



## **A Study on the Antibacterial Effect of Aloe Vera Leaf Extract Compared to That of Standardized Antibiotics Against Bacterial Isolated From Biotech Park Lucknow**

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### **ABSTRACT**

*The antimicrobial activity of aloe vera extract was tested against pathogenic bacteria like Staphylococcus aureus, Salmonella and E. coli at a different dose of Aloe vera extract. Aloe vera leaf powder was dissolved in sterile water for 24 hours to get the necessary extract from the leaf of this plant. The results expected to reveal that, extract at different dose was shown significant activity against Escherichia coli 25922, Salmonella typhi 14028, staphylococcus aureus 43300, staphylococcus aureus 25923, Shigella sonnei 25931, Shigella flexneri 12022, Pseudomonas aeruginosa 27853, Enterobacter faecalis 29212, Streptococcus pneumoniae 49619, Streptococcus pyogenes. The zone of inhibition was measured and compared with standard value of antibiotic discs against these bacteria. At the same time, standard value of Vancomycin(-); pure Aloe vera powder(-) and medium without antibiotic (+) were used as control. Aloe vera is also used in India topically to heal wounds and for various skin conditions, and orally as a laxative. Today, in addition to traditional uses, people take Aloe orally to treat a variety of conditions, including diabetes, asthma, epilepsy, and osteoarthritis, burns, and sunburns gastrointestinal diseases, sexual transmissible diseases, cardiac and respiratory infections. The Food and Drug Administration (FDA) has approved Aloe vera as a natural food flavoring (Natural Medicines Comprehensive Database Web site). This study reveals the importance of natural products to control antibiotic resistant bacteria, which have been a threat to human health. It is, therefore highly essential that medicinal plants whose properties have not been fully characterized should form a top agenda of top management in developing nations whose citizens are sometimes unable to afford expensive orthodox medicine.*

**Key words:** Aloe Vera plant, antimicrobial activity, traditional medicine, leave extracts, Inhibition zone.

### **INTRODUCTION**

Traditional medicine is in practice for many centuries by a substantial proportion of the population of many Countries. It is recognized that in some developing countries, plants are the main medicinal source to treat various infectious diseases. Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20% of the plants are found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources [1]. African traditional medicine is generally recognized as being organized into three levels of specialty [2]. These areas, which may overlap in some cases, are divination, herbalism, and spiritualism. The spiritualist is generally identified with a political role and public health duties; the diviner and herbalist are more involved with health delivery to the individual in traditional society. The diviner uses magic and supernatural powers to diagnose and treat diseases with the aid of ancestral spirits who endow amulet, animal parts, or herbs with the power to heal or drive away evil. The herbalist on the other hand applies knowledge attained by empirical observation [3]. This has been for a long time ignored or marginalized. In India, herbalists used to be for rural people. They were not allowed to publicly do their businesses regarding traditional medicines. Nowadays, it has been found that these people are not totally based on traditional powers, but according to the scientific researches there is a significant contribution to the health care or the wellbeing of Indian people in general. For this purpose, they have been allowed accordingly under ministry of health control. This plant

was for a long time cultured for ornamental purposes. In addition to that, its adaptation to any kind of soil contributed a lot to its propagation in India even if the scientific importance was still ignored.

*Aloe barbadensis* (Aloe vera) has a long history of use as a therapeutic agent with many reported medicinal properties. Amongst its therapeutic properties, it has been shown to have anti-inflammatory activity [4] immune stimulatory activity [5], and cell growth stimulatory activity [6,7]. Furthermore, activity against a variety of infectious agents has been attributed to Aloe vera; for instance, antibacterial [8], antiviral [9] and antifungal [10]. As we move forward, toward the brighter future we must also as scientists, give an important place to traditional healers and try to be specific on what they are making as medicines. In fact, this could help in working hand in hand to fight against different types of diseases, because there are so many which have been left behind according to different researches which are taking place and they are now significant in underdeveloped countries.

## MATERIALS AND METHODS

### MATERIALS

#### Plant materials

The plant materials used in this study were aloe vera leaves that were collected from Biotech Park.

Lucknow - Uttar Pradesh – INDIA. This was done by cutting them using the knife

#### Other materials

Petri dishes, conical flask, mortar or a blender (grinder), knife, micropipette, test tubes, test tube holder, incubator, fridge, filter paper, filter, spreader etc.

#### Bacteria

The tested organisms for this study were: *Escherichia coli* 25922, *Salmonella typhi* 14028, *Staphylococcus aureus* 43300, *Staphylococcus aureus* 25923, *Shigella sonnei* 25931, *Shigella flexneri* 12022, *Pseudomonas aeruginosa* 27853, *Enterobacter faecalis* 29212, *Streptococcus pneumoniae* 49619, *Streptococcus pyogenes*. These test organisms used in the study of antibacterial effects of Aloe barbadensis leaf extracts are medical isolates used by NRL lab technician in bacteriology section in quality control and these which are Standard Naïve Bacteria known to be affected by antibiotics.

### METHODS

#### Aloe leaves extraction

Fresh Aloe leaves were collected from the healthy aloe plant Biotech Park greenhouse Jankipuram, Kursi Road, Lucknow -Uttar Pradesh. Cell second one from Central Institute of Medicinal and Aromatic Plants Lucknow -Uttar Pradesh. Aloe powder extraction process was done by washing the aloe leaves with tap water ethanol 70% and sterile water first removing the spines and cut it into small pieces to facilitate the drying step (Figures 1 and 2). Cut leaves were put in an oven at 70°C for 24 hours. The dried aloe pieces were grind by using a mortar, the obtained powder was soaked in ethanol for 24 hours.

Figure 1: Shading of A.vera leaves Figure 2: Washing of A. vera leaves



Figure 3: A.vera powder



Figure 4: A.vera powder mixed with methanol



Above figures describe well the process of leaf methanolic extraction. 30g of Aloe verapowder was mixed 100 ml of methanol 80% in a conical flask for 72hours. The last step were evaporation of methanol in extract using evaporator.

#### FRESH LEAF AND GEL EXTRACT

##### Gel extraction

Aloe fresh leaves were collected from the field washed well with the tap water, ethanol 70% and sterile water after removing the spines, envelop them, mortared and pour extract in a sterile bottle. And this was used as such without any modification.



Figure 5: Aloe leaves pieces in a mortar

Figure 6: Aloe fresh green juice

All of these different types of extracts were applied on 10 types of bacteria for antibacterial effect testing. For both extracts serial dilution was done from 1/1; 1/10; 1/100; 1/1000; 1/10000

## RESULT

### Antimicrobial susceptibility testing

Standard antibiotic discs: ciprofloxacin and chloramphenicol

The most standard antibiotic discs used to kill or inhibit growth of these 10 bacterial strains are ciprofloxacin and chloramphenicol. Action of these antibiotics against these 10 bacteria is shown in diagrams below.

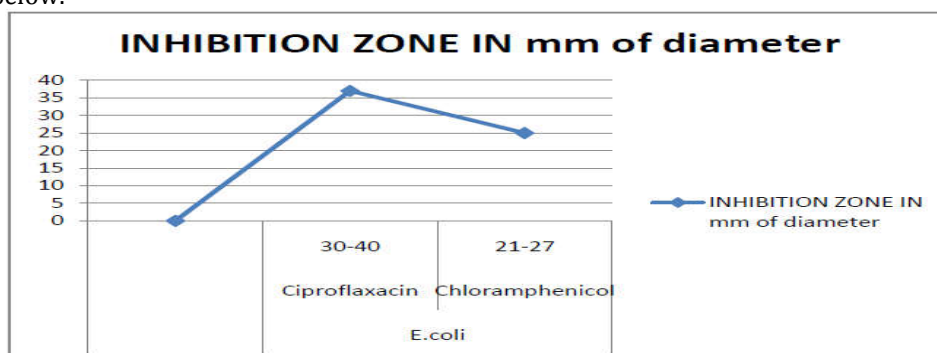


Fig 1: Effect of Chloromphenicol & Ciproflaxacin against *E.coli*

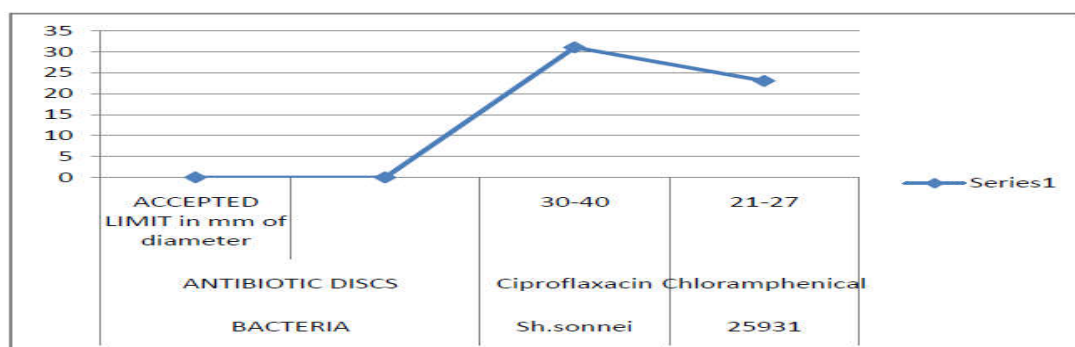


Fig 2: Effect of Chrolomphenicol & Ciproflaxacin against *S.Sonnei*

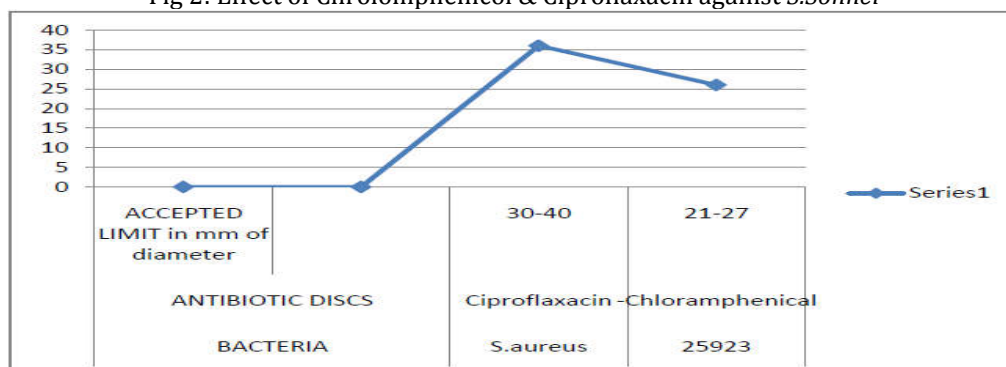


Fig 3: Effect of Chrolomphenicol & Ciproflaxacin against *S.aureus* 25923

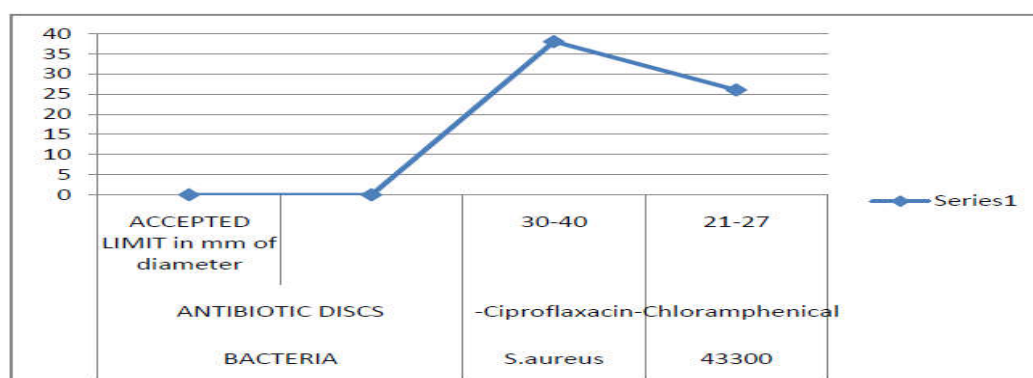


Fig 4: Effect of Chrolomphenicol & Ciproflaxacin against *S.aureus* 43300

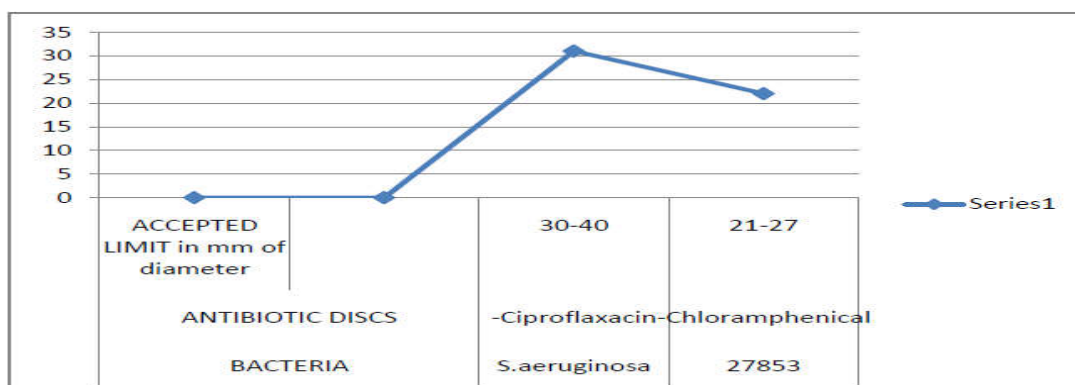


Fig 5: Effect of Chrolomphenicol & Ciproflaxacin against *S.aeruginosa*

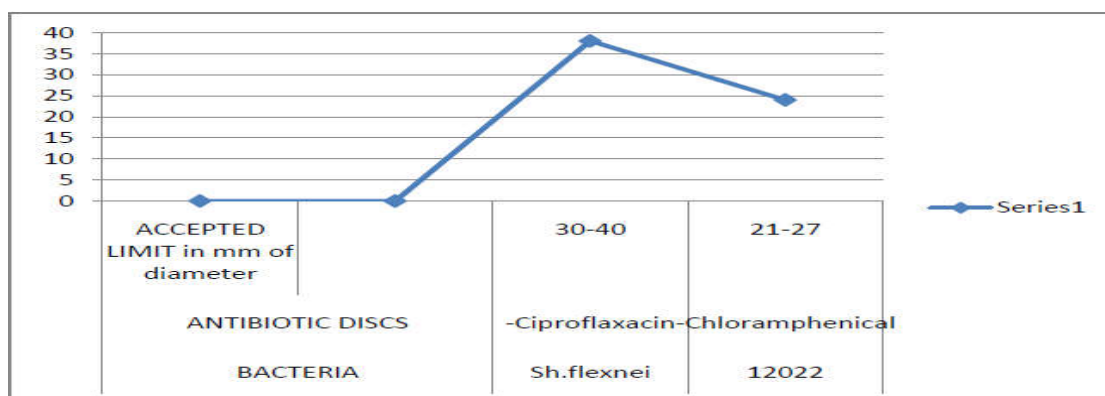


Fig 6: Effect of Chloromphenicol & Ciproflaxacin against *Sh. flexnei*

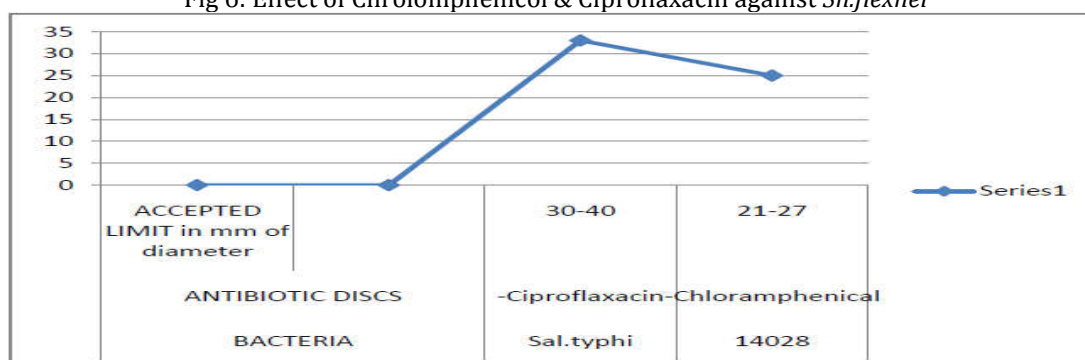


Fig 7: Effect of Chloromphenicol & Ciproflaxacin against *Sal. typhi*

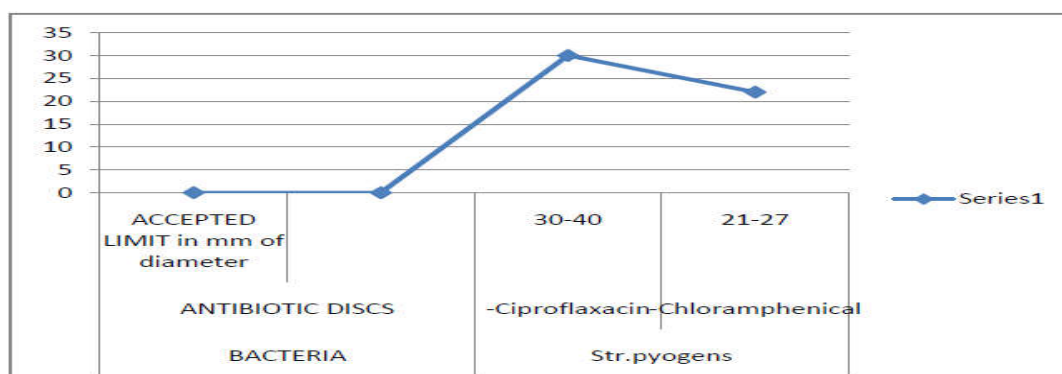


Fig 8: Effect of Chloromphenicol & Ciproflaxacin against *Str. pyogens*

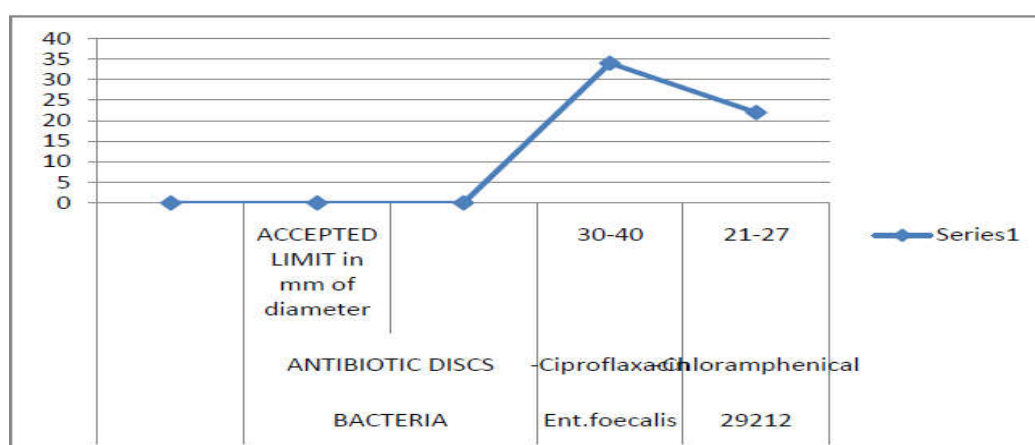


Fig 9: Effect of Chloromphenicol & Ciproflaxacin against *Ent. faecalis*

### Inhibition zones by methanolic leaf extract



As *A. vera* effects on studied bacteria were observed through the clear zones, some concentrations that were used in this study such as 1/10; 1/100; 1/1000; 1/10000 No inhibition zone observed, is only observed at high concentration (without dilution) Table above summarize result about the diameters measured in millimeters and describes well different concentrations of aloe extract and those of standard antibiotic discs applied on 10 different.

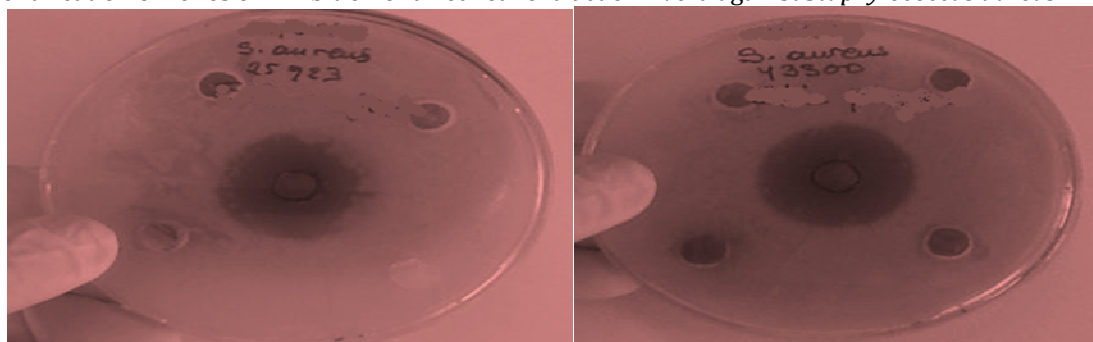
Bacteria Dilution number	Dried <i>Aloe vera</i> leaf extract				
	1/1	1/10	1/100	1/1000	1/10 000
<i>E.coli</i> 25922	22mm	0mm	0mm	14mm	20mm
<i>Sh.sonnei</i> 25931	17mm	0mm	0mm	0mm	0mm
<i>Sh.flexnei</i> 12022	15mm	0mm	0mm	0mm	0mm
<i>S.aureus</i> 25923	27mm	0mm	0mm	0mm	0mm
<i>S.aureus</i> 43300	28mm	0mm	0mm	0mm	0mm
<i>Ps.aeruginosa</i> 27853	24mm	0mm	0mm	0mm	0mm
<i>Sal.typhi</i> 14028	20mm	0mm	0mm	0mm	0mm
<i>Ent.foecalis</i> 29212	26mm	0mm	0mm	0mm	0mm
<i>Strp.pneumoni</i> a49619	23mm	0mm	0mm	0mm	0mm
<i>Strp.pyogens</i>	0mm	0mm	0mm	0mm	0mm

Table 1: Effect of *A.vera* leave extract against above bacteria

These tested bacteria are highly sensitive to *A.vera* extract at maximum concentration as it is to standard antibiotic discs as it is shown below by the pictures.

These tested bacteria are highly sensitive to *A.vera* extract at maximum concentration as it is to standard antibiotic discs as it is shown below by the pictures.

#### 1. Identification of zones of inhibition of dried leaf extract of *A.vera* against *Staphylococcus aureus*

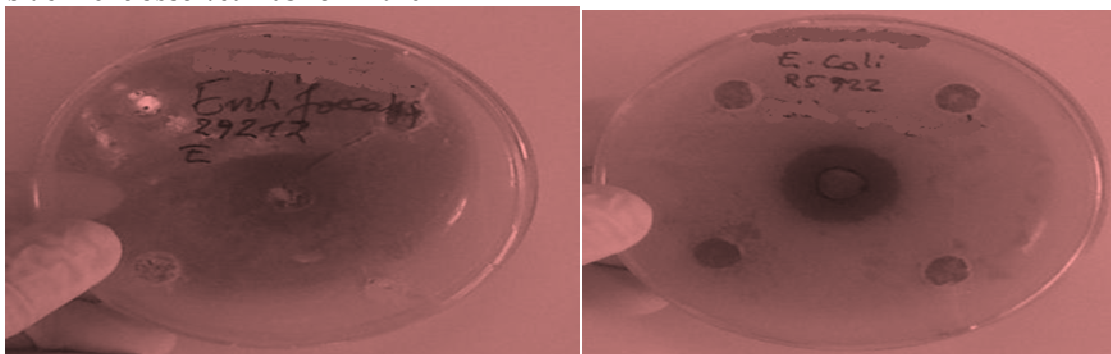


**Figure 10: Inhibition zone on *S.aureus* plates**

Inhibition zone varied from 27 to 28 mm of diameter.

2. Identification of zones of inhibition of dried leaf extract of *A.vera* against *Ent.foecalis* and *E.coli*.

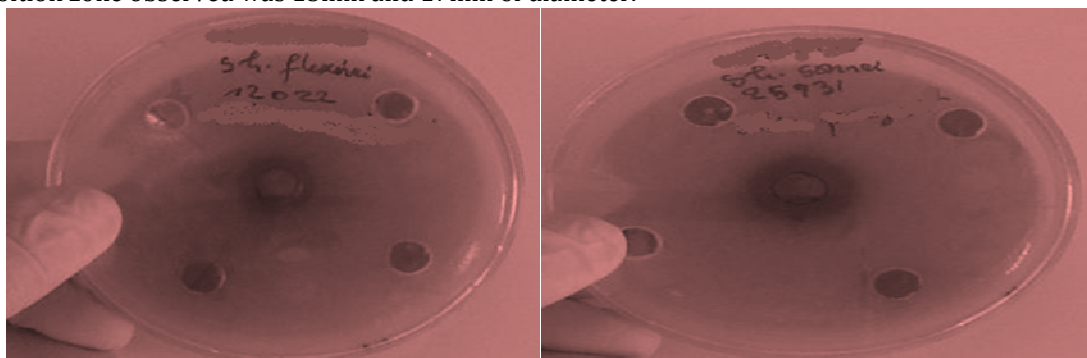
Inhibition zone observed was 26mm and 22mm.



**Figure11: TInhibition zone on *Ent.foecalis* and *E.coli***

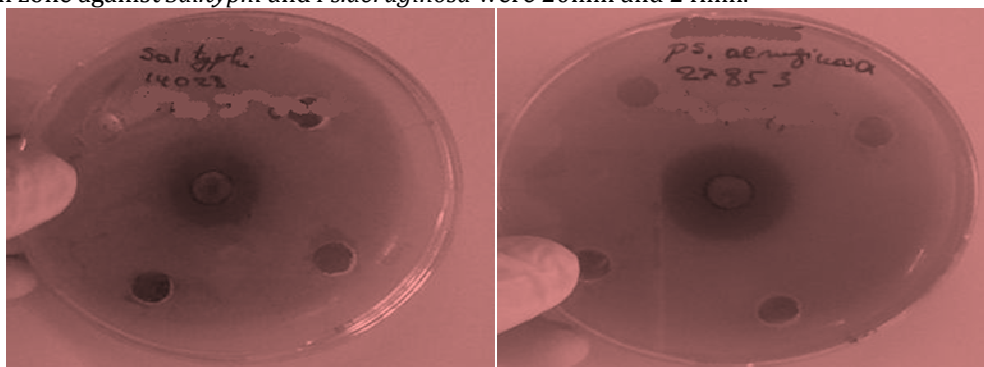
3. Identification of zones of inhibition of dried leaf extract of *A.vera* against *Sh.flexnei* and *Sh.sonnei*.

Inhibition zone observed was 15mm and 17mm of diameter.



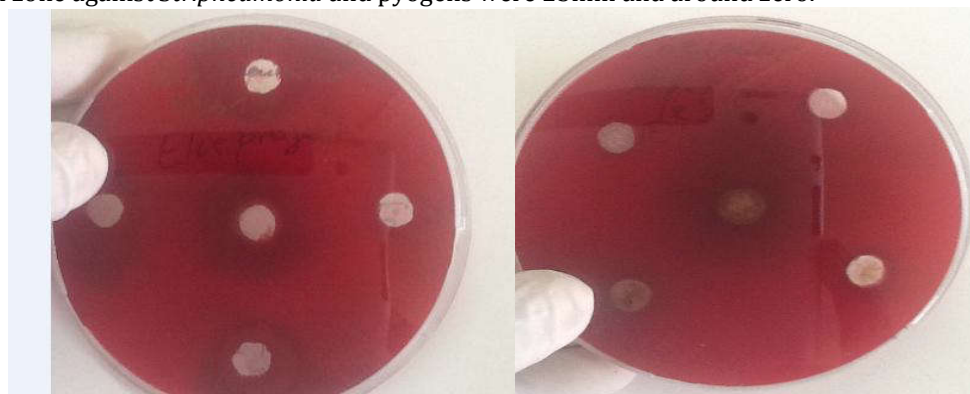
**Figure12: Inhibition zone on *Sh,sonnei* & *Sh.flexnei***

Inhibition zone against *Sal.typhi* and *Ps.aeruginosa* were 20mm and 24mm.



**Figure13: Inhibition zone on *Sal.typhi* & *Ps. aeruginosa***

Inhibition zone against *Str.pneumonia* and pyogens were 23mm and around zero.



**Figure 14: T Inhibition zone on *Str. pyogens* and pneumonia**

## DISCUSSION

Many researchers have been carried out on this plant specifically on this species, to determine its effect on different bacteria. Compared to for example, the work done by Msoffe and Mbilu in 2009 on the antibacterial effect of *Aloe secundiflora* sp, where the results showed that it has inhibitory effect on *S. aureus* and *Escherchia coli*. The results of this study show that *Aloe barbadensis* leaf extract has inhibitory effect on *S.aureus* (table 1). Among the tested bacteria, Aloe vera possesses greatest inhibitory effect on *S. aureus*. This result could be responsible for the popular use of Aloe leaves to relieve many types of gastrointestinal irritations [11, 12].

Since *S.aureus* form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract [13, 14]. Also aloe leaf extract showed effect on E coli (both fresh and ethanolic extract), by different zones of inhibition (Fig1, 3, 4). The Aloe "juice "showed inhibitory effect on Staphilococcus and Salmonella, but not on E coli. This can mean the difference between Aloe juice and leaf extract. This may be caused by the heating effect by altering the components. However Aloe gel has an effect only on E coli (Fig 4). As E coli is among the gastrointestinal flora it may not be affected when the Aloe "juice" is administered promote wound. Also healing due to the presence gel is of some components like anthraquinone and some hormones, which possesses antibacterial, antifungal and antiviral activities.

Compared to the work done on this plant (*Aloe barbadensis* sp.) in Nigeria, by the department of microbiology Federal University of Technology, Akure, Nigeria by Agarry on comparative antimicrobial activities of Aloe gel and leaf, 25mg/ml of the leaf extract and gel applied on *S. aureus* showed respectively inhibition zones of 18mm and 4mm [15]. On this study's results 4 inhibition zone of 14mm on *S.aureus* while the gel showed nothing on this bacterium.

This may be due to different factors such as plant water content, area and the soil where the plant has been grown, the climate changes etc, as the study was made for the same variety. The growth of Salmonella was also inhibited by A. vera leaf but not affected by the gel. The inhibitory effect of A. vera leaf on *S. aureus* growth, gives an explanation on its reputation as a cosmetic plant. The methanolic leaf extract is more effective than the fresh leaf extract and the juice. In all cases where possible, the ethanol extracts of these plants should be used at a concentration up to 400mg/ml (Table 1) so as to give a better treatment margin (MIC).

The difference in antimicrobial properties of a plant extract is attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), contamination by field microbes, adulteration and substitution of plants, incorrect preparation and dosage [16-18].

The use of disk papers was not successful, because wherever disks were applied no inhibition zones were observed. Instead, the wells method was adopted. There were difficulties to the medicine of diffusing on the medium.

## CONCLUSION

In conclusion, more work should be also carried out on the leaf to reveal some of its potentials. This study shows that both the gel and leaf are useful and that they can complete one another in their medicinal capabilities. The high activity of the ethanolic extracts verifies the use of the ethanolic extraction method by local herbalists.

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## Genetic Parameters Studies in Aromatic Rice

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### ABSTRACT

*The present investigation consists of 38 rice genotypes and the experiment was conducted during Kharif-2015 in Randomized Block Design with three replications. The data were recorded for 11 quantitative characters to study genetic variability, heritability and genetic advance. On the basis of mean performance, high grain yield per plant were exhibited by the genotype Pusa-1460. Analysis of variance among 38 genotypes showed significant difference for all characters studied. Highest genotypic coefficient of variation (GCV) & phenotypic coefficient of variation (PCV) was observed for grain yield per plant followed by biological yield per plant, panicle length, number of grain per panicle, harvest index, test weight and number of productive tillers per plant indicating that these characters could be used as selection for crop improvement. On the basis of high estimates of heritability coupled with high genetic advance was observed for plant height, number of productive tillers per plant, panicle length, biological yield per plant, harvest index, test weight and number of grains per panicle indicating predominance of additive gene effects and possibilities of effective selection for the improvement of these characters.*

**Keywords:** Rice, Genetic variability, Heritability and Genetic Advance.

### INTERODUCTION

Rice (*Oryza sativa* L.) is principal food crop of India as well as one of the pivotal staple cereal crops feeding more than one-third of the world's population. Rice is the staple food of about 65% of Indian population. Our rice requirement by the year 2020 is estimated to be around 122 million tons as against the present production of about 100 million tons, thus leaving a gap of about 22 million tons rice. It accounts for about 43% of total food grain production and 46% of total cereal production in the country (FAO 2015). Low productivity of rice in India is a major concern for food and nutritional security of more than 60% population which is dependent on rice. The slogan "Rice is life" is most appropriate for Indian as this crop plays a vital role in our national food security and a means of livelihood for millions of rural people of India. Rice occupies a pivotal place in Indian agriculture and it contributes to 15 per cent of annual GDP and provides 43 per cent calorie requirement for more than 70 per cent of Indians. The country witnessed an impressive growth in rice production due to adoption of semi dwarf varieties coupled with intensive input based management practices. In order to keep pace with growing population the estimated rice requirement by 2025 is about 130 million tones. The success of breeding program depends upon the quantum of genetic variability available for exploitation and the extent to which the desirable characters are heritable. Variability refers to the presence of differences among the

individuals of plant population. Variation results due to difference either in genetic constitution of the individual of a plant population or in environment, they have grown. The existence of variability is essential for improvement of genetic material. Selection is also effective when there is significant amount of genetic variability among the individuals in breeding materials. (Sumanth, V. *et al.*, 2017)

## MATERIAL AND METHOD

The material for the present investigation consists of 38 rice genotypes were grown in Randomized block design with three replications during *khariif*- 2015 at the Crop Research center, Chirodi of Sardar Vallabhbhai Patel University of Agriculture, Technology, Meerut Twenty five days old seedlings raised in nursery were transplanted at 20 cm x 15 cm spacing. Five representative plants for each genotype in each replication were randomly selected to record observations on days to 50% panicle emergence, days to 50% flowering, days to maturity, plant height, number of productive tillers per plant, panicle length, biological yield per plant, harvest index, test weight, number of grains per panicle and grain yield per plant. The variability was estimated as per procedure for analysis of variance suggested by Panse and Sukhatme (1967). PCV and GCV were calculated by the formula given by Burton (1952). Heritability in broad sense ( $h^2$ ) by Burton and De Vane (1953) and genetic advance were calculated by using the procedure given by Johnson *et al.* (1955).

## RESULTS AND DISCUSSION

Genetic variability in any crop is pre-requisite for selection of superior genotypes over the existing cultivars. The analysis of variance for different characters indicated the existence of highly significant differences for all the eleven characters study at 1% level of significance suggesting each and every genotype are genetically divergent from each other and there is ample scope for selection of characters from these diverse sources for yield and its components (Table 1). These findings were in accordance with the findings of Bekele *et al.*, (2013), Sandhya *et al.* (2015), Mishue *et al.*, (2015) and Anis *et al.*, (2016). A wide range of variance was observed for all the eleven characters. In general the phenotypic coefficient of variance was higher than genotypic coefficient variance for all yield and its contributing characters indicate the influence of environmental factors on these traits. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) ranged between (6.097 and 6.386) to (29.062 and 29.949) for days to maturity and grain yield per plant respectively. The highest GCV and PCV was recorded for grain yield per plant (29.062 and 29.949) followed by biological yield per plant (28.647 and 29.054), panicle length (27.964 and 28.571) number of grain per panicle (26.849 and 27.034), harvest index (26.740 and 26.909), test weight (25.906 and 25.968) and number of productive tillers per plant (25.736 and 25.789). Similar results were also reported by Anjaneyulu *et al.*, (2010), Idris *et al.* (2012), Sandhya (2014), Sarma *et al.*, (2015) and Anis *et al.*, (2016). Coefficients of variation studies indicated that the estimates of PCV were slightly higher than the corresponding GCV is presenting table-2 among the all traits and high values of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for these traits suggested the possibility of yield improvement through selection of these attributes. Closely relationship between GCV and PCV was found in all the characters and PCV values were slightly greater than GCV, revealing very little influence of environment for their expression. The amount of genetic variation considered alone will not be of much use to the breeder unless supplemented with the information on heritability estimate, which gives a measure of the heritable portion of the total variation. It has been suggested by Burton and Devane (1953) that the GCV along with heritability estimate could provide a better picture of the amount of advance to be expected by phenotypic selection. Since genetic advance is dependent on phenotypic variability and heritability in addition to selection intensity, the heritability estimates in conjunction with genetic advance will be more effective and reliable in predicting the response to selection (Johnson *et al.*, 1955). Heritability in broad sense includes both additive and non-additive gene effects. While, narrow sense heritability includes only additive components (Johnson *et al.*, 1955). In the present study, heritability in broad sense was estimated. Highest heritability in broad sense (> 60%) was recorded in the case of test weight (98.900) followed by number of grains per panicle (98.600), plant height (96.000), biological yield per plant (93.300), days to 50% flowering (92.800), days to 50% panicle emergence (92.200), grain yield per plant (92.000), days to maturity (91.100), panicle length (89.700), harvest index (89.100) and number of productive tillers per plant (69.2). The high heritability denotes high proportion of genetic effects in the determination of these traits and can be adopted for improving grain yield. Studied have been reported earlier also by Fiyaz *et al.* [7], Dhanwani *et al.*, [6], Sarma *et al.*, [17] and Islam *et al.*, [10]. Maximum genetic advance expressed as percentage of mean was revealed high (>20%) for number of grains per panicle

(54.925), grain yield per plant (41.633), harvest index (38.392), plant height (26.980), number of productive tillers per plant (25.259), test weight (24.402), biological yield per plant (23.184) and panicle length (21.401). Moderate genetic advance as percentage of mean (10-20%) was observed for days to 50% panicle emergence (16.725), days to 50% flowering (15.645) and days to maturity (11.990). High heritability ( $h^2$ ) coupled with high genetic advance was observed for plant height, number of productive tillers per plant, panicle length, biological yield per plant, harvest index, test weight and number of grains per panicle. High heritability coupled with high genetic advance for some of these traits have also been reported earlier by Baswaraja *et al.*, [3], Rahman *et al.*, [14], Tiwari, [19] and Islam *et al.*, [10]. This indicates substantial contribution of additive genetic variance in the expression of these traits and can be more useful in hybridization and selection for higher grain yield and these characters are largely controlled by additive gene action.

**Table-1 Analysis of variance (ANOVA) for eleven characters of rice**

Source of variation	d. f.	Replication	Treatment	Error
Grain yield per plant (g)	2.463	427.084**	11.909	
No. of grains per panicle	5.670	5507.418**	25.487	
Test weight (g)	0.023	27.131**	0.093	
Harvest index (%)	1.907	157.820**	6.164	
Biological yield per plant (g)	6.620	1015.799**	23.508	
Panicle length (cm)	0.893	27.165**	0.992	
No. of productive tillers per plant	2.888	20.619**	2.659	
Plant height (cm)	32.567	821.521**	11.149	
Days to maturity	1.719	161.700**	5.079	
Days to 50% flowering	6.482	172.264**	4.329	
Days to 50% panicle emergence	10.798	169.210**	4.636	

\*, \*\* significant at 5% and 1% level, respectively

**Table-2 Estimates of variability parameters for eleven characters in rice.**

Character	Mean	Range		Coefficient of variation		Heritability % (broad sense)	Genetic advance	Genetic advance (% of mean)
		Lowest	Highest	GCV (%)	PCV (%)			
Days to 50% panicle emergence	87.59	73.00	109.00	8.455	8.805	92.2	14.651	16.725
Days to 50% flowering	94.91	80.00	116.66	7.882	8.182	92.8	14.849	15.645
Days to maturity	118.50	106.66	136.66	6.097	6.386	91.1	14.209	11.990
Plant height (cm)	122.97	86.46	154.56	13.365	13.638	96.0	33.179	26.980
No. of productive tillers per plant	16.60	12.03	22.80	25.736	25.789	69.2	4.194	25.259
Panicle length (cm)	26.93	20.26	32.56	27.964	28.571	89.7	5.765	21.401
Biological yield per plant (g)	156.13	113.73	187.73	28.647	29.054	93.3	36.200	23.184
Harvest index (%)	36.01	23.90	54.06	26.740	26.909	89.1	13.827	38.392
Test weight (g)	25.21	21.00	31.00	25.906	25.968	98.9	6.152	24.402
No. of grains per panicle	159.21	72.40	241.66	26.849	27.034	98.6	87.451	54.925
Grain yield per plant (g)	55.85	33.70	78.33	29.062	29.949	92.0	23.253	41.633

## CONCLUSION

In the present investigation which included 38 genotypes of rice was carried out in order to study the nature and amount of variability, heritability and genetic advance for 11 quantitative characters. On the basis of mean performance, high grain yield per plant were exhibited by the genotype Pusa-1460. Analysis of variance among 38 genotypes showed significant difference for all characters studied. Highest genotypic coefficient of variation (GCV) & phenotypic coefficient variation (PCV) was observed for number of productive tillers per plant, panicle length, biological yield per plant, harvest index, test weight, number of grains per panicle and grain yield per plant indicating that these characters could be used as selection for crop improvement. High heritability coupled with high genetic advance as percent of mean estimated for plant height, number of productive tillers per plant, panicle length, biological yield per plant, harvest index, test weight, number of grains per panicle and grain yield per plant indicating predominance of additive gene effects and possibilities of effective selection for the improvement of these characters.

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