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Meraj Ali
Faculty of Gaya Prasad Institute of Human Excellence for Pharmacy, Malihabad, Uttar Pradesh, India

Urmila Nishad
Faculty of Gaya Prasad Institute of Human Excellence for Pharmacy, Malihabad, Uttar Pradesh, India

Vijay Kumar Yadav
Department of Pharmacy Dr. B.R. Ambedkar University, Agra, Uttar Pradesh, India

Corresponding Author:
Urmila Nishad
Faculty of Gaya Prasad Institute of Human Excellence for Pharmacy, Malihabad, Uttar Pradesh, India

Evaluation of phytochemical & antibacterial activity on some Indian medicinal plants (Kateli, Datura, Makoi)

Meraj Ali, Urmila Nishad and Vijay Kumar Yadav

Abstract
Objective: To prepare agar plates (cultures) of Gram-positive *Staphylococcus aureus (SA) and* Gram-negative *Pseudomonas aeruginosa* (PA), *Klebsiella pneumonia* (KP) and *Mycobacterium tuberculosis* (MTB) individually.
Methods: Collection of plant, Microbial strain and culture preparation, Preparation of antimicrobial extract by solvent extraction, antimicrobial analysis, determination of inhibitory concentration, phytochemical analysis, purification of secondary metabolism.
Result: Antibacterial screening of Solanum S, Solanum nigrum and Datura stramonium: Anti bio gram analysis of Acetone, 80%Methanol, Chloroform and petroleum ether extracts of *Solanum virginianum, Solanum nigrum* and *Datura stramonium* extract against *S. aureus (SA), Pseudomonas aeruginosa (PA), K. pneumoniae (KP) and Mycobacterium tuberculosis (MTB*).
Conclusion: Project was done in the end of May throughout this project, we come up to a point where we can state that *Solanum nigrum* (makoi) and *Datura stramonium* (Datura) plants are good source of antimicrobial compound and can give to be a good source of natural medicine. They activity of antimicrobial can be enhanced by using metal ions, tested in different temperature and pH and can be tested in various other solvent. It has demonstrated to be powerful against bacteria and used at raise temperatures. So, we can terminate that drugs made out of would not be based on what conditions that are stored, this gives an edge in hold the drugs build out of this fruit.

Keywords: Makoi, dhatura, kateli, phytochemical studies

Introduction
Natural productsare always good for health. Plants have a broad diversity in the world, plants Contains phytochemicals like a tannin, flavonoids, phenol etc. which are good for the treatment of infectious diseases. Today herbal products are increasing fast because of low cost, high effectiveness and low chance to cause side effects. The herbal products are safer today in contrast to synthetic product because synthetic product causes different types of side effects. India and China contributed about 80% of total natural drugs production on the other hand developed countries like United States contributed about 25% of total herbal drugs production so India was economically important for the herbal drugs production. Today infectious diseases increase very rapidly and harm lots of people every day in the world, mostly high-tech and large population areas and treatment of these infectious diseases by the synthetic compounds is highly cost effective and high chance to cause side effects.
Today business of herbal products in India increase fast in different fields like a beauty products and different companies increase their business like a Pat Anjali Ayurveda making different types daily use herbal products. Phytochemical is a Chemical compound that only present in plants. Phytochemical is use to making traditional medicines that medicines used against different types of microbial strains that cause different types of infectious diseases. Today was developed country like a United State and Europe in was increase their interest in herbal products in contrast to synthetic products because synthetic products initiate the formation of different types cancer. Plant phytochemical is obtained from the medicinal plants and different part of the medicinal plants like flower, fruit, leaves, stem and root, phytochemicals such as alkaloids, tannins, terpenoids and phenolic compounds, was proved to suppress experimental beginning of cancer formation various types in human body. In research field phytochemicals is only a chemical compound because it is not scientifically proven essential food and health effects. Herbal product is one of the great approaches to control initiation of cancer formation by the chemoprevention in different organ of human body. Some

medicinal plants have anti-oxidant properties that could be help to treatment and prevention of complex type's diseases like a stroke, diabetes and cancer.

Application of secondary metabolites: In the past history all peoples takes their daily requirement food from the plants not only food they were treat different diseases by the help plants. Different industries like a flavor, agrochemicals, and pharmaceuticals used mostly secondary metabolites. According to survey agencies about 70% population of the world depend on herbal medicines. Chemical have a more complex structure that impossible to chemically synthesis was produced by the help plants. Chemical production by the help of is multi billion business. Plant cell culture laboratories are useful to chemical production by the plants.

Material and Method

1. Collection of plant sample

Table 1: Three different plant sample fresh leaves, stem, fruit and flower were collected.

S.no	Sample names	Location
1.	*Solanum virginianum*, Kateli	Ahemamau,Arjunganj Lucknow
2.	*Solanum nigrum*	Ahemamau,Arjunganj Lucknow
3.	*Datura stramonium*	Ahemamau,Arjunganj Lucknow

The leaves and Stem were then washed and fresh leaf and stem dipped into organic solvents methanol 80%, acetone etc.

2. Microbial strain and culture preparation

We used bacterial Gram positive, *Staphylococcus aureus (SA)* and Gram negative, *Pseudomonas aeruginosa* (PA), *Klebsiella pneumonia* (KP) and *Mycobacterium tuberculosis* (MTB) as my test pathogen. Pathogens used sub-culture plates of the pathogen and streaked them in new agar plates to revive them. The revived culture worked as a source of pathogen broth.

3. Preparation of antimicrobial extract by solvent extraction

Requirement: Plant leaves, stem, flower and fruit, organic solvents 50ml Methanol, 50ml Chloroform, 50ml Acetone and 50ml Petroleum ether. Whatman filter paper, funnel, bowls, plastic, bottles, micro-centrifuge tube etc.

Principle: Secondary metabolites or the bio active compound and phytochemicals present in the plants render them with the antimicrobial properties. These secondary metabolites are soluble in organic solvent are used for the Methanol, Chloroform, Acetone and Petroleum ether. these solvents are used as extraction of phytochemicals these compound such as terpenoids, tannin, Steroid, flavonoids, carbohydrates, from plants extract.

Procedure

- Frist fresh plant sample washed than dry it for 5 min and cut samples into small pieces.
- 50ml of different organic solvents were measured in different containers and 5gm of fresh plant sample was added it.
- Now, the containers were stored in dark for 48 hours.
- After 48 hours the extracts were filtered using Whatman paper No.1 on a pre-weighed bowls.
- The bowls were carefully covered with aluminium foil and small-small holes were made in to it shows as to allow evaporation of solvents in hot air oven at 50 ℃.
- For hot water extracts, 5gm plant extract was soaked in 50ml water and heated at 100 ℃ for 60 minutes in water bath. The container was shaking in every 15 minutes so as to avoid settling of particles at base of the container.
- Extracts were filtered after 60 minutes using a what's man paper No.1 pre weighed bowl.
- The bowls were carefully covered with aluminium foil and small holes were made in to it, so as to allow evaporation of the solvents in the hot air oven at 50℃.
- When the solvents evaporate and the extracts dried, bowls were weighed again.
- To the extracts were scratched, in 1ml of DMSO (dimethyl sulfoxide).
- Working solution of concentration 100µg/ml was prepared using stoichiometric calculation.

4. Antibiogram analysis

Requirements: Petriplates, nutrient agar media (NA), microbial culture, micropipettes, plant extract, glass spreader, working solutions and tetracycline etc.

Principle: Agar well diffusion method was used for the antimicrobial screening of the plant extracts against the test pathogens.

Procedure: For antimicrobial screening:

- Autoclaved (sterile) nutrient agar media was prepared and poured 20ml into each sterile petri plates.
- The media was then allowed to solidify.
- After solidify 20µl of pathogen culture was then spread on the plates labeled as *Staphylococcus aureus (Sa), Pseudomonas aeruginosa (Pa), Klebsiella pneumoniae (Kp) and Mycobacterium tuberculosis (MTB).*
- After 3-4 minutes of spreading 5 wells of 8mm diameter were bored used a sterile borer and 50µl samples were loaded to each well.
- Bacterial plates were incubated at 37°Covernight.
- ZOI was calculated.

5. Determination of minimum inhibitory concentration

Requirements: Test tubes, test tubes stand, beaker, working plant extract, pathogen.

Principle: Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the growth of the microorganism after an overnight incubation.

Procedure

- The 3ml NB was prepared and sterilized.
- 500µg antimicrobial extract, was serially diluted by taking the 0.5ml respective volume.
- 20µl pathogens were added to each of first five test tubes, and the last test tube was kept as blank.
- Incubated at 37℃ for 24 hours in shaker.
- OD was taken at 620 nm.

6. Phytochemical analysis

1. Flavonoids

- 10% lead acetate/ lead nitrate was prepared.
- 1ml of extract was added with 1ml of lead nitrate.

- A yellow precipitate was observed that determined positive result for flavonoids.

2. Saponins
- 1ml of extract with 3ml of distilled water was taken, mixed.
- Froth denotes positive result for saponin

3. Tannin
- Few drops of lead nitrate were added in 1ml of extract.
- Precipitate was observed for positive result.

4. Steroids
- 1ml of extract was added with 2ml of Chloroform and 2ml of H_2SO_4.
- Reddish brown interface shows the positive result.

5. Terpinoids
- 0.1ml of chloroform was added with 0.1ml of extract.
- 0.1ml of H_2SO_4.
- 3Few drops of acetate shows Red color indicating positive result.

6. Carbohydrates
- 0.1ml of extract was added with 0.1ml of Fehling A.
- 0.1ml of Fehling B was added in the solution and boil for 5 minutes.
- A red precipitate indicates positive result.

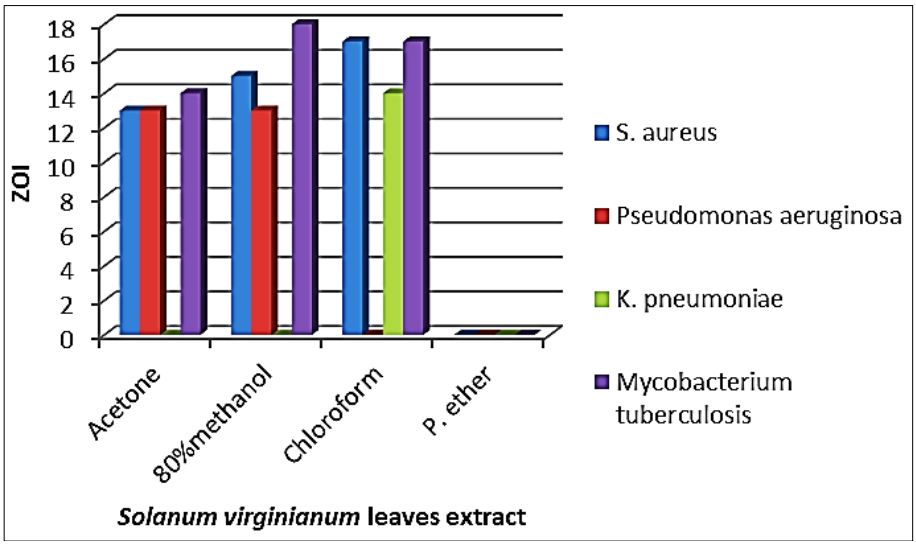
7. Purification of secondary metabolites:

Thin layer chromatography: Thin layer chromatography is technique of screening of phytochemicals present in plants extracts. TLC was done with fractions of polar and non-polar solvents were gave positive results. The fractions of solvents were run through TLC slides with Acetone: and Hexane: Acetone with increasing polarity and with 100% Hexane or Acetone through with increasing polarity.

Results

Table 1: Antibiogram analysis of *Solanum virginianum* leaves extract of Acetone, 80%methanol, Chloroform, P.ether.

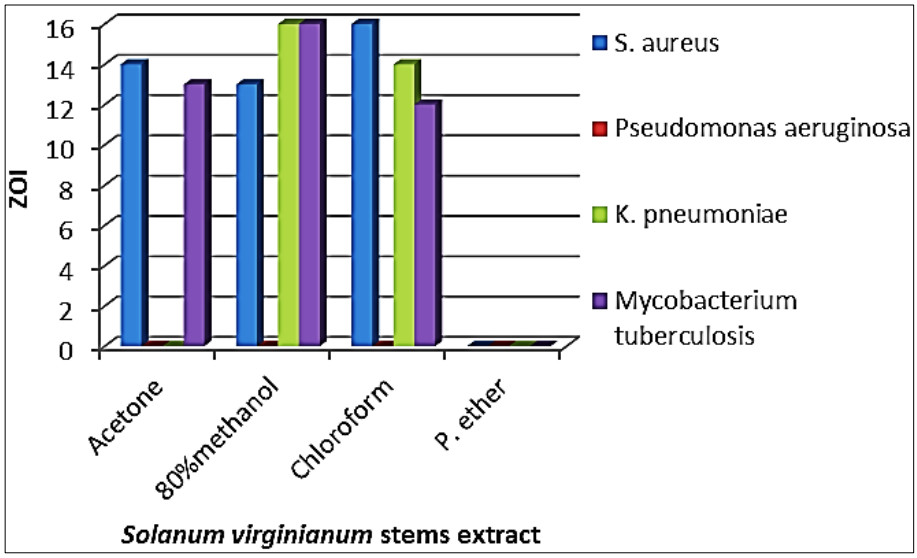
S.NO	Pathogen	Zone of inhibition (mm)			
		Acetone	80% methanol	Chloroform	P. ether
1	*S. aureus*	13	15	17	-
2	*Pseudomonas aeruginosa*	13	13	-	-
3	*K. pneumonia*	-	-	14	-
4	*Mycobacterium tuberculosis*	14	18	17	-



Graph 1: *Antibiogram analysis of Solanum virginianum stems extract of Acetone, 80%methanol, Chloroform, P. ether.*

Table 2: Antibiogram analysis of *Solanum virginianum* stems extract of Acetone, 80%methanol, Chloroform, P. ether.

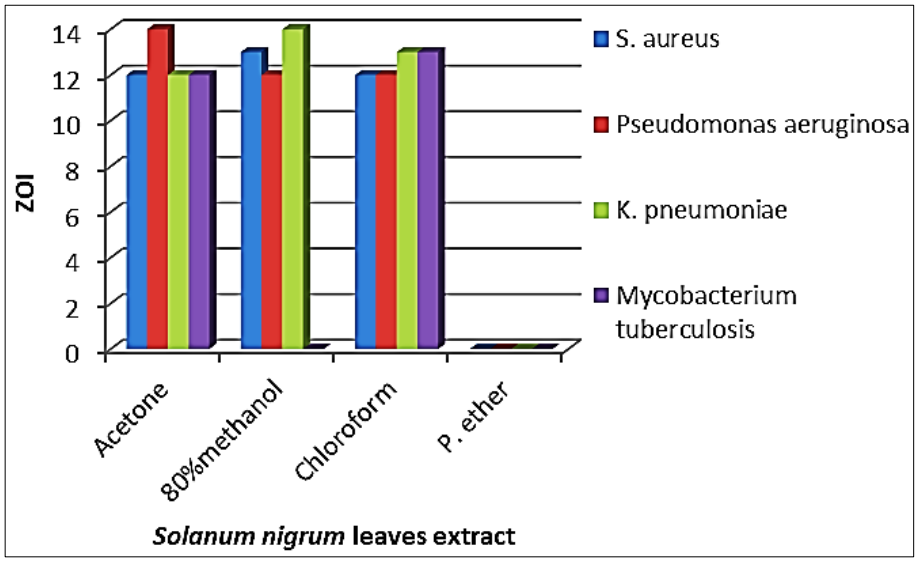
S. No	Pathogen	Zone of inhibition (mm)			
		Acetone	80%methanol	Chloroform	P. ether
1:	*S. aureus*	14	13	16	-
2:	*Pseudomonas aeruginosa*	-	-	-	-
3:	*K. pneumoniae*	-	16	14	-
4:	*Mycobacterium tuberculosis*	13	16	12	-



Graph 2: *Antibiogram analysis of Solanum virginianum stems extract of Acetone, 80%methanol, Chloroform, P. ether.*

Table 3: Antibiogram analysis of *Solanum nigrum* leaves extract of Acetone, 80%methanol, Chloroform, P. ether.

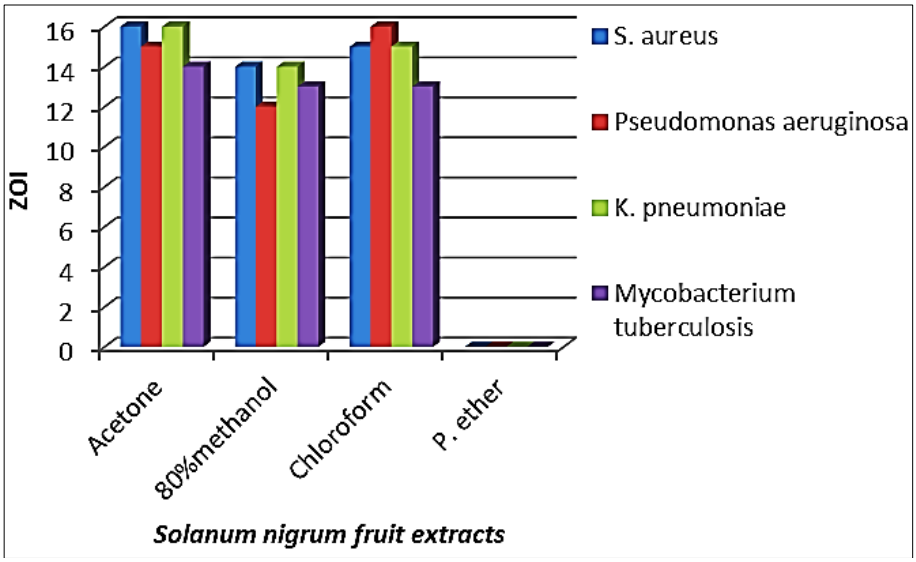
S. No	Pathogen	Zone of inhibition (mm)			
		Acetone	80%methanol	Chloroform	P. ether
1:	*S. aureus*	12	13	12	-
2:	*Pseudomonas aeruginosa*	14	12	12	-
3:	*K. pneumoniae*	12	14	13	-
4:	*Mycobacterium tuberculosis*	12	-	13	-



Graph 3: *Antibiogram analysis of Solanum nigrum leaves extract of Acetone, 80%methanol, Chloroform, P. ether*

Table 4: Antibiogram analysis of *Solanum nigrum* fruit extracts of Acetone, 80%methanol, Chloroform, P. ether.

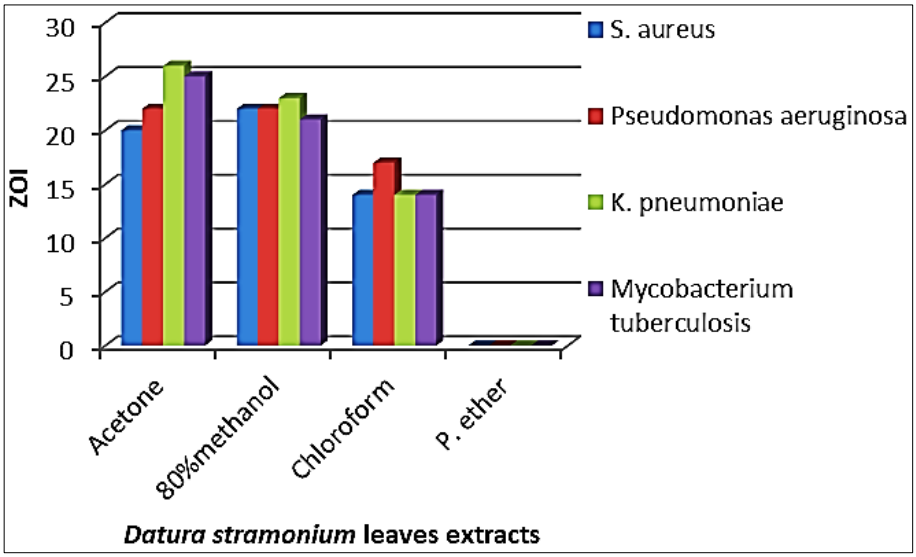
S.NO	Pathogen	Zone of inhibition (mm)			
		Acetone	80%methanol	Chloroform	P. ether
1:	*S. aureus*	16	14	15	-
2:	*Pseudomonas aeruginosa*	15	12	16	-
3:	*K. pneumoniae*	16	14	15	-
4:	*Mycobacterium tuberculosis*	14	13	13	-



Graph 4: Antibiogram analysis of Solanum nigrum fruit extracts of Acetone, 80%methanol, Chloroform, P. ether.

Table 5: Antibiogram analysis of *Datura stramonium* leaves extracts of Acetone, 80%methanol, Chloroform, P. ether.

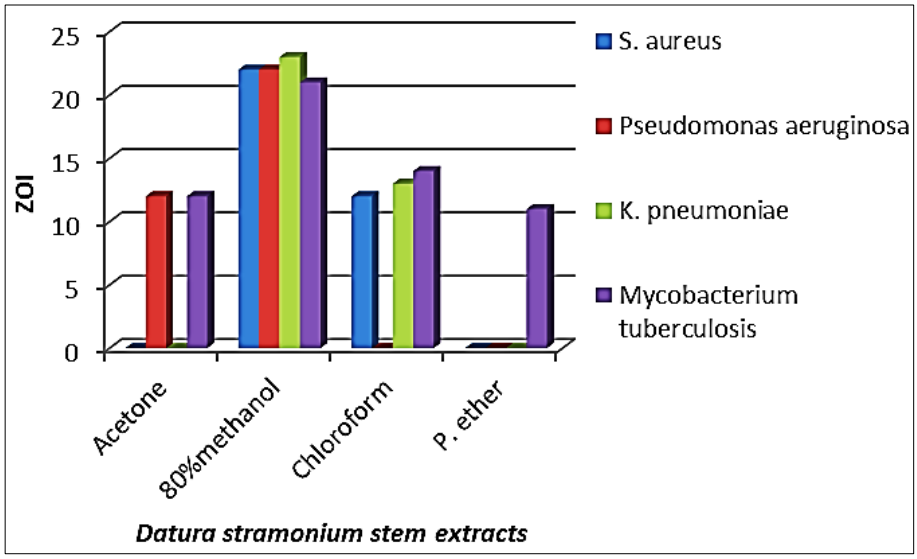
S.NO	Pathogen	Zone of inhibition (mm)			
		Acetone	80%methanol	Chloroform	P. ether
1:	*S. aureus*	20	22	14	-
2:	*Pseudomonas aeruginosa*	22	22	17	-
3:	*K. pneumoniae*	26	23	14	-
4:	*Mycobacterium tuberculosis*	25	21	14	-



Graph 5: Antibiogram analysis of Datura stramonium leaves extracts of Acetone, 80%methanol, Chloroform, P.ether.

Table 6: Antibiogram analysis of *Datura stramonium* stem extracts of Acetone, 80%methanol, Chloroform, P. ether.

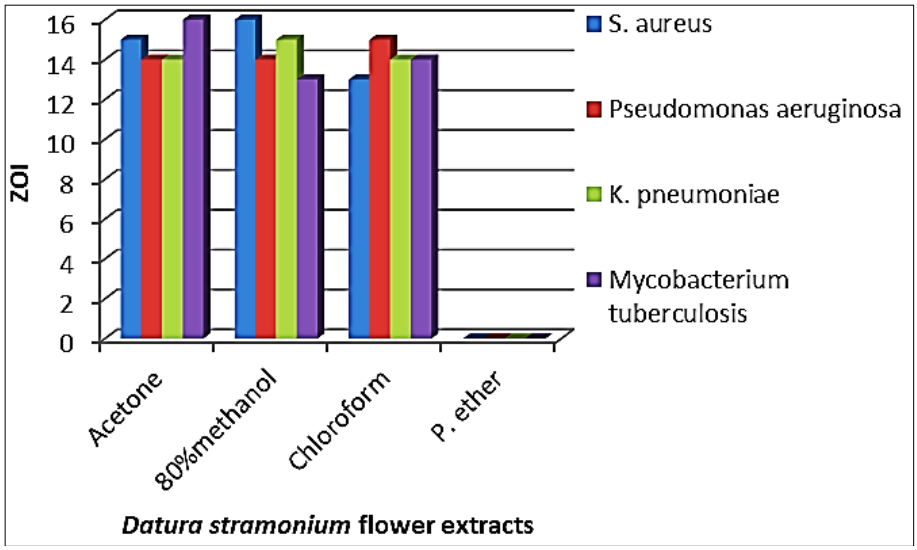
S.NO	Pathogen	Zone of inhibition (mm)			
		Acetone	80%methanol	Chloroform	P. ether
1:	*S. aureus*	-	22	12	-
2:	*Pseudomonas aeruginosa*	12	22	-	-
3:	*K. pneumoniae*	-	23	13	-
4:	*Mycobacterium tuberculosis*	12	21	14	11



Graph 6: Antibiogram analysis of Datura stramonium stem extracts of Acetone, 80%methanol, Chloroform, P.ether.

Table 7: Antibiogram analysis of *Datura stramonium* flower extracts of Acetone, 80%methanol, Chloroform, P. ether.

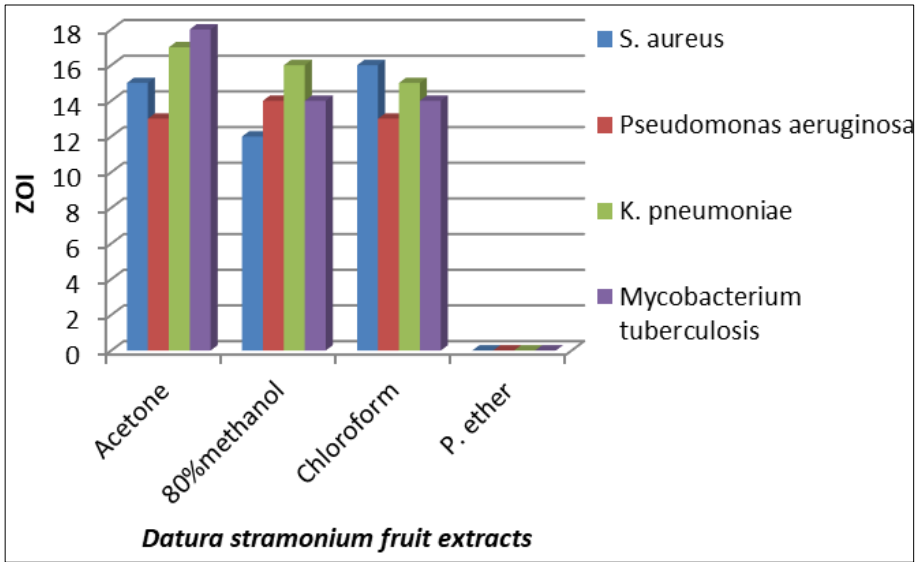
S.NO	Pathogen	Zone of inhibition (mm)			
		Acetone	80%methanol	Chloroform	P. ether
1:	*S. aureus*	15	16	13	-
2:	*Pseudomonas aeruginosa*	14	14	15	-
3:	*K. pneumoniae*	14	15	14	-
4:	*Mycobacterium tuberculosis*	16	13	14	-



Graph 7: Antibiogram analysis of Datura stramonium flower extracts of Acetone, 80%methanol, Chloroform, P. ether.

Table 8: Antibiogram analysis of *Datura stramonium* fruit extracts of Acetone, 80%methanol, Chloroform, P. ether.

S.NO	Pathogen	Zone of inhibition (mm)			
		Acetone	80%methanol	Chloroform	P. ether
1:	*S. aureus*	**15**	12	16	-
2:	*Pseudomonas aeruginosa*	**13**	14	13	-
3:	*K. pneumoniae*	**17**	16	15	-
4:	*Mycobacterium tuberculosis*	**18**	14	14	-



Graph 8: Antibiogram analysis of Datura stramonium fruit extracts of Acetone, 80%methanol, Chloroform, P. ether.

Minimum inhibitory concentration test

Table 9*: Datura stramonium*leaves (acetone) extractagainst *K. pneumonia*

S. no.	Conc.	OD 620 nm
1	33.33	0.03
2	2.22	0.13
3	0.14	0.42
4	0.09	0.49
5	0.06	0.50

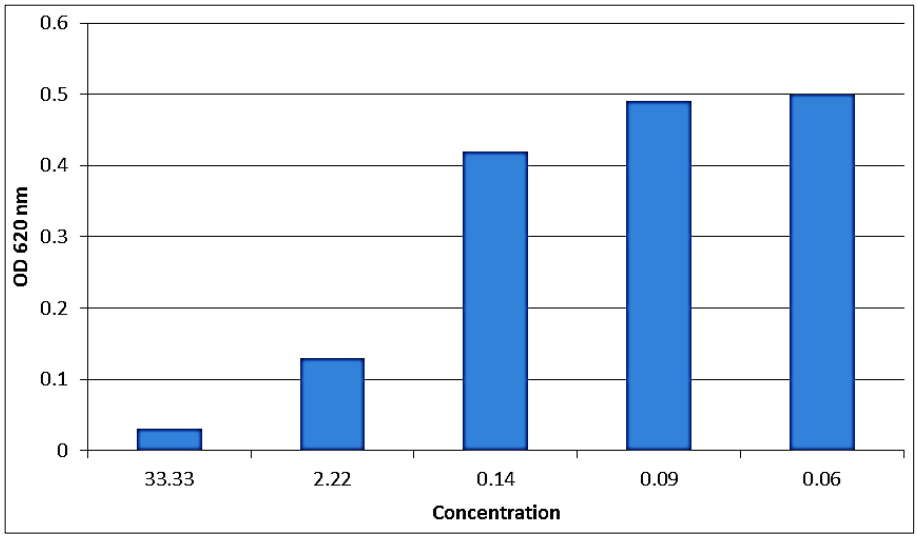


Fig 1: Concentration

Table 10*: Datura stramonium* flower (acetone) extract against *K. pneumonia*

S no.	Conc.	OD 620 nm
1	33.33	0.14
2	2.22	0.40
3	0.14	0.45
4	0.09	0.46
5	0.06	0.48
6	00	00

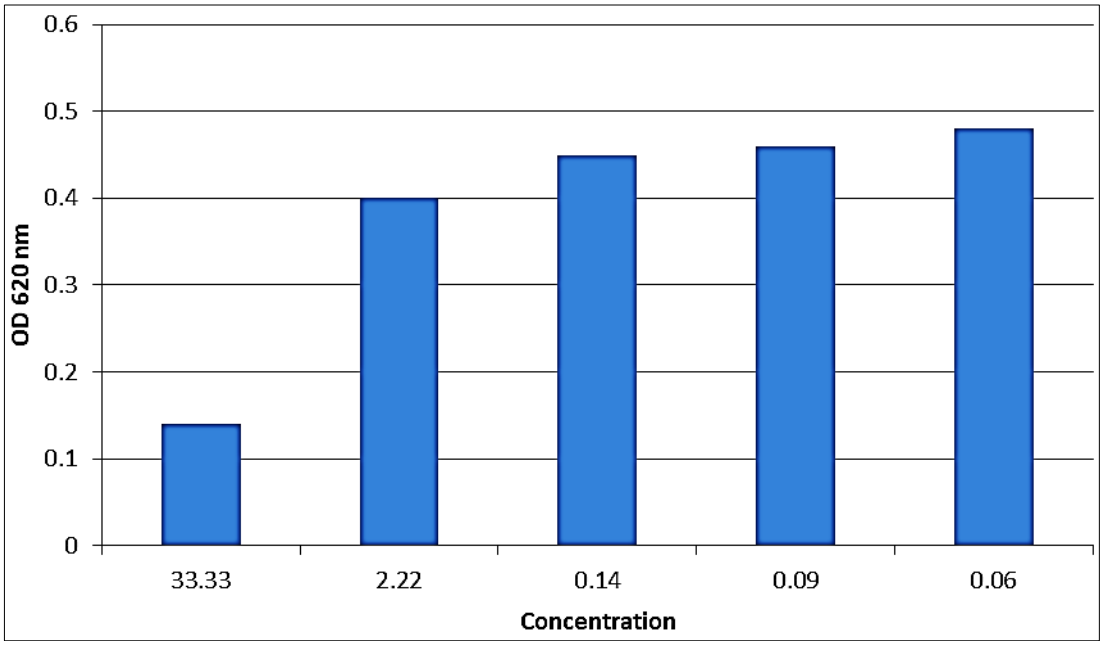


Fig 2: Concentration

Table 11: Datura stramoniumfruit (acetone) extract against *K. pneumonia*

S no.	Conc.	OD620 nm
1	33.33	0.08
2	2.22	0.33
3	0.14	0.49
4	0.09	0.57
5	0.06	0.96
6	00	00

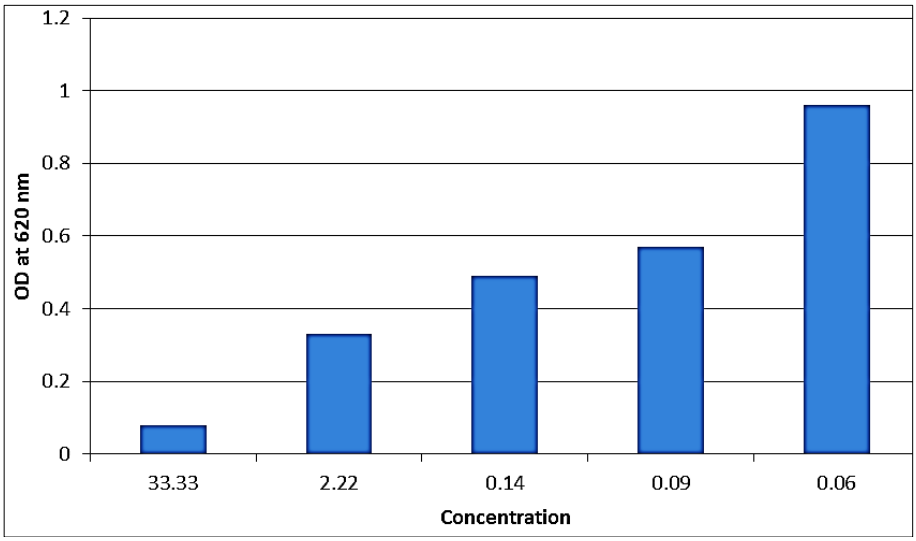


Fig 3: Concentration

Phytochemical Analysis

Table 12: Test for different phytochemical analysis was done and results were observed.

S no.	Phytochemical	Leaf (Acetone) extract *Datura stramonium*	Flower (Acetone) extract *Datura stramonium*	Fruit (Acetone) extract *Datura stramonium*
1.	Steroids	Positive	Positive	Positive
2.	Flavonoids	Positive	Positive	Positive
3.	Terpenoids	Positive	Positive	Positive
4.	Saponin	Positive	Positive	Positive
5.	Tannin	Positive	Positive	Positive
6.	Carbohydrate	Negative	Positive	Positive

Thin Layer Chromatography

Test for different phytochemical screening was done and results were observed.

Table 13: *Datura stramonium* leaves acetone extract in Acetone (0.5%, 1.0%, 1.5%, 2.0% and 2.5%) with hexane.

S.NO	Solvent %	No of spots	Colour	RF. value
1.	0.5	3	Yellow, green, blackish green	1.01, 3.6, 0.67
2.	1.0	2	Yellow, green	1.01, 3.9
3.	1.5	1	Yellow	1.04
4.	2.0	2	Yellow, green	1.01, 5.5
5.	2.5	2	Yellow, green	1, 5.5

Table 14*:* *Datura stramonium* leaves acetone extract in Hexane (0.5%, 1.0%, 1.5%, 2.0% and 2.5%) with Acetone.

S.NO	Solvent %	No of spots	Color	RF. value
1.	0.5	2	Yellow, green	1, 7.14
2.	1.0	2	Yellow, green	1, 5.5
3.	1.5	2	Yellow, green	1, 5
4.	2.0	4	Yellow, green, dark green, dark gray	1.01, 6.25, 1, 0.13
5.	2.5	2	Yellow, green	1, 7.33

Table 15: *Datura stramonium* flower acetone extract in acetone (0.5%, 1.0%, 1.5%, 2.0% and 2.5%) with hexane

S.NO	Solvent %	No of spots	Color	RF. value
1.	0.5	2	Yellow, green	1, 7.14
2.	1.0	2	Yellow, green	1, 5.5
3.	1.5	3	Yellow, green, light green	1, 5, 0.37
4.	2.0	2	Yellow, green	1.01, 6.25
5.	2.5	2	Yellow, green	1, 7.33

Table 16: *Datura stramonium* flower acetone extract in Hexane (0.5%, 1.0%, 1.5%, 2.0% and 2.5%) with acetone

S.NO	Solvent %	No of spots	Color	RF. value
1.	0.5	3	Yellow, green, violet	1, 6.14, 0.9
2.	1.0	2	Yellow, green	1.14, 5.5
3.	1.5	2	Yellow, green	1, 5.6
4.	2.0	1	Yellow	1.01
5.	2.5	2	Yellow, green	1.1, 7.33

Table 17: *Datura stramonium* fruit acetone extract in acetone (0.5%, 1.0%, 1.5%, 2.0% and 2.5%) with hexane

S.NO	Solvent %	No of spots	Color	RF. value
1.	0.5	2	Yellow, green	1, 6.14
2.	1.0	2	Yellow, green	1, 6.5
3.	1.5	2	Yellow, green	1.02, 5
4.	2.0	3	Yellow, green, dark green	1, 6.25, 0.96
5.	2.5	2	Yellow, green	1.01, 7.33

Table 18: *Datura stramonium* fruit acetone extract in haxane (0.5%, 1.0%, 1.5%, 2.0% and 2.5%) with acetone

S.NO	Solvent%	No of spots	Color	RF. value
1.	0.5	2	Yellow, green	1.01, 7.14
2.	1.0	4	Yellow, green, violet, dark green	1, 2.01, 4.05, 7.36
3.	1.5	2	Yellow, green	1, 5.05
4.	2.0	3	Yellow, green, dark green	1.01, 6.25, 7.01
5.	2.5	2	Yellow, green	1.01, 7.33

Discussion

All what creation gives us the way for natural medication and we need search to benefit of human being and entering to this final way I selected three different plants the assign its antimicrobial activity and finally *Datura stramonium* plant acetone leaf, flower and fruit choose for antimicrobial activity because leaves of *Datura* give highest ZOI but *Solanum virginianum* and *Solanum nigrum* not give good results. Acetone leaves (*D. stramonium*) showed an average zone of 26mm against *K. pneumonia*. The current study indicate that *D. stramonium* leaves extracts inhibit the activity of common pathogenic bacteria *S. aureus*, *E. coli*, *S. pneumoniae* and *K. pneumoniae* which is line with outcomes obtained by Obi *et al*. (2002). Then I have increased the activity of secondary metabolites with the help of metal ion, different temperature and different pH level. Zn^{++} have increase the activity of all the plant extracts and combination of Zn^{++} and plant extract obtained a good result respected to normal plant extract. In 4 °C temperature plant extracts give high ZOI in compare to normal plant extract. In different pH level activity of plant extract is not increased but in previous data metal ion and temperature variation not increase activity of any plant extracts (Solomon B., Nega B. *et al*, 2017). For finding the phytochemicals which are present in the plants are qualitatively analyzed with phytochemical tests regarding the phytochemicals. For phytochemical detection I support to

previous work all phytochemical are present in *Datura stramonuium* (Samier A. and Prashant *et al*, 2014). In previous study *Datura stramonium* leaves extract give highest antibacterial activity against S. aureus 18.2 mm but my study according leaves extract give highest activity against *K. pneumoniae* and MIC was recorded against *K. pneumoniae* (Solomon B., Nega B. *et al*, 2017).

Conclusion

Project was done in the end of May throughout this project, we come up to a point where we can state that *Solanum nigrum* (makoi) and *Datura stramonium* (Datura) plants are good source of antimicrobial compound and can give to be a good source of natural medicine. They activity of antimicrobial can be enhanced by using metal ions, tested in different temperature and pH and can be tested in various other solvent. It has demonstrated to be powerful against bacteria and used at raisetemperatures. So, we can terminate that drugs made out of would not be based on what conditions that are stored, this gives an edge in hold the drugs build out of this fruit.

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