface was formed to show the presence of terpenoides.

Test for tannins

About 0.5gm of the dried sample was boiled in 20ml of water in a test tube & then filtered. A few drops of ferric chloride was added & observed for brownish green or a blue black coloration.

Test for phenols

A few drops of alcohol & ferric chloride solution were mixed with the plant extract. A blue green or red colour indicates the presence of phenols.

Test for amino acid & proteins

Take 1ml extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added & heated. Blue colour indicates the presence of proteins.

Test for quinines

A few drops of sodium hydroxide mixed with plant extract & shaken vigorously. A blue green or red colour indicates the presence of quinones.

RESULTS

Antibiotic optimization test result

Table 1: The tabular representation for antibiotic optimization test showing the zone of inhibition against bacterial strain

Sl. No.	Pathogens	ZOI in mm of ofloxacin	ZOI in mm of tetracycline	ZOI in mm of amikacin
1	<i>E.coli</i>	16.0	-	13.0
2	<i>P.aeruginosa</i>	18.0	-	12.5
3	<i>S.aureus</i>	20.0	-	11.5

Out of the three antibiotics (Amikacin,tetracycline&ofloxacin) used for antibiotic optimization test, ofloxacin had the highest activity against the pathogens. So in further work ofloxacin was used.

Antibiogram results of aloe vera leaves with effect of metal ion against bacterial pathogens:

Table 2: Antibacterial activity of Methanolic extract of leaves

Sl. No.	Pathogens	ZOI(mm) of ofloxacin	ZOI(mm)of Zn	ZOI(mm) of Zn+PE
1.	<i>E.coli</i>	22.5	30.5	10
2.	<i>P.aeruginosa</i>	20.0	28	21
3.	<i>S.aureus</i>	23.0	21	34

Table show the ZOI of aloe vera with methanol extract in the presence of metal ion, the best result was obtained in the presence of Zn against *S.aureus*.

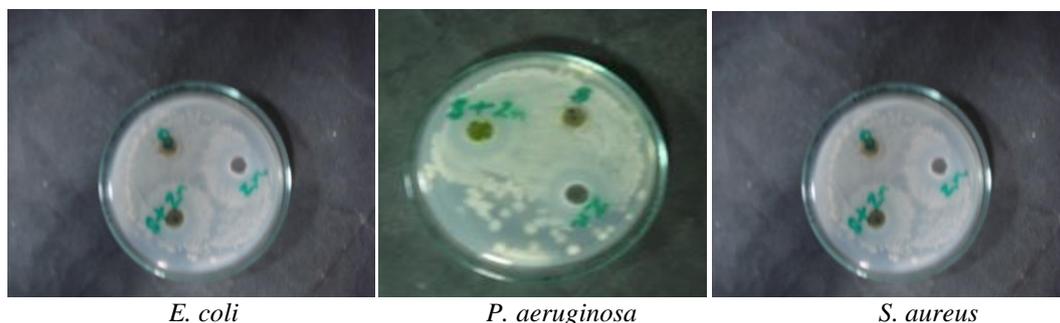


Fig. 1: Antibacterial activity of Methanolic extract of leaves of aloe vera with metal ions

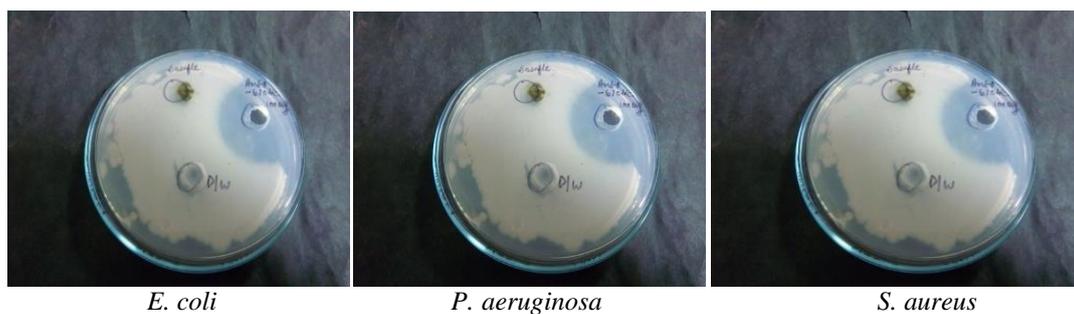


Fig. 2: Antibacterial activity of Methanolic extract of leaves of aloe vera without metal ions

Fig 1& 2 showed that the best antibacterial activity was obtained in the presence of Zn against *S.aureus*.

Table 3: Antibacterial activity of leaves+Ethanol extract

S.No	Pathogens	ZOI(mm)of ofloxacin	ZOI(mm) of Zn	ZOI(mm)of PE+Zn
1.	<i>E.coli</i>	22.0	-	24
2.	<i>P.aeruginosa</i>	23.0	30	27
3.	<i>S.aureus</i>	21.5	22	24

Table show the ZOI of aloe vera ethanol extract in the presence of metal ion was best for *P.aeruginosa*

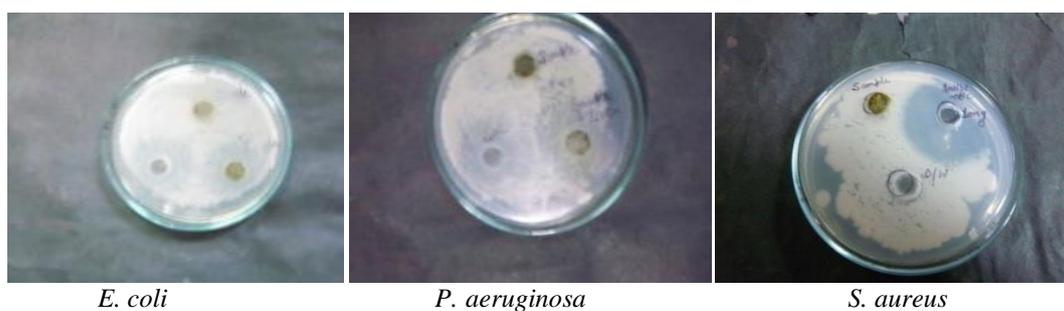


Fig. 3: Antibacterial activity of ethanolic extract of leaves of aloe vera with metal ions

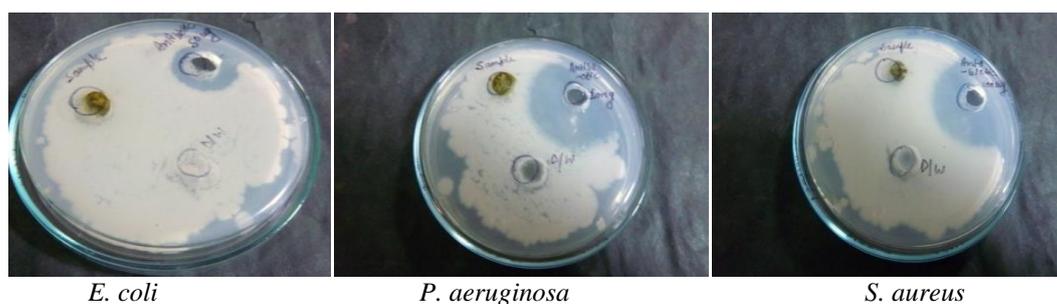


Fig. 4: Antibacterial activity of ethanolic extract of leaves of aloe vera without metal ions

Table 4: Antibacterial activity of leaves +Ethyl acetate extract

Sl. No.	Pathogens	ZOI (mm) of ofloxacin	ZOI (mm)of Zn	ZOI (mm) of Zn+PE
1.	<i>E.coli</i>	20.5	30	30
2.	<i>P.aeruginosa</i>	19.0	46	33
3.	<i>S.aureus</i>	25.0	31	29

Table show the best result in the form of ZOI of aloe vera ethyl acetate extract in the presence of metal ion.



Fig. 5: Antibacterial activity of ethyl acetate extract of leaves of aloe vera with metal ions

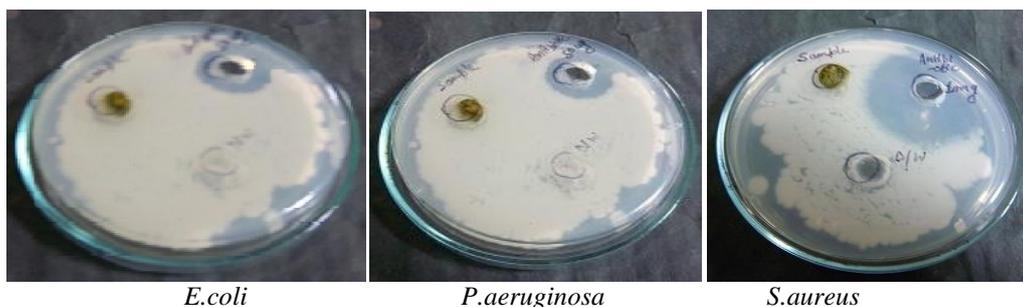


Fig. 6: Antibacterial activity of ethyl acetate extract of leaves of aloe vera without metal ions

Table 5: Antibacterial activity of leaf+Chloroform

Sl. No.	Pathogens	ZOI (mm) of ofloxacin	ZOI (mm) of Zn	ZOI (mm) of Zn+PE
1.	<i>E.coli</i>	23.0	21	20
2.	<i>P.aeruginosa</i>	21.0(partial)	10	10.5
3.	<i>S.aureus</i>	26.0	24	21

Table show the ZOI of aloe vera chloroform extract in the presence of metal ion was best against *S.aureus*.

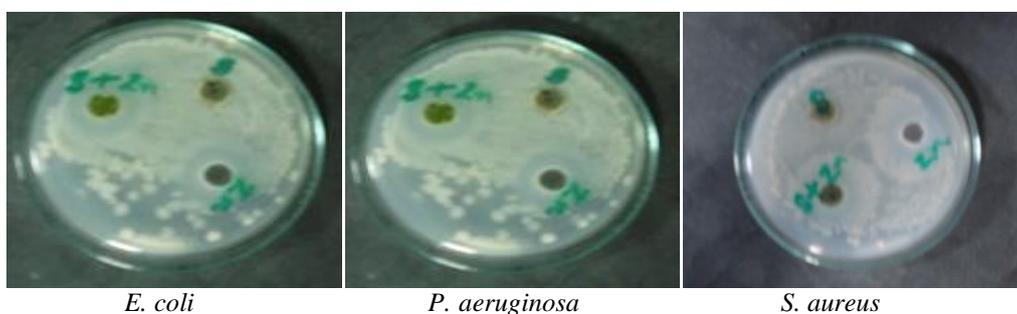


Fig 7: Antibacterial activity of chloroform extract of leaves of aloe vera with metal ions

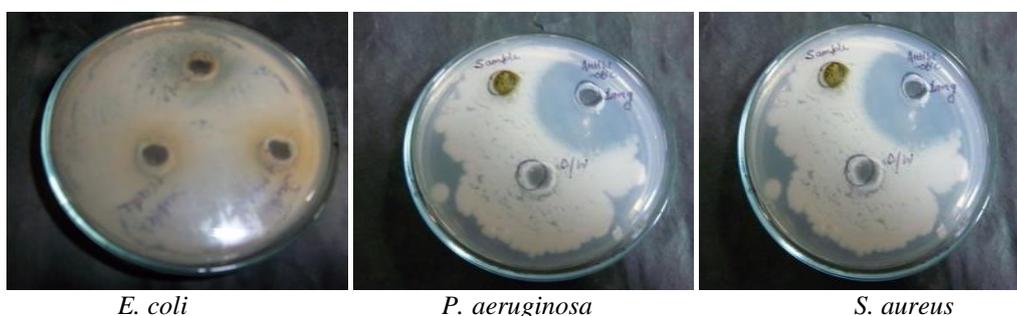


Fig. 8: Antibacterial activity of chloroform extract of leaves of aloe vera without metal ions

Table 6: Antibacterial activity of leaf+Hot water

Sl. No.	Pathogens	ZOI(in mm)of ofloxacin	ZOI(In mm)of Zn	ZOI(In mm)of Zn+PE
1.	<i>E.coli</i>	20.0	26	22
2.	<i>P.aeruginosa</i>	21.5	26	22
3.	<i>S.aureus</i>	23.0	20	31

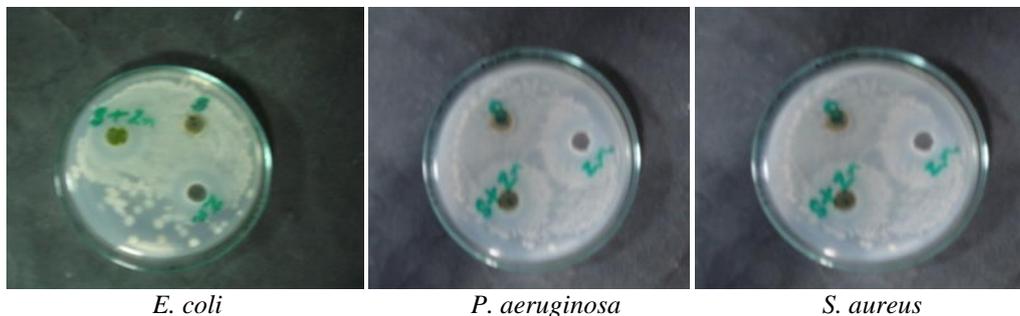


Fig. 9: Antibacterial activity of leaf+Hot water extract of aloe vera without metal ions

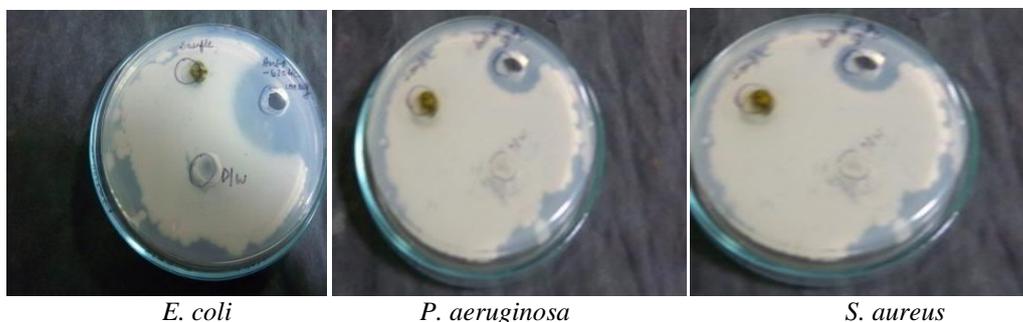


Fig. 10: Antibacterial activity of leaf+Hot water extract of aloe vera without metal ions

Antibiogram Results of Coriander With Metal Ion

Table 7: Antibacterial activity of leaf +Methanol extract

Sl. No.	Pathogens	ZOI (mm) of ofloxacin	ZOI(mm)of Zn	ZOI(mm)of Zn+PE
1	<i>E.coli</i>	23.5	26	32
2	<i>P.aeruginosa</i>	20.5	21	20
3	<i>S.aureus</i>	23.0	25	41

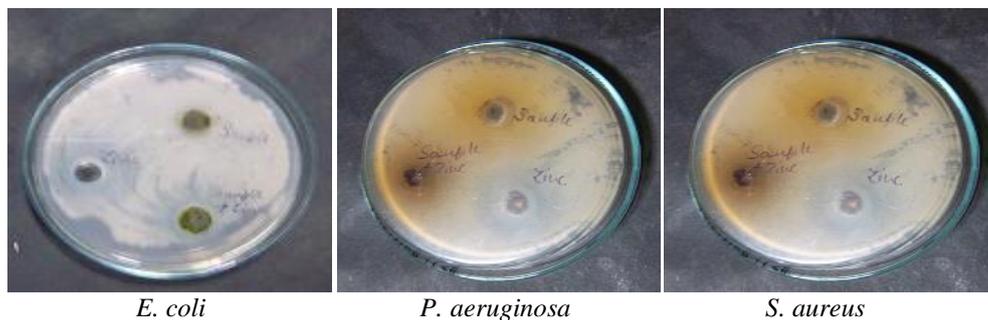


Fig. 11: Antibacterial activity of leaf +Methanol extract of Coriander with metal ions

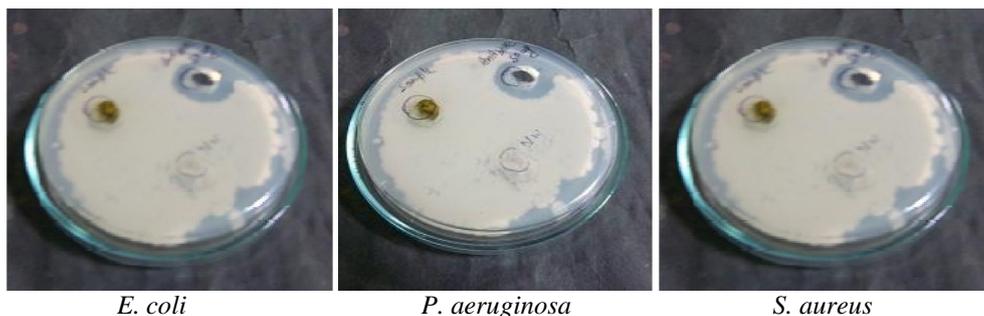


Fig. 12: Antibacterial activity of leaf +Methanol extract of Coriander without metal ions

Table 8: Antibacterial activity of leaf+Ethanol

Sl. No.	Pathogens	ZOI(mm) of ofloxacin	ZOI (mm)of Zn	ZOI (mm)of Zn+PE
1	<i>E.coli</i>	23.0	14	16
2	<i>P.aeruginosa</i>	21.0	21	20
3	<i>S.aureus</i>	24.5	21	31



Fig. 13: Antibacterial activity of leaf +ethanol extract of Coriander with metal ions



Fig. 14: Antibacterial activity of leaf +ethanol extract of Coriander without metal ions

Table 9: Antibacterial activity of leaf+Ethyl acetate

Sl. No.	Pathogens	ZOI(in mm)of ofloxacin	ZOI(In mm)of Zn	ZOI(In mm)of Zn+PE
1	<i>E.coli</i>	20.0	27	28
2	<i>P.aeruginosa</i>	21.5	31	28
3	<i>S.aureus</i>	23.0	-	-

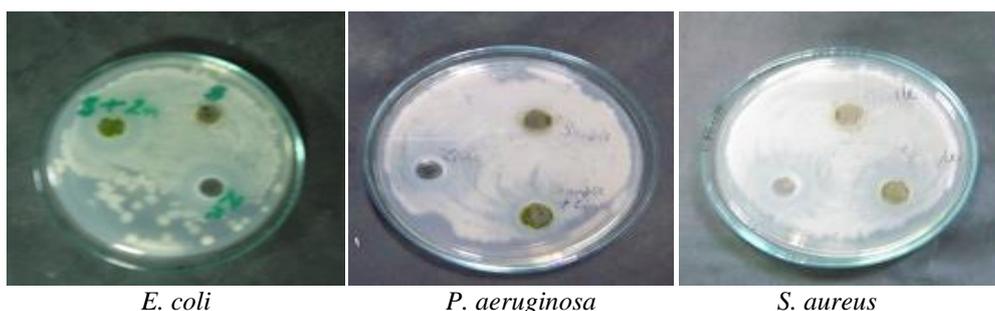


Fig 15: Antibacterial activity of leaf + Ethyl acetate extract of Coriander with metal ions



E. coli

P. aeruginosa

S. aureus

Fig 16: Antibacterial activity of leaf + Ethyl acetate extract of Coriander without metal ions

Table 10: Antibacterial activity of leaf+Chloroform

Sl. No.	Pathogens	ZOI (mm) of ofloxacin	ZOI (mm) of Zn	ZOI (mm) of Zn+PE
1	<i>E.coli</i>	25.0	26	25
2	<i>P.aeruginosa</i>	20.5	21	20
3	<i>S.aureus</i>	21.0	28	41

Table show the ZOI of coriander chloroform extract in the presence of metal ion.



E .coli

P. aeruginosa

S. aureus

Fig 17: Antibacterial activity of leaf + chloroform of Coriander with metal ions



E. coli

P. aeruginosa

S. aureus

Fig 18: Antibacterial activity of leaf + chloroform extract of Coriander without metal ions

Table 11: Antibacterial activity of leaf+Hot water

Sl. No.	Pathogens	ZOI (mm) of ofloxacin	ZOI (mm) of Zn	ZOI (mm)of Zn+PE
1	<i>E.coli</i>	19.0	30	20
2	<i>P.aeruginosa</i>	23.0	29	-
3	<i>S.aureus</i>	21.5	29	24

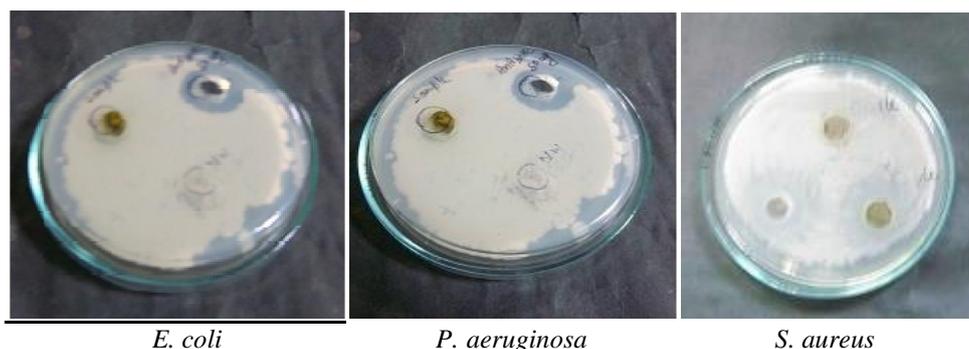


Fig 19: Antibacterial activity of leaf+Hot water of Coriander with metal ions

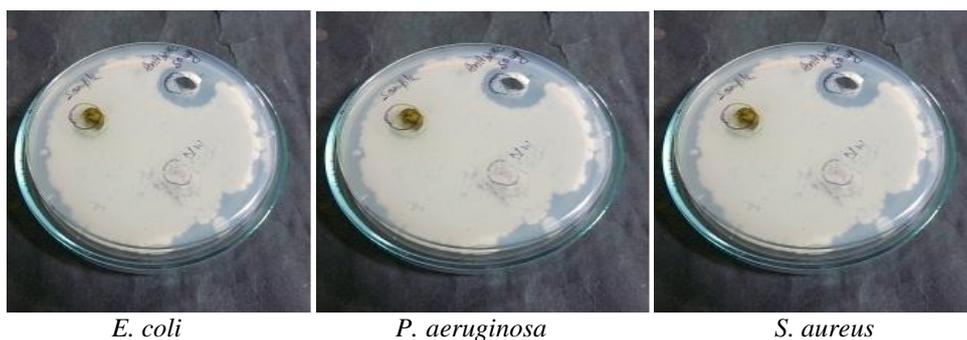


Fig 20: Antibacterial activity of leaf+Hot water of Coriander without metal ions Minimum inhibitory concentration (MIC)

Table 12: MIC results of aloe vera leaf with different solvents & pathogens

Sl. No.	Conc. (mg/ml)	Methanol Extract OD Against <i>S.aureus</i>	Ethanol Extract OD Against <i>P.aeruginosa</i>	Ethyl acetate Extract OD Against <i>S.aureus</i>	Hot water Extract OD Against <i>S.aureus</i>
1.	20.83	0.01	0.07	0.03	0.08
2.	3.47	0.14	0.13	0.01	0.10
3.	0.57	0.36	0.01	0.09	0.01
4.	0.96	0.06	0.14	0.16	0.0
5.	0.016	0.13	0.14	0.10	0.05
6.	.0027	0.11	0.15	0.16	0.01

Table 12-Table show the MIC results of aloe vera with different solvents. The MIC value of that extract for methanol 0.06 mg/ml, for ethanol 0.01, for ethyl acetate 0.01 & for hot water 0.0mg/ml.

Table 13: MIC results of coriander leaf with different solvents & pathogen

Sl. No.	Conc. (mg/ml)	Methanol Extract OD against <i>S.aureus</i>	Ethanol Extract OD against <i>S.aureus</i>	Ethyl acetate Extract OD against <i>E.coli</i>	Chloroform Extract OD Against <i>S.aureus</i>
1.	20.83	0.46	0.18	0.64	1.06
2.	3.47	0.02	0.04	0.10	0.17
3.	0.57	0.06	0.05	0.38	0.02
4.	0.96	0.10	0.18	0.26	0.11
5.	0.016	0.13	0.19	0.33	0.07
6.	.0027	0.14	0.02	0.24	0.33

Table show the MIC result of coriander with different solvents. Note: The value show in bold is the MIC value of that extract means for methanol 0.02, for ethanol 0.02, for ethyl acetate 0.10 & for chloroform 0.02mg/ml.

Table 14: Phytochemical analysis (Aloevera)

Sl. No.	Phytochemicals	Colour indication	Result (+ve or -ve)
1.	Cardio glycosides	Brown ring	Positive
2.	Reducing sugar	-	Positive
3.	Saponin	Emulsion formed	Positive
4.	Oil & fats	Oil strain	Positive
5.	Terpenoides	Reddish brown	Positive
6.	Tanins	Blue black	Positive
7.	Phenols	-	Negative
8.	Amino acid & Proteins	-	Negative
9.	Quinones	-	Negative

Table 15: Phytochemical analysis (Corriander)

Sl. No.	Phytochemicals	Colour indication	Result (+ve or -ve)
1.	Cardio glycosides	Brown ring	Positive
2.	Reducing sugar	-	Negative
3.	Saponin	Emulsionformed	Positive
4.	Oil & fats	-	Negative
5.	Terpenoides	Reddish brown	Positive
6.	Tanins	Blue black	Positive
7.	Phenols	-	Negative
8.	Oil & fats	-	Negative
9.	Quinones	-	Negative

DISCUSSION

An antimicrobial is a substance that kills or inhibits the growth of microorganism such as bacteria, fungi, protozoan's etc. Antimicrobial agents either kill or inhibit the growth of microbes. The aloevera and coriander have also antimicrobial properties. The present study was carried out to analyze the antimicrobial activity of both aloevera & coriander with the effect of metal ion & also identify the minimum inhibitory concentration & presence of secondary compounds by phytochemical analysis [10, 11, 12].

The samples were collected in the Lucknow region & after performing the antibiogram, MIC & Phytochemical analysis the overall result show the increased activity of both *Aloe barbedensis mill.* & *Coriandrum sativum* in the presence of metal ion. The sample are used with five solvents methanol, ethanol, ethyl acetate, chloroform & hot water for preparing there extracts, out of which first four are organic solvents. The maximum zone of inhibition was recorded in the case of aloevera with solvent methanol against *S. aureus* 34mm & also with solvent ethyl acetate against *P. aeruginosa* 33mm. The coriander show the maximum zone of inhibition with solvent methanol & chloroform against *S. aureus* 41mm. The lowest concentration of the extract regarded as MIC. Among the tested sample with different solvents the least MIC value was obtained at the concentration ranging from 3.47-0.57mg/ml. The lowest MIC value was .01 at conc. 3.47mg/ml of aloevera with ethanol extract against

S. aureus & in the coriander with chloroform extract against *S. aureus* was .02 at conc. 0.57mg/ml [13,14].

The phytochemical analysis reveals the presence of secondary compounds in aloevera is cardio glycosides, saponin, tannins, terpenoides and oil or fats. In the coriander presence of cardioglycosides, tannins, saponin, terpenoides were analyzed.

CONCLUSION

After doing all the work it was concluded that both *Aloe barbadensis mill.* & *Coriandrum sativum* have antimicrobial properties. The antimicrobial activity of both the sample was increased in the presence of metal ion. Preliminary phytochemicals analysis reveals the presence of secondary metabolites which is responsible for its antimicrobial activity. 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 process of research & evaluation. One big advantage of using aloevera & coriander for medicinal purpose is that it is widely available and thus future drug agent will be cheap. Being a herbal plants, drug obtained will have no side effects.

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