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EVALUATION OF ANTI MICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF KALANCHOE GASTONIS BONNIERI AND KALANCHOE DELAGOENSIS

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ABSTRACT:
The objective of the study is to evaluate the possible antimicrobial properties of ethanolic extract of leaves of Kalanchoe gastonis bonnieri and Kalanchoe delagoensis. The antimicrobial activity of ethanolic leaf extract of Kalanchoe gastonis bonnieri was tested by Agar well diffusion method. The antimicrobial activity of ethanolic leaf extract of Kalanchoe delagoensis was test by Agar dilution method. The mixtures of Kalanchoe gastonis bonnieri and Kalanchoe delagoensis was tested by Time kill method against six pathogenic bacterial culture.

Key words:

Introduction, Materials and methods, Methods for evaluation of anti-microbial activity of plant extract, Result, Discussion and Conclusion.

I. INTRODUCTION

- An Antibiotic is a type of Antimicrobial substance active against bacteria.
- They may either kill or inhibit the growth of bacteria.
- Antibiotics are usually grouped together based on mechanism of action.
- Each type of antibiotic only works against certain types of bacteria or parasites.
- This is why different antibiotics are used to treat different types of infection.

II. MATERIALS AND METHODS:

Table 1; Required chemicals and apparatus

S.No	Chemical or Apparatus required	SUPPLIER DETAILS
1.	Oflaxacin	OF*-200(J.B Chemicals & Pharmaceuticals LTD), Neelam centre, Mumbai.
2.	Ethanol(500ml)	K.C.P. Sugar and Industries Corporation. Vuyyuru, Andhra Pradesh.
3.	Soxhlet apparatus	Goel Scientific Glass Works Ltd, Vadodara, Gujarat

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4.	Kalanchoe gastonis bonnieri	From college botanical garden
5.	Kalanchoe delagoensis	From college botanical garden
6.	Agar medium	
	a. Beef extract	Loba Chumie Pvt. Ltd, Jehangir village, Mumbai
	b. Peptone	Nice chemicals Pvt. Ltd, Kochi, Kerala
	c. Agar and sodium chloride	Finar limitated, chacharwadi village Ahmedabad, Gujarat.
7.	Glass ware a. Petri dishes b. Pipettes c. Empty test tubes	Goel Scientific Glass Works Ltd, Vadodara, Gujarat.
8.	Antibiotic zone reader	Bio techno Labs, Tamilnadu.

Plant material:

1. Kalanchoe gastonis bonnieri-

The leaves of kalanchoe gastonis bonnieri and kalanchoe delagoensis is collected from Narasaraopeta institute of pharmaceutical sciences, Andhra Pradesh, and were cleaned, shade dried at room temperature for 10-15 days.

2. The collected plant materials were coarsely powdered and stored in air tight containers.

Extraction:

- We have collected the plant extract by using soxhlet extraction apparatus using ethanol as a solvent.
- Soxhlet extraction process is also known as Hot continuous percolation.
- Soxhlet extractor extracts the components using the condensed vapours of the solvent. The condensed vapours come in contact with sample powder and soluble part in the powder get mixed with the solvent.

Preparation of standard solution:

- The stock solution of test compounds was prepared by dissolving the dried extracts of methanolic extracts of leaves of Kalanchoe gastonis bonnieri and Kalanchoe delagoensis.
- The stock solution of reference standard antibiotic (Ofloxacin) was prepared at a concentration of 10µg/ml in sterile water.
- Antimicrobial activity was screened by adding 0.05ml stock solution to each cup by micro pipette using suitable evaluation method.

Test organisms:

The test organisms used are,

- Escherichia coli
- Bacillus subtilis
- Staphylococcus aureus
- Salmonella typhi
- Klebsiella pneumonia
- Pseudomonas aeruginosa

Culture media:

- Beef extract – 0.2g
- Sodium chloride – 0.5g
- Agar – 1.5g
- Peptone – 0.5g, PH is adjusted to 7.

Inoculum preparation:

- One loop full of micro- organisms were inoculated into 100ml of sterile medium and incubated for 24hrs at 37℃ for bacterial culture.
- After 24hrs of incubation 1ml of broth containing micro -organisms was added to 9ml of peptone water.
- 10 fold serial dilutions were made in the range of 10^{-1} to 10^{-10} were spread over the sterile nutrient agar plates and kept at 37^{o} and 27℃. For 24hrs respectively.
- The number of colony forming units(CFU) was counted and number of micro organisms per 1ml of stock culture was calculated

Methods for evaluation of anti-microbial activity of plant extract:

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Agar well diffusion method

• Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

Agar well dilution method

• The agar dilution method involves the incorporation of varying desired concentrations of the antimicrobial agent into an agar medium (molten agar medium), habitually using serial two-fold dilutions, followed by the innoculation of a defined microbial inoculum onto the agar plate surface. The MIC endpoint is recorded as the lowest concentration of antimicrobial agent that completely inhibits growth under suitable incubation conditions

Time kill test

• The time-kill kinetic assay measures the rate of killing while being exposed to a single drug concentration, usually one that is physiologically achievable at standard dosage.

• The time- kill kinetic assay is currently taught to be the best method to study in-vitro drug synergy.

III. RESULT:

Table2; Extractive values of powdered kalanchoe gastonis bonnieri& kalanchoe delagoensis(100g)

S.NO.	Extract	%w/w yield
1.	Ethanolic extract of Kalanchoe gastonis bonnieri	20
2.	Ethanolic extract of Kalanchoe delagoensis	18

Table 3; Phytochemical analysis of Kalanchoe gastonis bonnieri and Kalnchoe delagoensis

S.NO.	Test	Observation	Result
1.	Test for Alkaloids a)Wagner's test	Reddish brown colour precipitate	+
2.	Test for proteins a)Biuret test	Dark violet colour	+
3.	Test for steroids a)Liebermann-burchard test	Dark red colour	+
4.	Test for glycosides Keller-kelliani	Reddish brown colour	+
5.	Test for phenols a)Ferric chloride test	Bluish black	+
6.	Test for flavonoids a)Shinoda test	Dark pink colour	+

Table4; Antimicrobial activity of leaf extract of Kalanchoe gastonis bonnieri - Agar well difussion method

S.NO	Microorganisms	Standard drug Ofloxacin (10µg/ml)	Zone of inhibition (mm)
			Ethanolic extract of Kalanchoe gastronisbonneri (A)
1.	Escheritia coli	37± 0.92	35±1.02
2.	Bacillus subtilis	32± 1.85	32± 1.5

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3.	Staphylococcus aureus	36± 0.92	33.72± 0.32
4.	Salmonella typhi	28± 1.07	30± 1.68
5.	Klebsiella pneumonia	35± 1.74	37.06± 0.3
6.	Pseudomonas aeruginosa	26± 0.63	26± 1.34

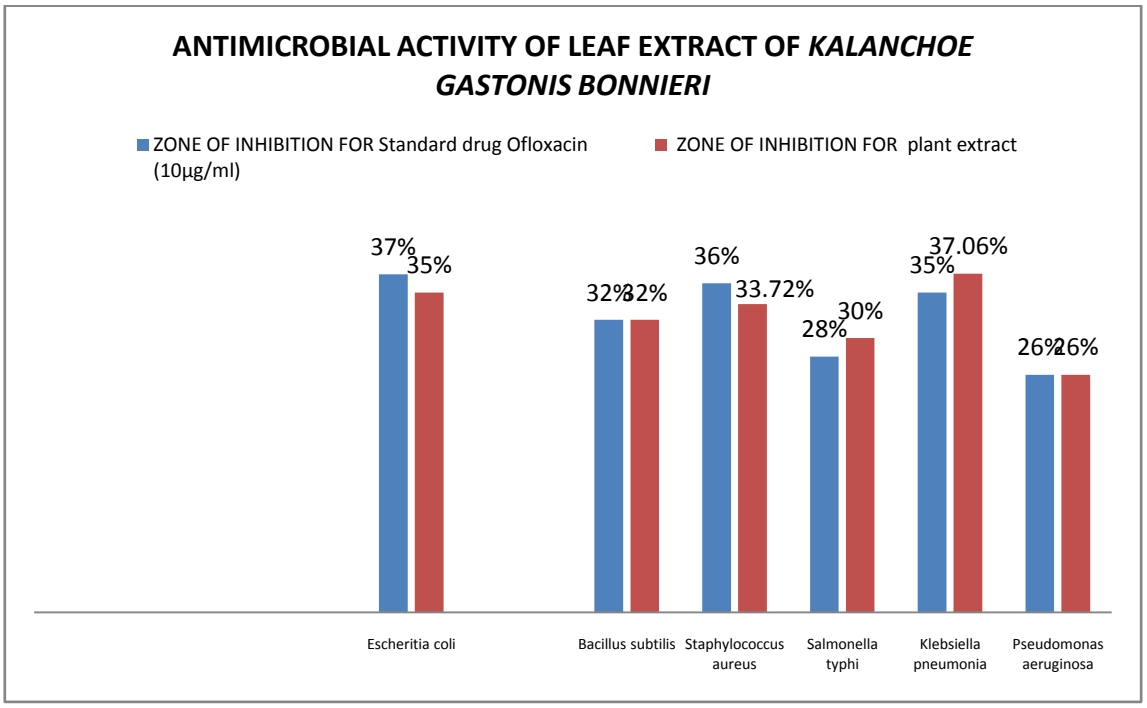

Figure1; Antimicrobial activity of leaf extract of Kalanchoe gastonis bonnieri

Table5; Antimicrobial activity of leaf extract of Kalanchoe delagoensisby Agar dilution method

S.NO	Microorganisms	Standard drug Ofloxacin (10µg/ml)	Zone of inhibition (mm) Ethanolic extract of Kalanchoe delagoensis (B)
1.	Escheritia coli	37 ± 0.92	35 ± 1.2
2.	Bacillus subtilis	32 ± 1.85	30± 1.05
3.	Staphylococcus aureus	36 ± 0.92	36.2 ± 0.53
4.	Salmonella typhi	28 ± 1.07	31 ± 1.7
5.	Klebsiella pneumonia	35 ± 1.74	35± 1.23
6.	Pseudomonas aeruginosa	26 ± 0.63	28± 1.7

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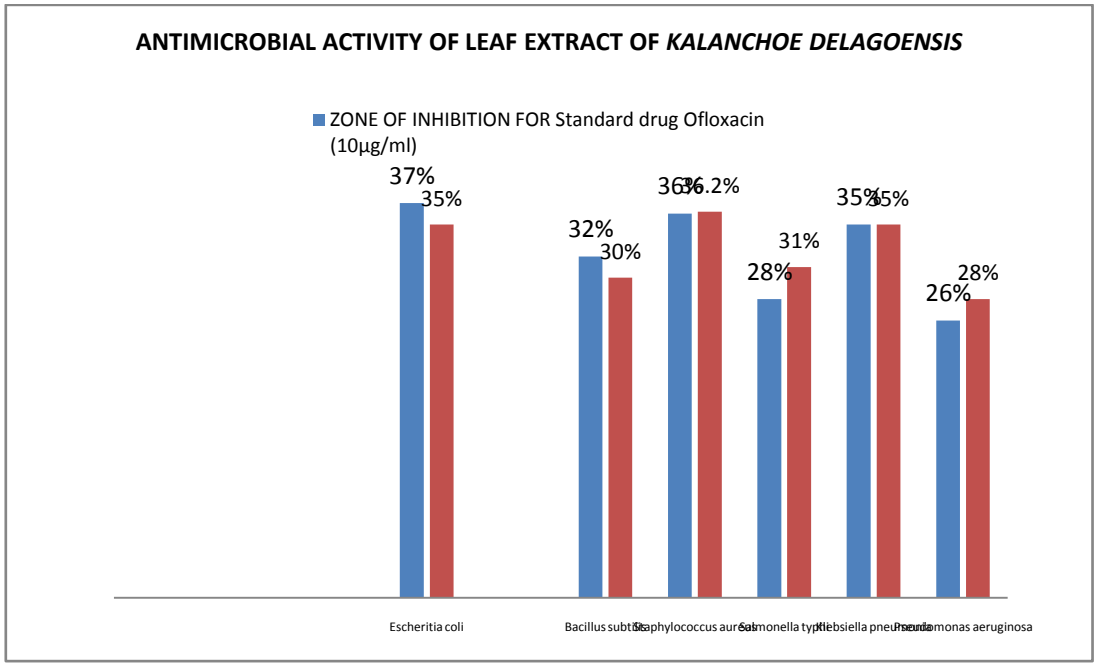

Figure2; Antimicrobial activity of leaf extract of Kalanchoe delagoensis

Table6; Antimicrobial activity of leaf extract of Kalanchoe gastronis bonneri and Kalanchoe delagoensis mixture.

S.NO	Microorganisms	Standard drug Ofloxacin (10µg/ml)	Zone of inhibition (mm)
			Ethanolic extract of A+B
1.	Escheritia coli	37 ± 0.92	38.6 ± 1.5
2.	Bacillus subtilis	32 ± 1.85	31± 1.0
3.	Staphylococcus aureus	36 ± 0.92	33.67± 0.53
4.	Salmonella typhi	28 ± 1.07	28.5 ± 1.07
5.	Klebsiella pneumonia	35 ± 1.74	35± 1.5
6.	Pseudomonas aeruginosa	26 ± 0.63	28± 1.07

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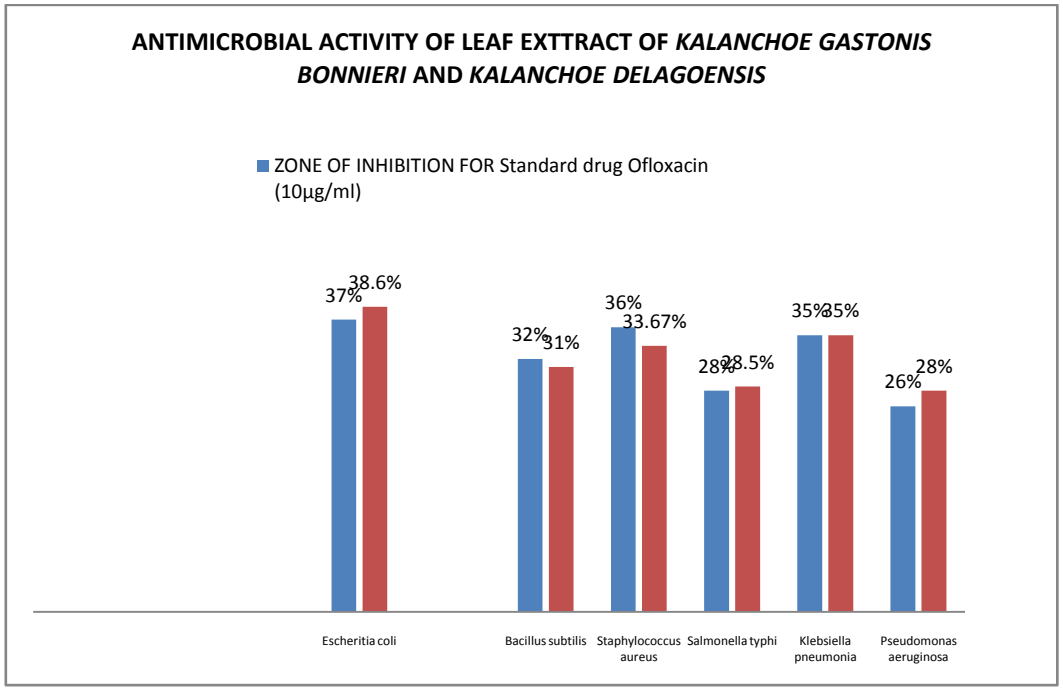

Figure3; Antimicrobial activity ofKalanchoe gastonis bonnieri&Kalanchoe delagoensismixture

IV. DISCUSSION:

The results for the evaluation of antimicrobial activity of selected plant extracts kalanchoe gastonis bonneri, kalanchoe delagoensis and mixture of both plant extract of selected bacterial cultures using standard antibiotic are presented in the above tables. It was measured by agar disc diffusion method against 6 bacterial pathogens. The test organisms included both grampositive and gram negative bacteria. Among the taken three kinds of ethanolic plant extracts of kalanchoe species the mixture of kalanchoe gastonis bonnieri and kalanchoe delagoensis ethanolic plant extract shows highest antimicrobial activity.

The test organisms taken to measure anti microbial activity include Escheritia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia and pseudomonas aeruginosa. The antimicrobial activity of ethanolic leaf extract of mixture of kalanchoe gastonnis bonneiri and kalanchoe delagoensis shows highest anti microbial activity against Staphylococcus aureus. kalanchoe delagoensis alone shows second highest anti microbial activity followed by kalanchoe gastonnis bonnieri.

After Staphylococcus aureus, the mixture of kalanchoe gastonis bonnieri and kalanchoe delagoensis shows highest anti microbial activity against Escheritia coli and followed by kalanchoe delagoensis and kalanchoe gastonis bonnieri. Pseudomonas aeruginosa shows similar order of antimicrobial activity of ethanolic leaf extracts.

The mixture of kalanchoe gastonis bonnieri and kalanchoe delagoensis is more active against Klebsiella pneumonia when compared to other leaf extracts but the ethanolic leaf extracts of kalanchoe delagoensis and kalanchoe gastonis bonnieri had shown similar zone of inhibition against the microorganism Klebsiella pneumonia.

The zone of inhibition of ethanolic leaf extract of mixture of kalanchoe gastonis bonnieri and kalanchoe delagoensis against the Salmonella typhi is more when compared to the ethanolic leaf extracts of plants individually.

Among all the microorganisms taken Bacillus subtilis had shown less antimicrobial activity compared to other microbes. The mixture of leaf extracts of kalanchoe gastonis bonnieri and kalanchoe delagoensis showed more zone of inhibition against Bacillus subtilis. The antimicrobial activity of Kalanchoe delagoensis is less than that of kalanchoe gastonis bonnieri.

V. CONCLUSION

From all the plants taken and investigated for antimicrobial potential, all the leaf extracts showed positive results against all the tested microorganisms.

The ethanolic leaf extracts such as kalanchoe

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gastonisbonnieri and kalanchoe delagoensis exhibited mild to moderate anti microbial activity when they are tested individually against selected microorganisms.

The ethanolic leaf extract of mixture of kalanchoe gastonis bonnieri and kalanchoe delagoensis found to exhibit highest antimicrobial activity when they are tested as a mixture against selected microorganisms. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin which does not have conventional ADR like hypersenstivity, diarrhoea and immune compromisation.

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