*International Research Journal of Environment Sciences*_____ ISSN 2319–1414
Vol. **3(10),** 75-78, October **(2014)** *Int. Res. J. Environment Sci.*

Antibacterial Properties of Various Medicinal Plants Extracts against *Klebsiella Sp.*

Sneh Lata, Geetika Sharma and Harmanjot Kaur Sandhu
Department of Biotechnology, University Institute of Pharma Science, Chandigarh University, Gharuan, SAS Nagar, Punjab, INDIA

Available online at: www.isca.in, www.isca.me
Received 30th July 2014, revised 21st August 2014, accepted 30th September 2014

Abstract

The present study involves the checking of antibacterial properties of various medicinal plants like lemon grass, stevia, jatropha against Klebsiella sp. as these bacteria can cause many diseases. Firstly culture was authenticated by Gram staining and also biochemical characterization was done which confirms the strain of bacteria. After that extracts of these plants like methanol extract, chloroform extract and hot water extract were prepared by using respective chemical .These extracts were prepared from leaves of respective plant. Zone of inhibition of each plant extract against Klebsiella sp. was measured and antibacterial activity of each extract of plant was compared for the results.

Keywords: *Klebsiella sp*., Lemon Grass, Stevia, Jatropha, Chloramphenicol.

Introduction

Klebsiella is a gram negative, rod shape bacteria. It leads to a range of diseases like pneumonia, urinary tract infection and soft tissue infection etc. Many methods are now discovered to avoid these diseases. But naturally some plants are there which show antibacterial properties to this bacterium. Stevia is a herb used in many parts of the world as a non caloric sweetener [1]. Stevia can also be used in bakery, beverage and soft drink industries. Stevia produces decrease in blood pressure [2]. Lemon grass belongs to Poaceae family which is also a herb has antifungal properties [3].The plant is a native herb from india and is cultivated in other tropical and subtropical countries[4]. Lemon grass is used in different parts of the world in the treatment of digestive disorders, fevers, menstrual disorder, rheumatism and other joint pains[5]. Jatropha is perennial shrub belongs to Euphorbiaceae family same as rubber tree and cassava[6]. Oil content in the seed is about 30-40%[7-8]. All these medicinal plants are tested for present research.

Material and Methods

Authentication of culture was done by gram staining and by biochemically characterising the culture. Biochemical characterization was done with the help of primary biochemical tests which included: indole test, urease, oxidase, catalase, nitrate and citrate tests. For biochemical tests standard procedures were used [9].

Culture Condition: The microorganism was cultured in an incubator maintained at temperature of 37˚C. The culture was hand shaken once a day to ensure proper distribution of nutrient to the cell for better growth and was microscopically examined. The bacterial culture was maintained by transferring it to the autoclaved flasks containing nutrient broth.

Table-1
Preparation of NB (Nutrient Broth)

Chemicals	Quantity (per 100 ml)
Peptone	0.5g
Beef Extract	0.3g
NaCl	0.8g

Growth Curve: Growth of culture was checked by preparing100 ml of nutrient broth and inoculating with *Klebsiella sp.* Readings were taken on spectrophotometer after different time intervals and O.D of the bacterial culture was plotted against time intervals.

Preparation of Different Plant Extract, Preparation of Methanol Extract: Methanol extract was prepared by crushing leaves with methanol in a pestle mortar and then filtered the extract by using the filter paper and was stored in a flask.

Preparation of Hot Water Extract: Hot water extract was prepared by crushing leaves with distilled water in a flask and place them at100˚C and then filtered the extract by using filter paper and stored in a flask.

Preparation of Chloroform Extract: Chloroform extract was prepared by crushing leaves with chloroform in a pestle mortar and then filtered the extract by using filter paper and stored in a flask.

Preparation of Acetone Extract: Acetone extract was prepared by crushing leaves in acetone with pestle mortar and then filtered the extract by using filter paper and stored in flask.

Table-2
Preparation of NAM (Nutrient Agar Medium)

S.No.	Chemicals	Quantity(per 100 ml)
1.	Peptone	0.5g
2.	Beef Extract	0.3g
3.	NaCl	0.8g
4.	Agar	2.0g

Antibiotic Sensitivity: Discs of whatmann filter paper were prepared. Nutrient agar medium was poured in three different petri plates and allowed to solidify for 15-20 minutes. The antibiotic solution (chloramphenicol) was prepared in three different dilutions i.e. 50 mg/ml, 5mg/ml and 0.5mg/ml and was poured in the above three solidified petri plates. Dipped two discs in each of these three petri plates and waited for 1-2 minutes for absorbance of antibiotic solution. The 0.5ml of the bacterial culture was poured in each of the three petri plates. With the help of swabbing stick swabbed the culture on them from first petri plate in which 50mg/ml antibiotic solution was added. Take two discs one by one and set in the second petri plate. After 24hrs a zone of inhibition was measured.

Antimicrobial Assay Using Different Plant Extract: The antimicrobial activity of different extract of lemon grass, stevia and jatropha was tested on microorganism using the paper disc diffusion method [10].

Results and Discussion

Gram Staining: In the present study Gram staining of the culture showed Gram negative, rod shaped bacteria. Table-3 showing results of gram staining below.

Table-3
Results of Gram staining of the culture

S.No	Colour	Shape	Type
1.	Pink	Rod Shaped	Gram -ve
2.	Pink	Rod Shaped	Gram -ve
3.	Pink	Rod Shaped	Gram -ve

Biochemical Characterization of Isolates: After gram staining, biochemical characterization of culture was done. Results for biochemical characterization are depicted in table-4 showing the positive results for catalase, citrate, urease and nitrate tests while negative results were observed for oxidase and indole tests [9].

Table-4
Results of Biochemical Characterization

Culture	Catalase Test	Oxidase Test	Indole Test	Citrate Test	Urease Test	Nitrate Test	Strains
1.	+Ve	-Ve	-Ve	+Ve	+Ve	+*Ve*	*Klebsiella*
2.	+Ve	-Ve	-Ve	+Ve	+Ve	+*Ve*	*Klebsiella*

Table-5
Growth Curve Readings of *Klebsiella sp.*

S. No.	Time	O.D.
1.	0 hr	0.015
2.	30 min	0.018
3.	60 min	0.018
4.	90 min	0.012
5.	120 min	0.027

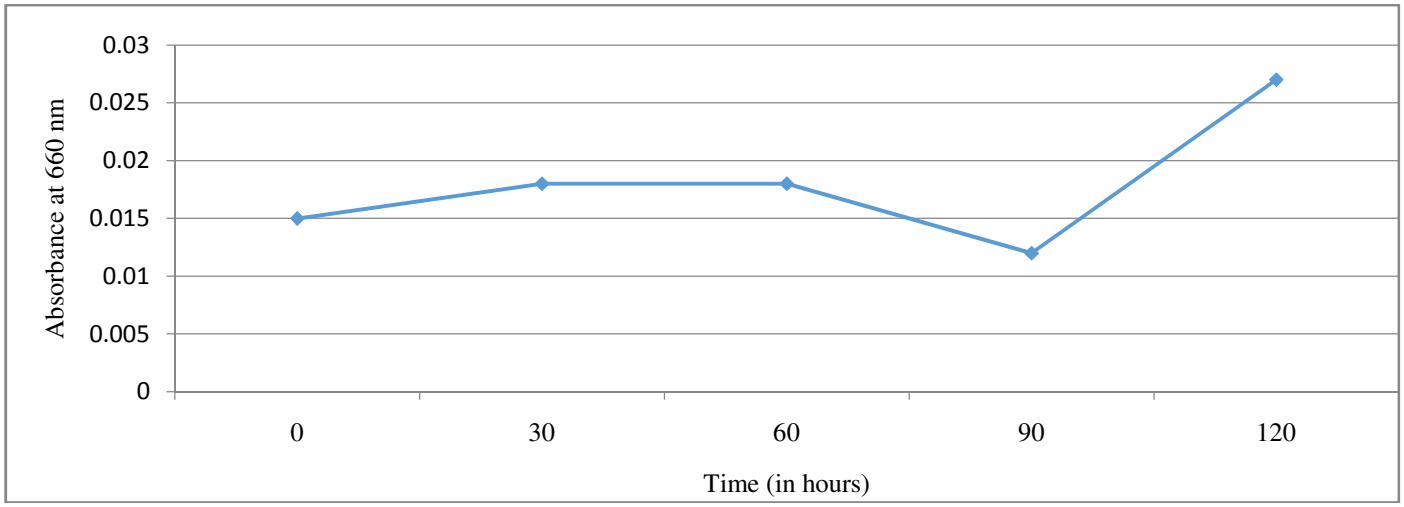

Figure- 1
Growth curve of *Klebsiella sp.*

Results of antimicrobial assay of different extracts against *Klebsiella:* Measurement of zone of inhibition of chloramphenicol against *Klebsiella sp*. was done. Results are depicted in table-6

Table-6
Zone of inhibition of chloramphenicol against *Klebsiella.*

S. No.	Concentration of Chloramphenicol	Zone of Inhibition
1.	0.5mg	10mm
2.	5mg	15mm
3.	50mg	20mm

Antibacterial activity of Jatropha: Highest antibacterial activity of jatropha against *Klebsiella* was exhibited by hot water extract (10mm zone of inhibition) followed by chloroform extract, methanol extract and acetone extract.Table-7 showing results.

Table -7
Zone of inhibition of different extract of Jatropha against *Klebsiella*

S. No.	Extract of Jatropha	Zone of Inhibition
1.	Hot Water	10mm
2.	Methanol	7mm
3.	Chloroform	5mm
4.	Acetone	7mm

Antibacterial activity of Stevia: Highest antibacterial activity of stevia against *Klebsiella* was exhibited by methanol extract (10mm zone of inhibition) followed by acetone extract and chloroform extract and hot water extract. Results are depicted in table-8

Table-8
Zone of inhibition of different extract of stevia against *Klebsiella*

S.No.	Extracts of Stevia	Zone of Inhibition
1.	Hot Water	6mm
2.	Methanol	10mm
3.	Chloroform	7mm
4.	Acetone	9mm

Antibacterial activity of Lemon grass: Highest antibacterial activity of lemon grass against *Klebsiella* was exhibited by chloroform extract (9mm zone of inhibition) followed by hot water extract, methanol extract and acetone extract.

Table -9
Zone of inhibition of different extract of lemon grass against *Klebsiella*

S.No.	Extracts of Lemon Grass	Zone of Inhibition
1.	Hot Water	9mm
2.	Methanol	7mm
3.	Chloroform	10mm
4.	Acetone	6mm

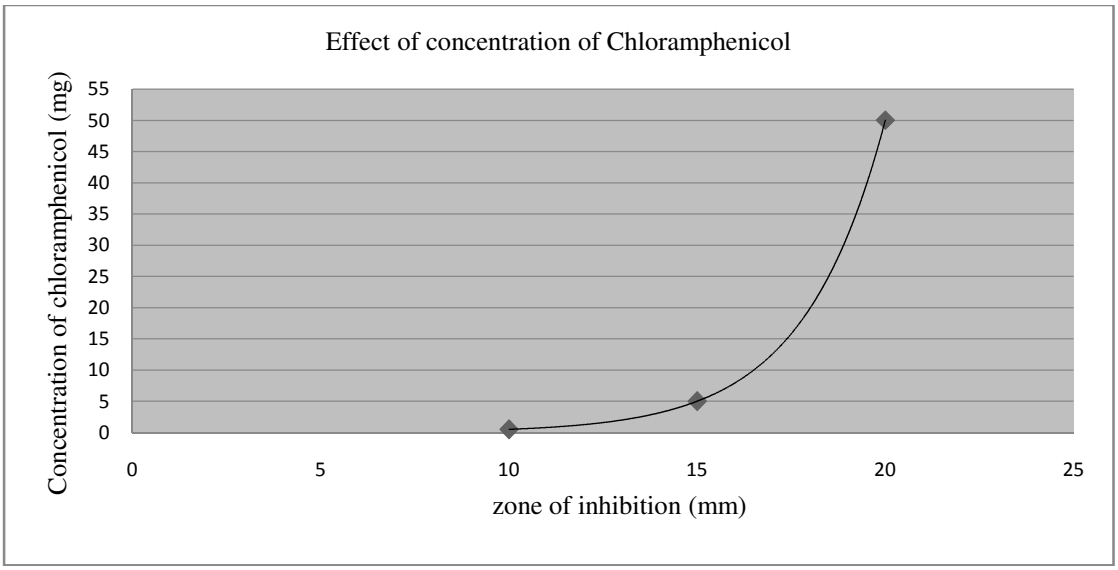

Figure-2
Effect of concentration of chloramphenicol against *Klebsiella*

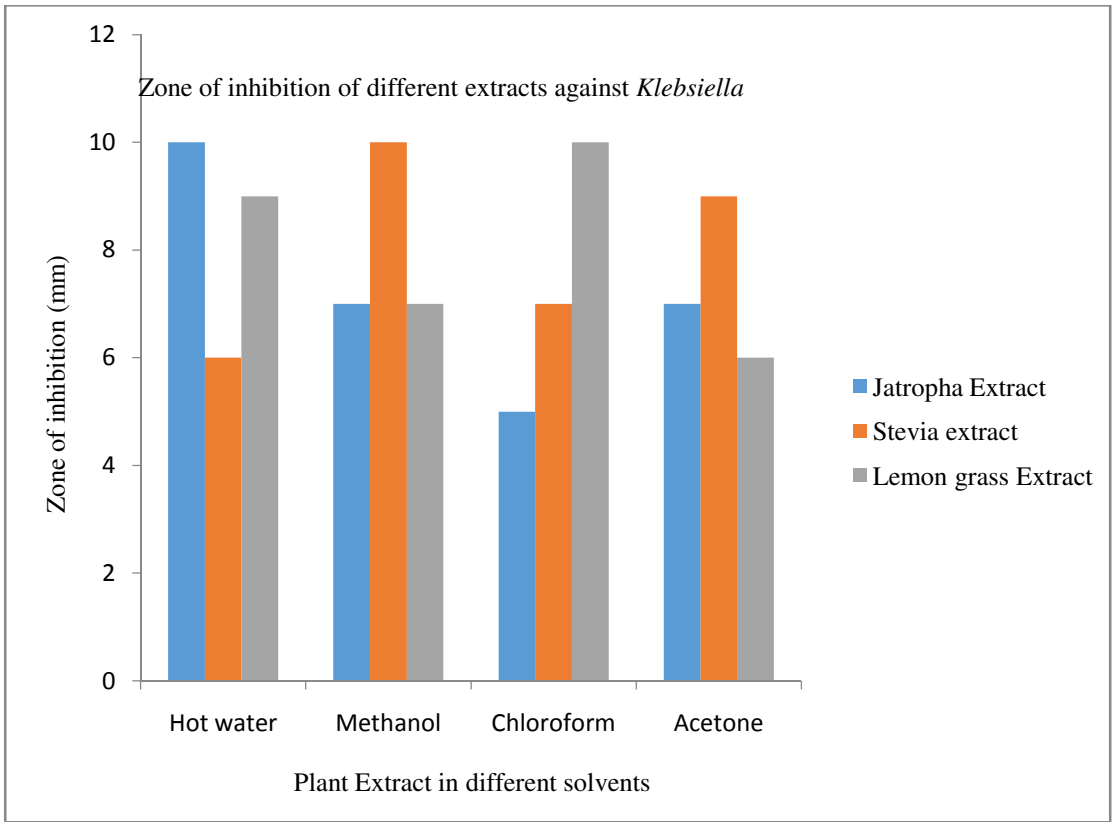

Figure-3
Zone of inhibition of different plant extracts against *Klebsiella*

Conclusion

On observing the overall antibacterial activity of different extracts it was found that hot water extract of jatropha, methanol extract of stevia and chloroform extract of lemon grass showed the highest antibacterial activity (10mm) against *Klebsiella sp.*

References

1. Cardello HM, Da Silva MA and Damasio MH., Measurement of relative sweetness Of stevia extract, aspartame and Cyclamate/Saccharin blend as compared to sucrose at different concentrations, *Plant Foods Hum Nutr*, **54**, 119-130 **(1995)**

2. Melis MS., Chronic administration of aqueous extract of *Stevia Rabaudiana* in rats: renal effects, *J Ethnopharmacol*, **47**,129-134 **(1995)**

3. Shadab Q., Hanif M. and Chaudhary F.M., Antifungal activity by Lemongrass essential oils, *Pak. J. Sci. Ind. Res*, **35,** 246-249 **(1992)**

4. Figueirinha A., Paranhos A., Perez-Alonso J.J., Santos-Buelga C., and Batista M.T. Cymbopogon Citratus leaves. characterisation of flavonoids by HPLC-PDA-ESI/MS And an approach to their potential as a source of bioactive polyphenols, *Fd. Chem,***110**, 718-728 **(2008)**

5. Simon J.E., Chadwick A.F. and Cracker L.E., The scientific literature on selected herbs and medicinal plants of the remperate zone, *Arch. Int. Med*, **148**, 36-39 **(1984)**

6. N. Carels., *Jatropha Curcas*: A Review, Adv. Biot. Res. **50**, 39-86 **(2009)**

7. Fairless. D., The Little Shrub that could-may be, *Nature*, **449,** 652-655 **(2007)**

8. Kandpal J.B. and Madan M., *Jatropha Curcas*-A renewable source of energy for meeting future energy needs, *Renew Energy*, **6**,159-160 **(2006)**

9. R. Cruickshank., Medical Microbiology, 12th eds. (revised reprient) edinburg, *Churchill Livingstone*, **35**, 170-189 **(1980)**

10. Vanden Berghe D.A. and Vlietinck A. J., Screening methods for antibacterial and antiviral agents from higher plants. In: Dey, P.M., Harbone, J.D. (eds), Methods in Plant Biochemistry, *Academic Press, London*, **24**, 47-69 **(1991)**