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Comparative analysis of antibacterial activity of four *Piper betel* varieties

Tarun Agarwal*, Rachana Singh, Amar Deep Shukla, Imran Waris and Ankita Gujrati

Amity Institute of Biotechnology, Amity University, Lucknow

ABSTRACT

The present study aims at a comparative study of antimicrobial properties of four varieties of Piper betel; namely Desawari, Desi, Bangladeshi and Jaleswar, cultivated in India. Cold Aqueous, Methanolic, Ethanolic, and Ethyl Acetate extracts of dried leaves of all the four varieties of Piper betel at a final concentration of 500 mg/ml were tested against pathogenic microorganisms such as Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli using agar well diffusion method.

Key words: Antibacterial properties, *Piper betel* varieties, Pathogens, Solvent extraction, Agar Well Diffusion.

INTRODUCTION

Widespread use of drugs is leading to the development of resistance against them in the pathogen [1] and also the side effects associated with them is urging people not to use them. Therefore there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious disease from medicinal plant [2].

The best solution to such a problem is to use traditional method of fighting against pathogens. The herbal medicines, medicinal plants have been used since time immemorial for the treatment of uncountable diseases. Plant based natural constituents can be derived from any part of the plant like bark, leaves, roots, fruits, seed, fruit rind, etc [3].

Piper betel L., an indigenous medicinal plant has a folk (Siddha and Ayurvedha) reputation in the rural areas of southern India, a member of the piperaceae. The plant is dioeciously, shade loving perennial root climber with glossy heart-shaped leaves. Significance of *P. betel* leaves have been explains in relation to each and every plethora of human life from the dawn of civilization [4].

Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, hysteria, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, ringworm, swelling of gum, rheumatism, abrasion, cuts and injuries etc as folk medicine while the root is known for its female contraceptive effects [5][6]. The leaves are very nutritive and contain substantial amount of vitamins and minerals. The leaves also contain the enzymes like diastase and catalase besides a significant amount of all the essential amino acids except lysine, histidine and arginine, which are found only in traces [7][8].

The four varieties of *Piper betel* taken into account are namely; Desawari, Desi, Bangladeshi and Jaleswar. The varieties show many morphological similarities and dissimilarities, as described in [4]. Along with these morphological differences, the also differ in their smell and taste. The name of varieties and their Origin is shown in Table 1.

The present paper focuses onto the comparative characterization of extent of antimicrobial properties present in the chosen four varieties of *Piper betel*.

Table 1: Origin of *Piper betel* varieties

S. No.	Name of Variety	Origin
1	*Piper betel* var. Desawari	Uttar Pradesh
2	*Piper betel* var. Desi	Uttar Pradesh
3	*Piper betel* var. Bangladeshi	West Bengal
4	*Piper betel* var. Jaleswar	Madhya Pradesh

MATERIALS AND METHODS

Sample Collection

Fresh leaves of *Piper betel* of all the four varieties namely Desawari, Desi, Bangladeshi and Jaleswar were collected from Lucknow after proper identification. The characteristics for the identification of the *Piper betel* leaves were confirmed from [4]. The *Piper betel* leaves of all four varieties were washed with tap water followed be distilled water and then dried. Dried sample was grinded into fine powder by the help of motar pestle.

Bacterial strains and culture preparations

Three pathogenic strains namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, available at Amity University, Lucknow were subcultured and used throughout the study.

Preparation of Plant Extract

Antimicrobial metabolites from the dried leaves of all the four varieties of *Piper betel* were extracted using various solvents such as Cold Aqueous, Methanol (80%), Ethanol (70%), and Ethyl Acetate (80%). 2 gm of powdered dried sample was soaked in 20ml of the respective solvents (1:10) and kept in dark for 3-4 days so that secondary metabolites diffuse out into the solvents. It was then filtered in weighed petri plate and dried in hot air oven at 50^{o}C, so that solvents get evaporated. The dried metabolite extract was dissolved in double volume of DMSO (Dimethyl Sulfoxide) thus giving the final concentration of extract to 500 mg/ml.

Antibacterial Susceptibility Assay

Antibacterial susceptibility assay was carried out by well diffusion method [9] wherein sterile Nutrient agar plates were prepared and spreaded with 60µl of the available bacterial cultures against which antibacterial activity was tested. There after 3 wells of 8 mm diameter were dug with the help of sterile borer. Two plates were prepared for each microbial strain and for each variety of *Piper betel* used in the studies.

In the plate 1; the 1st, 2nd and 3rd well was filled with 60µl of standard antibiotic Tetracycline, Methanolic and Ethanolic extract respectively. In the plate 2; the 1st, 2nd and 3rd well was filled with 60µl of standard antibiotic Tetracycline, Cold Aqueous and Ethyl acetate extract respectively.

Plates were incubated at 37ºC for 24 hours. The antibacterial activity of each extract was expressed in terms of mean of diameter of Zone of Inhibition (in mm) produced by each extract at the end of incubation period.

RESULTS

Antibiogram analysis:

In order to check the antimicrobial activity of extracted plant samples, agar well diffusion method was used. Table 2-5 below, shows the results of zone of inhibitions (ZOI) observed for the antimicrobial properties in the extracts of all the four varieties namely Jaleswar, Bangladeshi, Desawari and Desi against the standard antibiotic tetracycline used in the study.

Table 2: Antibacterial Susceptibility Assay of *Piper betel* var. Jaleswar leaves Extracts.

S.NO.	EXTRACTS	ZONE OF INHIBITION (ZOI) AGAINST (in mm)					
		Escherichia coli		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1	Ethanol	26.0	22.0	11.5	13.0	31.0	21.0
2	Methanol	31.5	22.0	11.0	13.0	33.5	21.0
3	Ethyl Acetate	19.5	21.0	17.0	14.0	31.5	20.0
4	Cold Aqueous	24.5	21.0	19.0	14.0	31.0	20.0
Note: Well diameter = 8mm.							

Table 3: Antibacterial Susceptibility Assay of *Piper betel* var. Bangladeshi leaves Extracts.

S.NO.	EXTRACTS	ZONE OF INHIBITION (ZOI) AGAINST (in mm)					
		Escherichia coli		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1	Ethanol	18.5	16.0	26.5	12.0	29.0	20.0
2	Methanol	16.5	16.0	26.0	12.0	26.0	20.0
3	Ethyl Acetate	20.0	20.0	33.0	13.5	38.0	19.5
4	Cold Aqueous	18.0	20.0	15.0	13.5	29.5	19.5
Note: Well diameter = 8mm.							

Table 4: Antibacterial Susceptibility Assay of *Piper betel* var. Desawari leaves Extracts.

S.NO.	EXTRACTS	ZONE OF INHIBITION (ZOI) AGAINST (in mm)					
		Escherichia coli		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1	Ethanol	15.5	15.0	17.0	13.0	-	16.5
2	Methanol	16.5	15.0	17.5	13.0	-	16.5
3	Ethyl Acetate	17.5	20.0	16.0	13.5	27.0	15.5
4	Cold Aqueous	16.0	20.0	13.5	13.5	-	15.5
Note: Well diameter = 8mm.							

Figure 1-8 shows the photographs of the antibiogram of all the four varieties of *Piper betel* performed against available pathogens.

Figure 1: Ethanolic (E) and Methanolic (M) Extracts of *Piper betel* var. Jaleswar.

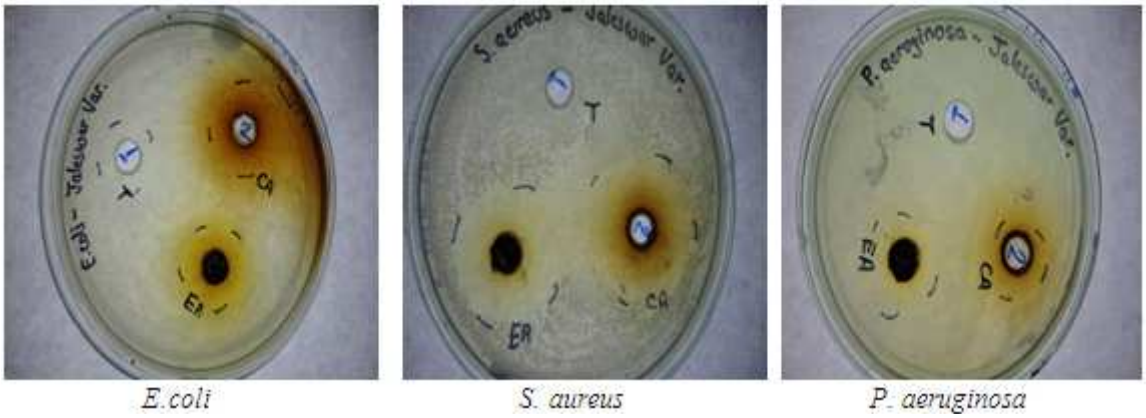


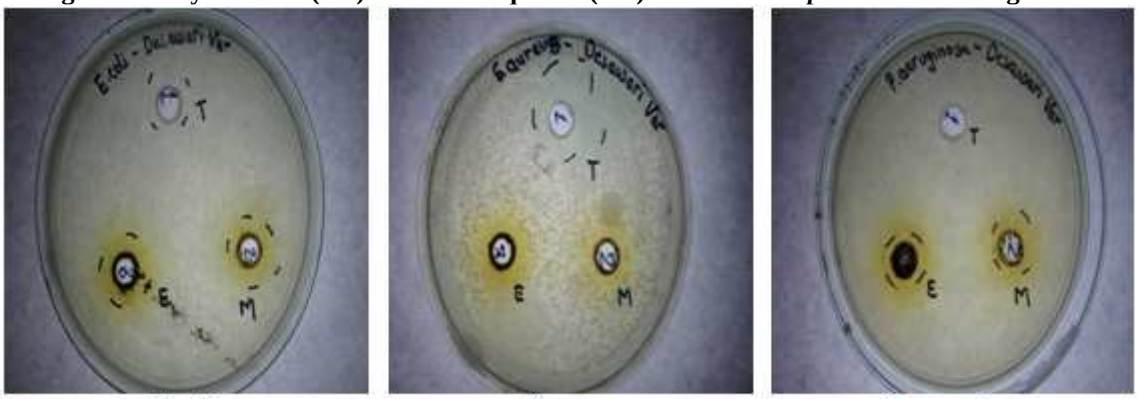
Figure 2: Ethyl Acetate (EA) and Cold Aqueous (CA) Extracts of *Piper betel* var. Jaleswar.

E.coli *S. aureus* *P. aeruginosa*

Figure 3: Ethanolic (E) and Methanolic (M) Extracts of *Piper betel* var. Bangladeshi.

E.coli *S. aureus* *P. aeruginosa*

Figure 4: Ethyl Acetate (EA) and Cold Aqueous (CA) Extracts of *Piper betel* var. Bangladeshi.



E.coli *S. aureus* *P. aeruginosa*

Figure 5: Ethanolic (E) and Methanolic (M) Extracts of *Piper betel* var. Desawari.

Table 5: Antibacterial Susceptibility Assay of *Piper betel* var. Desi leaves Extracts.

S.NO.	EXTRACTS	ZONE OF INHIBITION (ZOI) AGAINST (in mm)					
		Escherichia coli		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
		By Extract	By Tetracycline	By Extract	By Tetracycline	By Extract	By Tetracycline
1	Ethanol	15.5	14.0	17.0	16.0	23.5	18.0
2	Methanol	14.5	14.0	22.0	16.0	28.0	18.0
3	Ethyl Acetate	12.5	13.5	14.5	14.0	27.0	13.5
4	Cold Aqueous	14.5	13.5	15.5	14.0	20.5	13.5
Note: Well diameter = 8mm.							

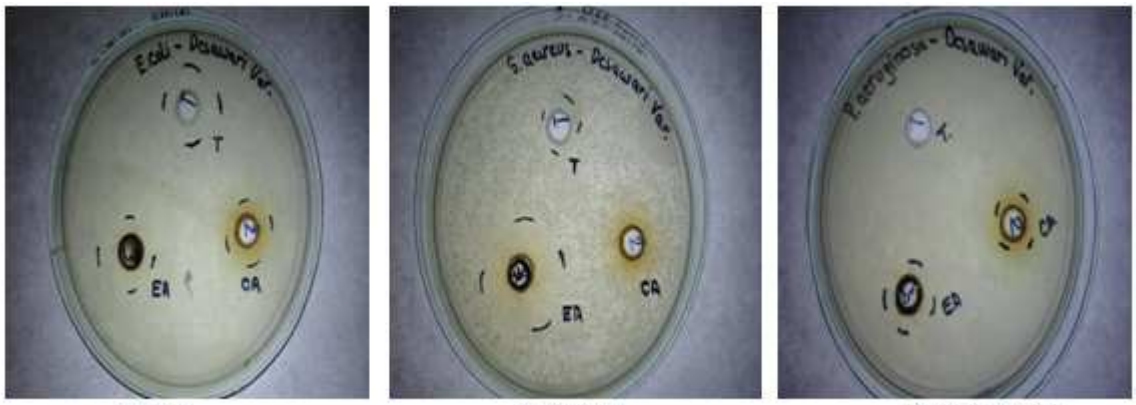


Figure 6: Ethyl Acetate (EA) and Cold Aqueous (CA) Extracts of *Piper betel* var. Desawari.

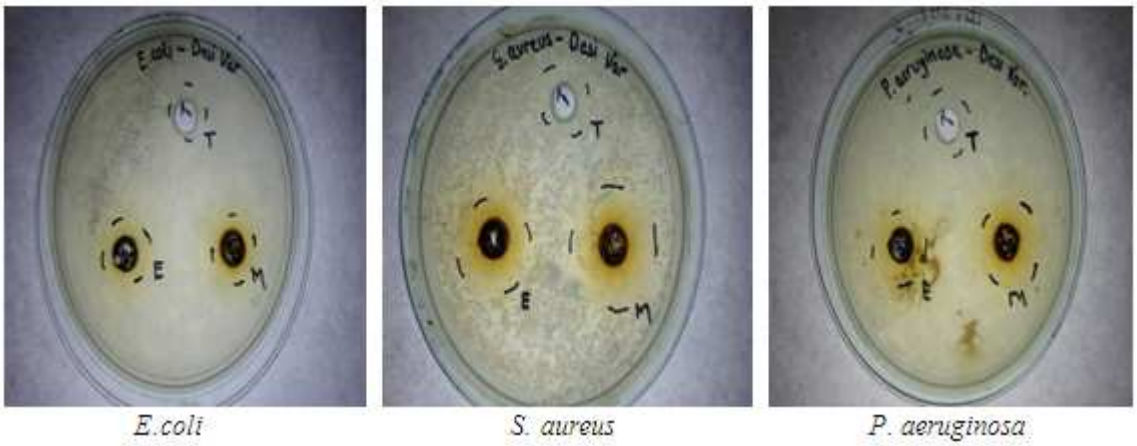


Figure 7: Ethanolic (E) and Methanolic (M) Extracts of *Piper betel* var. Desi.

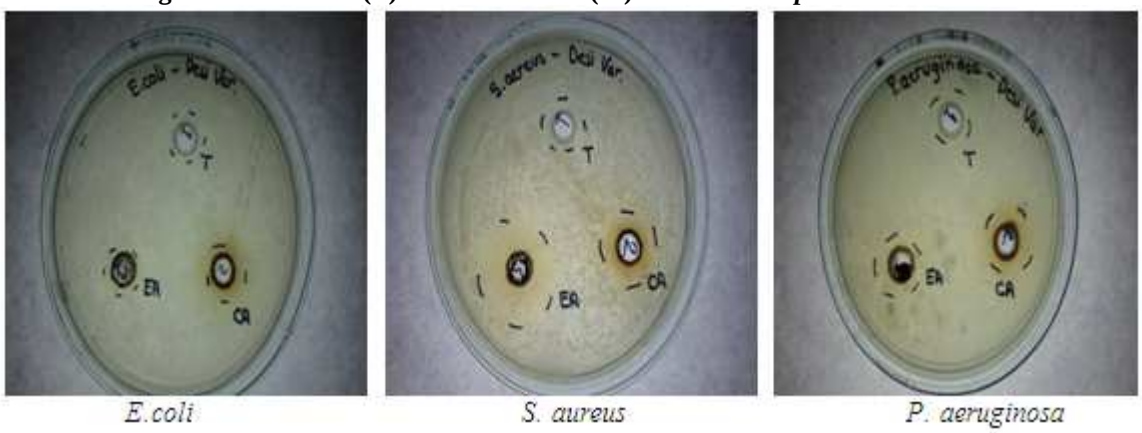


Figure 8: Ethyl Acetate (EA) and Cold Aqueous (CA) Extracts of *Piper betel* var. Desi.

DISCUSSION

Researchers have extensively studied the biological properties of *Piper betel* and their results showed that this plant is ethno-medically valuable. Aqueous, Ethanolic and Methanolic extracts of Piper betel leaves were taken for the antibacterial studies in the present research work conducted earlier [10][11].

The figure 9-11 shows the graphical comparisions made on the extent of antimicrobial properties possessed by the chosen four varieties of *Piper betel*.

Figure 9: *E. coli*: Comparative study of *Piper betel* varieties in various solvents.

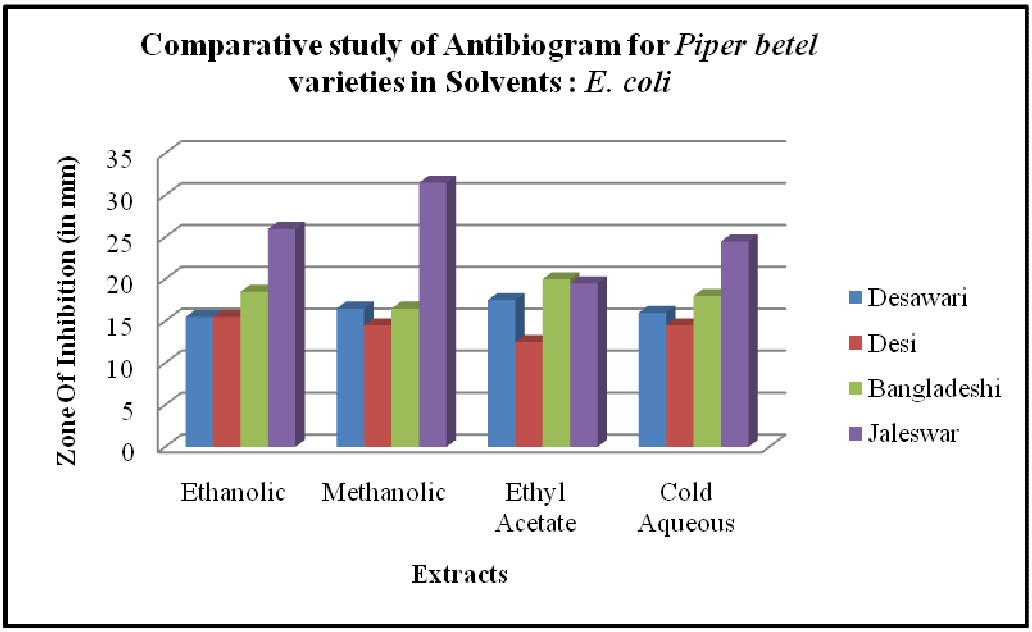
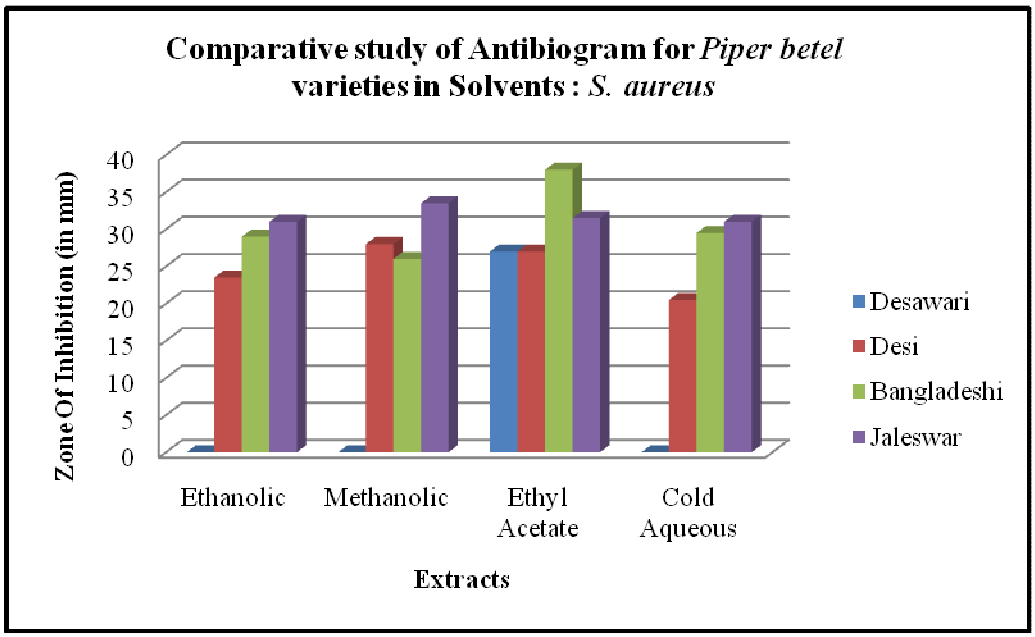


Figure 10: *S. aureus*: Comparative study of *Piper betel* varieties in various solvents.



Agar well diffusion method was used here in order to determine the anti microbial properties of the plant extracts against the pathogens performed earlier by [12].

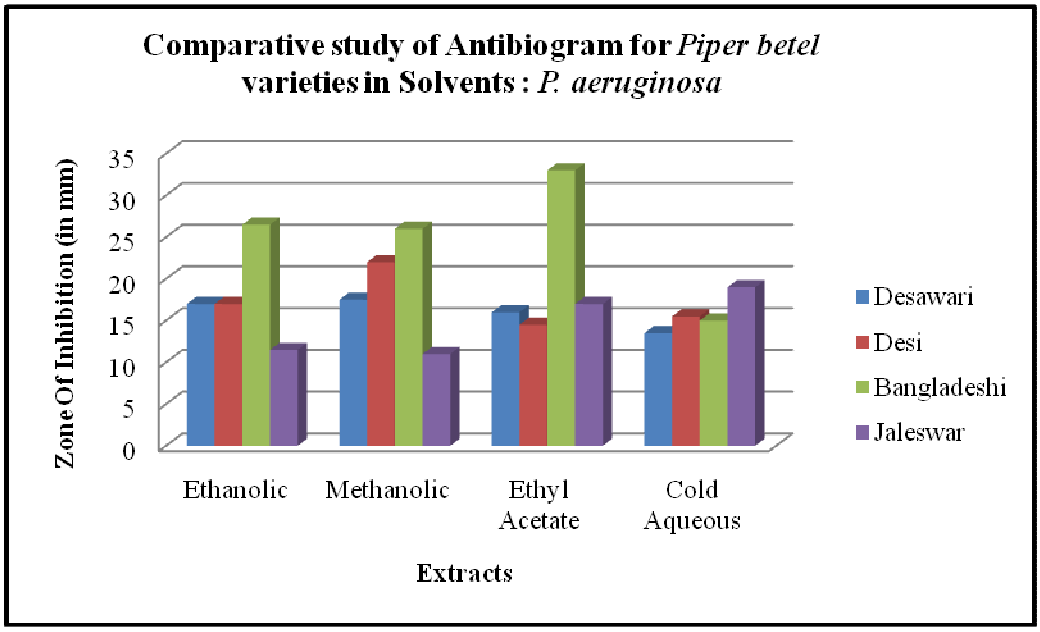
Ethyl Acetate extract of *Piper betel* var. Bangladeshi showed the maximum Zone of Inhibition (38 mm) against *Staphylococcus aureus*, followed by Zone of Inhibition (33 mm) against *Pseudomonas aeruginosa*.

The Ethyl Acetate extract of *Piper betel* of all the varieties showed the good Zone of Inhibition against *Staphylococcus aureus.*

The Ethanolic, Methanolic, Ethyl Acetate and Cold Aqueous extracts of *Piper betel* var. Jaleswar posses good antimicrobial properties against *Staphylococcus aureus* and *Escherichia coli.*

Similarly, the Ethanolic, Methanolic, Ethyl Acetate and Cold Aqueous extracts of *Piper betel* var. Bangladeshi posses good antimicrobial properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Figure 11: *P. aeruginosa*: Comparative study of *Piper betel* varieties in various solvents.



The Ethanolic, Methanolic and Cold aqueous extracts of *Piper betel* var. Desawari did not show a clear Zone of Inhibition against *Staphylococcus aureus.* The Ethyl Acetate extract of the same showed a clear Zone of Inhibition (27mm) against *Staphylococcus aureus.*

The antibacterial activity of leaves of *Piper betel* may be indicative of presence of metabolic toxins or broad spectrum antimicrobial compounds that act against gram +ve as well as gram –ve bacteria especially in solvents Ethanol, Ethyl Acetate and Methanol.

CONCLUSION

Based on the above research work it can be concluded that the leaves of *Piper betel* especially of variety Bangladeshi and Jaleswar, can be a very good source for herbal drugs especially in Ethanol, Ethyl Acetate and Methanol as a solvents. The extracts of both of these varieties in the above named solvents are effective against the above experimental bacteria. This can be explored further for the extraction of antimicrobial agents by more sophisticated procedures for extraction in order to increase the yield.

The future prospects of present research work includes isolation and purification of the therapeutic antimicrobial compounds from the active extract and there further pharmacological evaluation by several method such as – NMR, MS, GC-MS, TLC, HPLC.

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