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Evaluation of Mentha piperitaL Extracts against Biofilm Producing Bacteria

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ABSTRACT:
The objective of this study was to determine the antibacterial properties of the extracts of Mentha piperita.In current study four extracts in different solvent (chloroform, acetone, ethanol, and methanol)were examined for their antibacterial potential against different biofilm forming Gram-positive Staphylococcus aureus, Gram-negative bacteria Escherichia coli,Klebsiella pneumoniae andPseudomonas aeruginosaisolates. Antimicrobial efficacy of various extracts was assessed by using agar-well diffusion method.The zone of inhibition of plant extracts Mentha piperita with their respective solvents (ethanol, methanol, acetone and chloroform) ranged between 10- 38 mm.The ethanolic extract of plant was found to be efficient for the antibacterial activity.

KEYWORDS: Mentha piperita, plantextracts, antibacterial, agar-well diffusion method

I. INTRODUCTION:

Medicinal plants or herbal products are becoming increasingly popular as the first line of self-treatment in developing countries, such as India, China, South Africa, and the United States, working together to prevent certain diseases. In primary health care, 70–80 percent of the world's population uses unconventional medicine, often of herbal origin (W.H.O. 2002) and herbal medicines are the only choice for poor people in some parts of the world. In Ethiopia, herbal medicine is used by more than 85% of the population for primary health care (Meena et al., 2010).

According to the literature, plants were crucial in treating various illnesses, from mild cold to life-threatening diseases like tuberculosis and malaria (Abera, 2014; Chekole, 2017). Plants are widely used throughout the world in herbal medicine and the agricultural and nutritional industries. However, the antimicrobial activities of several medicinal plants have not yet been thoroughly investigated. Plants are considered the most significant source of obtaining new antimicrobials. They produce secondary metabolites, phytochemicals, which protect the plant against pathogens. Plant extracts from medicinal plants have been used to treat infectious diseases due to their availability and affordability (Kose et al., 2021). They are effective against urinary tract infections, gastrointestinal disorders, respiratory diseases, and cutaneous infections (Ali-Shtayeh et al., 2000).Mentha spp. is well-known genus, humans have used for thousands of years. Oral care goods, chewing gum, liquors, and fragrances are among the most popular uses. Its promise is based on its various properties as a carminative, antioxidant, antifungal, and antimicrobial agent (Desam et al., 2017; Santini et al., 2018). They identified it as a possible nutraceutical and functional food for disease prevention and treatment. Mentha piperita L. (peppermint) is a perennial herb that grows 50-90 cm tall and has square-shaped stems with opposite leaves (Briggs, 1993). These plants grow in temperate climates worldwide, mainly in Europe, North America, and North Africa, but are now grown in all parts of the globe. With its well-known absorbing minty odour and cooling taste, menthol is the dominant compound in peppermint (Pushpangadan & Tewari, 2006).

II. MATERIAL AND METHODS:

1) **Collection of plant materials:**The medicinal plantMentha piperita (peppermint) parts like leaves and flowers, were collected from different localities of Amravati region, East of Maharashtra state, India by standard method (Harnischfeger, 2000). The collected plant materials were soon transported to the research laboratory and washed with sterile water. Plant were identified and authenticated by a competent authority. Plant material was allowed for complete shade drying and then

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made into a fine powder with a mechanical grinder.

2) **Preparation of Extract:** Collected plant materials powder (20 gm) were extracted sequentially using a Soxhlet extractor with 250 mL of pure organic solvents separately to extract non-polar and polar compounds, with solvents of increasing polarity such as chloroform, acetone, ethanol, and methanol until the extract was clear or colourless. The obtained crude extracts were then filtered through Whatman No.1 filter paper, then evaporated to dry. Extracts were stored in sterile capped bottles under refrigeration conditions (4ºC) before use for subsequent assays.With the help of the following formula percent yield of plants crude extract, and the dry extract was calculated (Clifford et al., 1999).

$$\text{Dry extract percent yield } (\%) = 100 \text{ x } \frac{\text{weight of dry extract (g)}}{\text{weight of dry plant (g)}}$$

Figure: **Extraction of *Mentha piperita* (Peppermint)**

3) **Collection, Isolation and identification of clinical isolates**A total of 342 specimens from urine, catheter tip, pus, blood, and sputum were collected from government general hospitals and private pathology laboratories within Akola and Amravati city. The samples collected from Amravati and Akola regions were from individual patients and considered to include a distinct pathogenic strain. Hence all of the samples of urine, blood, sputum and catheter tips were used for bacterial isolation. The patients were primarily diagnosed for a probable causative pathogenic bacterium, which helped in presuming the type of media to be used. Though, every sample was spread on all the four selective media viz EMB media for E. coli and K. pneumoniae, Mannitol salt agar for S. aureus, and Cetrimide agar for P. aeruginosa. After spread plate, the selective isolates were made as pure cultures and used for routine cultural, morphological, biochemical characteristics, other tests using Bergey's Manual of Systematic Bacteriologyfurther confirmation of the isolated species.

4) **Biofilm Production Assay:** Biofilm production was assayed by the tube method: a loopful of isolated organisms was inoculated in 10 mL of trypticase-soy broth with 1% glucose in test tubes. Incubate the tubes for 24 hours at 37 °C. After incubation, tubes were decanted, washed with phosphate buffered saline (pH 7.3), and dried. Tubes were then stained with crystal violet (0.1%). Wash excess stains with distilled water. Tubes were dried in an inverted position. The scoring for the tube method was made corresponding to the results of the control. Biofilm formation was considered positive when a visible, thick film lined the wall and the bottom of the tube (Christensen et al., 1982).

5) **Antimicrobial activity testing of plant extracts:** The agar-well diffusion method was used for the screening of the antimicrobial potential of selected plant extracts against the test bacterial isolates. Mueller-Hinton agar plates were prepared by pouring about 25 mL of the sterilised medium into a Petri dish to a depth of 3–4 mm. The bacterial suspension was spread evenly over the surface of the Mueller Hinton agar plate. Wells were then bored into the agar medium with a 6 mm sterile cork borer. The stock solution of extracts (100 mg/ml) was prepared by dissolving crude extract with 2% DMSO. A hundred microliters of each solvent extract of

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plant materials, at a concentration of 100 mg/mL, were dispensed into each well in the Petri plate. Plates were incubated in an upright position, and after incubation at 37 ºC for 24 hours, the plates were checked for the zone of inhibition as per the CLSI. (2012). DMSO was used as a negative control. The assessment of antibacterial activity was done by measuring the diameter of the growth inhibition zone formed around the well. The test was performed in triplicates.

III. RESULTSAND DISCUSSION:

The Leaf extract preparation and yield

Table1 shows the yield of the plant extract using different solvents. These extract showed variable percentage of yield relative to the polarity of the solvents. The potential of various solvents to remove extractable components from plant parts went in this order: Ethanol > Methanol > Acetone > Chloroform. Ethanolic extract showed a higher extractive yield.The extractive yield of the plant with Chloroform that is weakly polar was between 4.0 to 4.6 % while that of Ethanol that is highly polar was between 8.6 to 11.7 %. It may be inferred that compounds having high polarity showed percent yields higher than that of less polar compounds. The extraction was consistent throughout the experiment as shown by a low standard error in the dry mass and percent yield. The resultant dry mass was dissolved in 1 ml DMSO.

Table:1 The yield of the plant extract using different solvents

Sr. no	Name of the plant	Solvent used	Dry mass yield (g)	Percentage Yield (%)
1	Mentha piperita (Peppermint)	Chloroform	0.81 ± 0.06	4.05
		Acetone	0.89 ± 0.06	4.45
		Ethanol	2.32 ± 0.06	11.60
		Methanol	2.3 ± 0.06	11.50

Collection, Isolation and identification of clinical isolates

A total of 342 samples, 94 urine samples (27.5 %), 83 sputum samples (24.3 %), 77 blood samples (22.5 %), 46 pus samples (13.4 %) and 42 (12.3 %) catheter tip specimens were sampled. From those, 184 samples showed bacterial growth to give pure isolates. Every sample corresponded to a separate infectious microbe. Hence all isolates were cultured and maintained for further investigations.

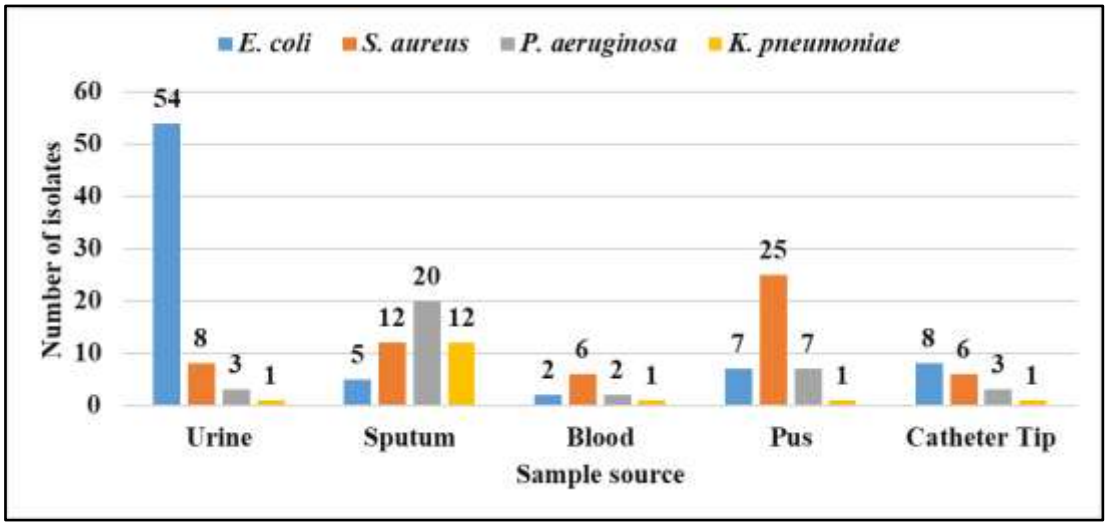

Figure 2: Comparative analysis of the distribution of isolates out oftotal 184 isolates

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Biofilm production potential of isolates

Out of 184 isolated 90 isolates (75 %) were shown to have biofilm production which included strong and moderate biofilm producers and 30 weak or non-biofilm producers. The highest biofilm producing percent isolates were of P. aeruginosa (100 %), followed by E. coli (74.07 %) and S. aureus (73.68 %). The lowest percentage of biofilm producers were of K. pneumoniae (25 %).

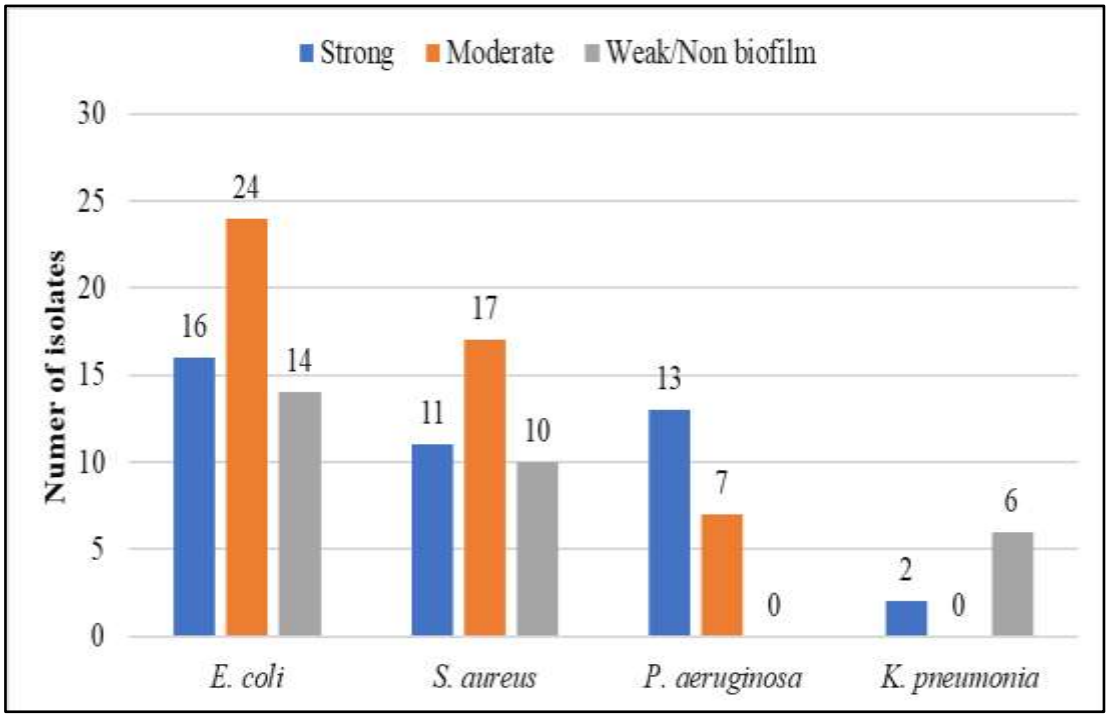

Figure 3: Comparative assessment of total biofilm producing isolates

Out of 184 bacterial isolates, total 90 biofilm producing were selected for susceptibility testing. The results of susceptibility testing of different solvent extracts of plantMentha piperitaagainst 90 biofilm producing isolates of E. coli, S. aureus, P. aeruginosa and K. pneumoniae are mentioned below in this section. Out of these, 40 E. coli, 28 S. aureus, 20 P. aeruginosa and 2 K. pneumoniae biofilm producing isolates have been studied and shown. The results of the susceptibility test of the leaf extracts were categorized similar to that done during the antibiotic testing (Resistant (≤ 13mm), Intermediate (14-16 mm), susceptible (≥ 17 mm). All the tested extracts demonstrated varying degrees of antibacterial activity against different isolates. DMSO was used as a negative control, which showed no growth inhibition of the bacterial isolates, denoting that it had no role in bacterial inhibition.

Antibacterial activity of plant extracts

Antibacterial activity of four solvent extracts of Mentha piperita against E. coli, S. aureus,P. aeruginosa, and K. pneumoniae was examined. Overall, M. piperita was shown to be moderately efficient against E. coli,.However, among these extracts, the ethanolic extract efficiently inhibits the isolates and the zone of inhibition is found to be in the range of 15- 26 mm. as showed in **Figure 4 & 8.**

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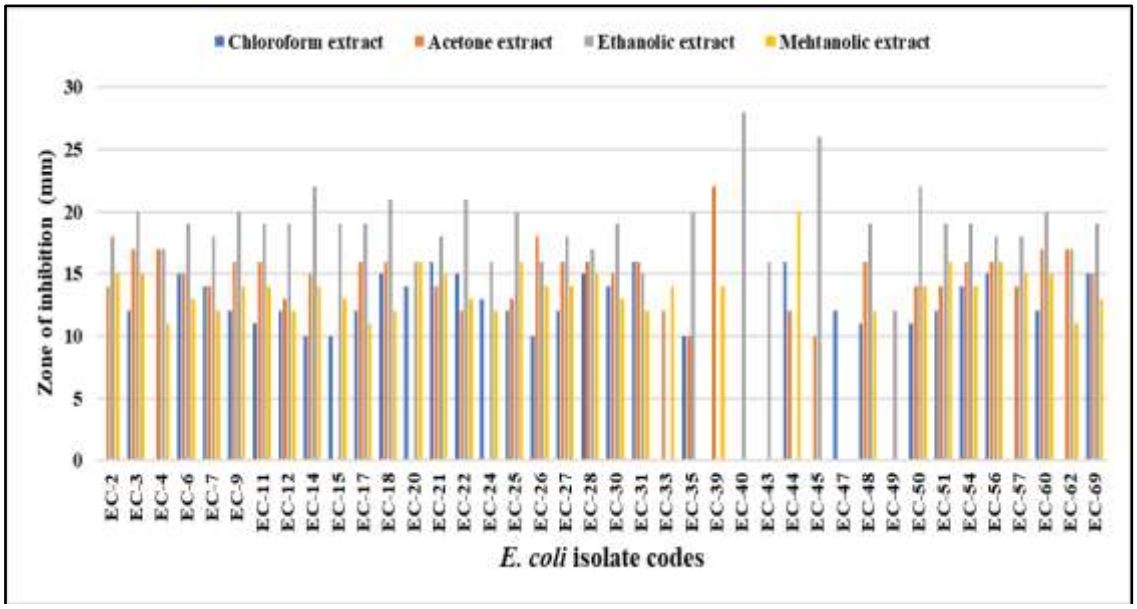

Figure 4: Antibacterial activity of solvent extract of M. piperita against biofilm-producing

E. coli isolates

S. aureus isolate was subjected to antibacterial assays using Mentha piperita. In these studies. Many isolates showed susceptibility against the ethanolic extract of M. piperita in the broader range of 10- 23 mm. The isolates that were susceptible to this extract were 93 %. The moderate zone of inhibition was shown by acetone extract in the range of 10- 24 mm with susceptibility of S. aureus isolates against acetone extract was 75 %. However, methanolic extract exhibited less inhibition against S. aureus and the susceptibility shown by the extractwas 40 %. The susceptibility and antibacterial activity of methanolic extract was found to be lower than acetone and ethanolic extract. The least performing was chloroform extract in the range of just 10- 15 mm in which merely 25 % isolates were inhibited. Details of the performance of the M. piperita extracts against S. aureus have been shown infigure 5& 8.

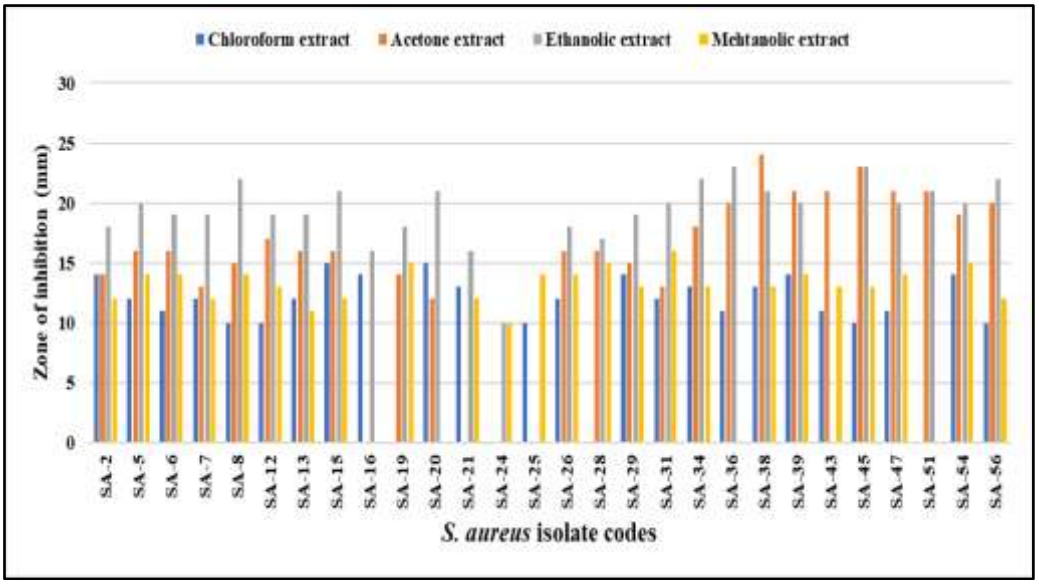

Figure 5: Antibacterial activity of solvent extract of Mentha piperita against biofilm-producing S. aureus isolates

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Mentha piperita acetone extract was efficient in inhibiting the P. aeruginosa isolates. The methanolic extract and chloroform extract shown the least activity towards the P. aeruginosa isolates with susceptibility found to be nearly just 5 %. Even the zone of inhibition was in the range of 10- 15 mm.Details of the performance of the M. piperita extracts against P. aeruginosa have been shown figure 6&8.

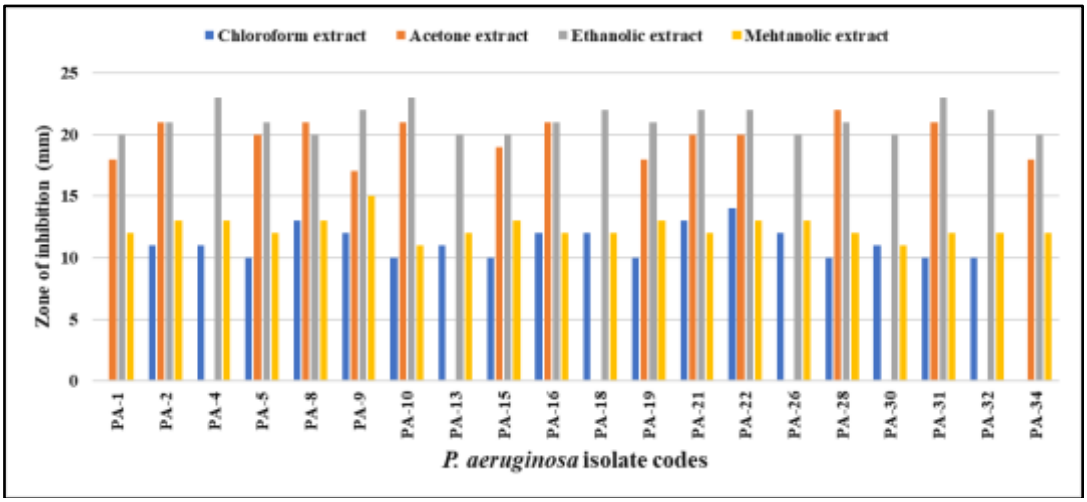

Figure 6: Comparison of antibacterial activity of solvent extract of Mentha piperita against biofilm-producing P. aeruginosa isolates

Two isolates of K. pneumoniae with biofilm formation were chosen for this experiment. Overall, the ethanolic extract showed stronger inhibition against K. pneumoniae isolates. KP- 06 was susceptible to both ethanolic and methanolic extract with 22 mm zone of inhibition. KP- 03 was found to be resistant to methanolic and chloroform extract. Details of the performance of the M. piperita extracts against K. pneumoniae have been shown in figure 7 & 8 .

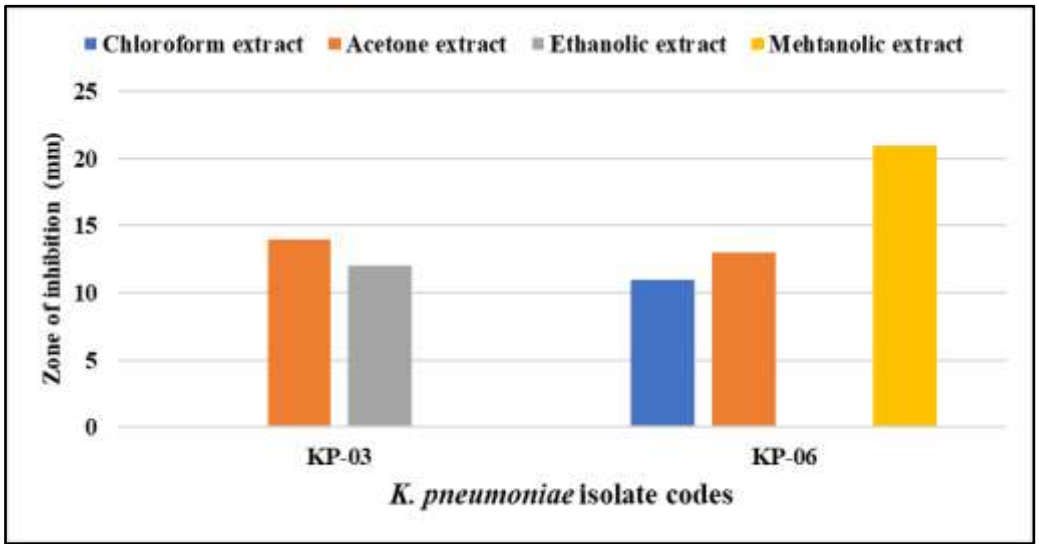

Figure 7: Comparison of antibacterial activity of solvent extract of Mentha piperita against biofilm-producing K. pneumoniae isolates

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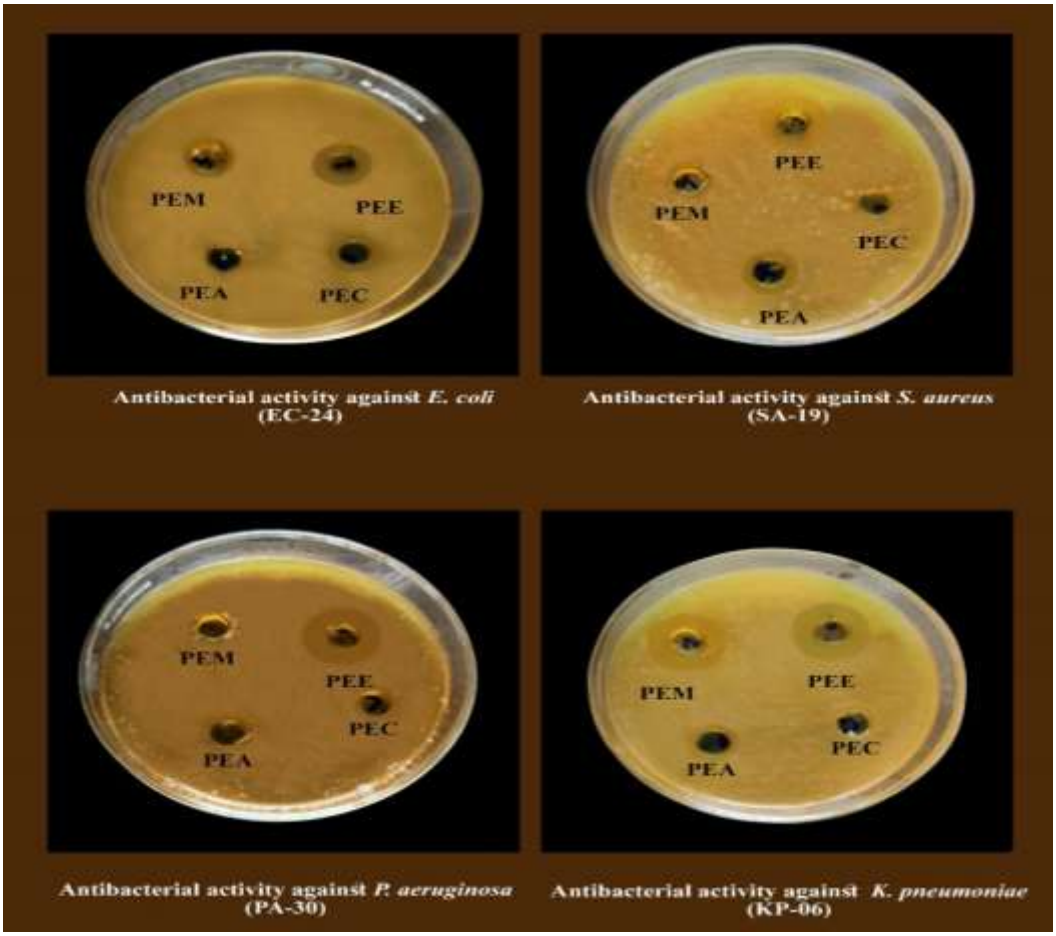

Figure 8: Antimicrobial activity of Mentha piperita extracts in solvents like Methanol, Ethanol, Acetone and Chloroform against biofilm producing bacterial isolates.

IV. CONCLUSION:

In this work, antimicrobial property of Mentha piperitaextract powder has been evaluated by agar well diffusion method. The zone of inhibition of plant extracts Mentha piperita with their respective solvents (ethanol, methanol, acetone and chloroform) ranged between 10- 38 mm. The ethanolic extract of plantwas found to be efficient for the antibacterial activity that was in the range of (20- 38 mm), followed by acetone, methanol and chloroform extracts respectively that exhibited less activity.The majorly of plant extracts of plants showed a very efficient antibacterial activity against E. coli (90 % inhibition),

A lack of detailed understanding of the mechanisms of the individual components of plant extracts thus accrediting our superficial consideration inhibitory activity. Future research should therefore explore the mechanisms of the individual components of plant extracts to combat against biofilm forming bacterial diseases. Therefore, innovative new strategies for drugs development could provide an interesting platform in the near future for this area of research.

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