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Phytochemical analysis and antimicrobial activity of *Eupatorium triplinerve* Vahl

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Abstract
Objective: The objective of the present work is to evaluate the presence of phytochemical constituents and antimicrobial activity of different extract of leaves of *Eupatorium triplinerve* Vahl.
Methods: The serial exhaustive extraction was the clones of serial solvents: Hexane, Chloroform, Ethyl acetate and Methanol with increasing polarity using soxhlet apparatus. The Phytochemical analysis was done by using the standard procedures. Anti microbial activity was evaluated by agar diffusion method of eight human pathogens.
Results: The results related that the leaf extracts contain a broad spectrum of secondary metabolites. Alkaloids, Flavonoids, Tannin, Saponin, Phenol, Ttriterpenes, steroids, in major proper against. Ethyl acetate extract was shown to be more effective against all the solvents followed by Hexane, Chloroform, Ethyl acetate and Methanol extracts. The *Staphylococcus aureus* (19.4mm-1000µg/mg) was found to be most sensitive organism followed by *Streptococcus phyogens* (17.8mm/1000µg/ml) and fungal strain *Aspergillus fumigates* (21.5mm 1000µg/ml) *Aspergillus niger* 19.8 mm-1000µg/ml). The stem extract of hight activity of *staphylococcus aureus* (19.0mm-1000µg/mg), gram negative bacteria *Klebsielia pneumonia* (14.2mm/1000µg/ml) and fungal pathogen *Aspergillus fumigates* (20.3 mm 1000µg/ml). Root extract of hight activity of *staphylococcus aureus* (20.8 mm-1000µg/mg), gram negative bacteria *Klebsielia pneumonia* (14.5mm/1000µg/ml) and fungal pathogen *Aspergillus fumigates* (21mm 1000µg/ml).
Conclusion: The present study concludes that the different extracts from *Eupatorium triplinerve* leave to contain a broad spectrum of secondary metabolites and also exhibits antimicrobial activity against all the tested microorganism. It can also be concluded that *Eupatorium triplinerve* plant can be exploited to discover natural products that may serve leads in the development of new pharmaceuticals.

Keywords: Solvent Extracts, Phytochemical Analysis, Antimicrobial Activity, Secondary Metabolites.

1. Introduction
The extensive use of natural as primary health remedies due to their pharmacological properties are quite common. Natural products are preferred for biologically screening based on ethno-medical use of plants, because many infections disease are known to have been treated with herbal remedies through the history of mankind [1]. The investigation into the efficacy of plant based drugs has been paid attention because of their few side effects, cheap and easy availability [2, 3]. The plants used in traditional medicine areas still a large source of natural antioxidant, antimicrobial, anticancer agents that might serve as leads for the development of novel drugs [4].
Natural crude drugs extracts and biological active compounds isolated from plant species used in traditional can be prolific resource for such new drugs. Microorganisms have been development resistant to many antibiotics due to the indiscriminate used of antimicrobial drugs inducing thus an increase in problems with clinical treatment of infectious disease. In addition antibiotic area sometimes associated with adverse effects of the host who includes hypersensitivity, depletion of gut and mucosal microorganism, immunosuppressant and Allergic reactions. Therefore, there is a need for alternative antimicrobial drugs for the treatment of infectious disease. One approach is to screen local medicinal plants for possible antimicrobial properties. Medicinal herbs represent a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained [5]. Due to alarming increase in the rate of infection with antibiotic resistant the microorganism and due to side

effects [6] of some synthetic antibiotics there is an increasing interest in medicinal plant accumulate to synthetic [7]. Many higher plants accumulate extractable organic substance in quantities sufficient to be economically useful as pharmaceuticals. Species of higher plants were less much surveyed for antibacterial activity [8]. *Eupatorium triplinerve* Vahl or *Eupatorium ayappana* familiarly known as *ayappana* in Malayalam language belongs to the family Asteraceae and is an ornamental plant. The essential oil from the plant has been reported to possess a number of medicinal properties such as Central nervous system (CNS) depressant, analgesic and sedative effects [9].

The methanolic extract of *Eupatorium triplinerve* is reported to have hepatoprotective effect and antioxidant effect against carbon tetrachloride induced hepatotoxicity in rats [10]. While the ethanolic extract had analgesic effect in inflammatory model of pain [11]. Antibacterial and antifungal activity [12, 13]. Antiseptic and in the treatment of various ulcers and hemorrhages [14].

2. Materials and Method

2.1 Collection of Plant material

Healthy plants of *Eupatorium triplinerve* Vahl were collected from State Forestry Research Institute Kolapakkam, Kanchipuram District, Tamilnadu. Deposited in the Herbarium of Department of Biotechnology, Periyar University, Salem (PU/BT/ *Eupatorium triplinerve* Vahl/ Voucher specimen No: 012/2010).

2.2 Plant sample extraction

100 g of air dried powder of leaf samples was extracted from 500 ml of solvents such as methanol with gentle stirring for 72 h. The sample was kept in the dark for 72 h with intermittent shaking. After incubation the solution was filtered through Whatman No. 1 filter paper and the filtrate was collected (crude extracts). It was then transferred to glass vials and kept at 4 °Cs before use.

2.3 Phytochemical Screening

The different chemical tests were performed for establishing profile of the extract from its chemical composition, the following chemical tests for various phytoconstituents in the powder, (Hexane, Chloroform, Ethyl Acetate, and Methanol)

(1) Test for alkaloids

i) **Dragendroff's Test:** In a test tube containing 1ml of extract, few drops of Dragendroff's reagent was added and the color developed was noticed. Appearance of orange color indicates the presence of alkaloids.

ii) **Wagner's Test:** To the extract, 2ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicates the presence of alkaloids.

iii) **Mayer's Test:** To the extract, 2ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

iv) **Hager's Test:** To the extract, 3 ml of Hager's reagent was added; the formation of a yellow precipitate confirmed the presence of alkaloids.

(2) Test for tri-terpenoids

i) Salkowski test: To 1 ml of extract, tin (one bit) and vinyl chloride were added. Appearance of pink color indicates the presence of tri-terpenoids.

ii) Horizon reaction: When a substance was heated with a trichloro acetic acid, red to purple color was observed.

(3) Test for coumarins

To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

(4) Test for steroids

i) Liebermann Burchard Test: To 1ml of extract, 1ml of glacial acetic acid and 1mL of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution becomes red, then blue and finally bluish green, indicates the presence of steroids.

(5) Test for tannins

i) To the extract, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

(6) Test for saponins

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

(7) Test for flavonoids

i) Shinoda Test: To the extract, a few magnesium turnings and 1-2 drops of conc. HCl Were added; formation of red color shows the presence of flavors.

(8) Test for Quinones

To 1 ml of the extract, 1 ml of concentrated sulfuric acid was added. Formation of red color shows the presence of Quinones.

(9) Test for flavanones

i) To the substance, 10% sodium hydroxide was added; yellow to orange color shows the presence of flavanones.

ii) To the substance can. Sulfuric acid was added, orange to crimson red color confirms the Presence of flavanones.

2.4 Test Organisms

The test pathogens used for screening efficacy of plant extracts were *Staphylococcus aureus*, *Streptococcus pyogens*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigates.*

2.5 Antimicrobial Activity

The antimicrobial assay was carried out using Agar well diffusion method [15, 16]. *Ciproflaxin* (5µg/ml disk, *Ketocazale* (10 µg/ml disk) is used as drugs and the corresponding solvents (Hexane, Chloroform. Ethyl acetate and Methanol) are used as positive controls. About 20 ml of Muller- Hinton agar medium for bacteria and potato dextrose agar for fungus was poured in the sterilized Petri dishes and allowed to solidify. The agar medium was spread with 24hrs cultured 108 CFU/ ml of microbial strains by a sterilized rod. Wells of 6mm in diameters were made in the culture medium using sterile cork borers. About 50µl of the plant extracts (1mg/ml) was added to the wells. Plates were then incubated at 37 ^{0}C for 24h. Antimicrobial activity was evaluated by measuring the inhibition zone diameter in mm into the well. The assay was carried out in triplicates and the result thus obtained is taken as the mean of the three readings for each concentration and no statistical tools were used to calculate the standard deviation.

3. Results

3.1 Phytochemical screening

Phytochemical evaluation of the various extracts from of the leaf, stem and root of *Eupatorium triplinerve* Vahl were done for the presence Alkaloids, Flavonoids, Saponin, Tannin, Phenol, Treterpinoid, Glycoside, Steroids, Coumarin, and Valatile oil the results are presented in (Table 1).

Table 1: Phytochemical screening of the leaf extracts of *Eupatorium triplinerve* Vahl

S. No	Chemical content	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Methanol Extract
1	Alkaloids	-	+	+	+
2	Flavonoid	+	-	+	+
3	Saponin	+	+	+	+
4	Tannin	+	+	+	+
5	Phenol	-	+	+	+
6	Triterpinod	-	+	+	+
7	Glycoside	+	-	+	+
8	Steroid	+	-	+	-
9	Coumarin	+	+	+	-
10	Volatile oil	+	-	-	-

Table 2: Phytochemical screening of the stem extracts of *Eupatorium triplinerve* Vahl

S. No	Chemical content	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Methanol Extract
1	Alkaloids	+	+	+	+
2	Flavonoid	-	-	-	-
3	Saponin	+	+	+	+
4	Tannin	+	-	-	+
5	Phenol	+	+	+	+
6	Triterpinoid	+	+	+	+
7	Glycoside	-	-	+	+
8	Steroid	+	+	+	+
9	Coumarin	+	+	+	+
10	Volatile oil	-	-	-	-

Table: 3 Phytochemical screening of the root extracts of *Eupatorium triplinerve* Vahl

S. No	Chemical content	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Methanol Extract
1	Alkaloids	-	-	+	-
2	Flavonoid	-	-	+	-
3	Saponin	-	-	-	+
4	Tannin	-	-	-	+
5	Phenol	-	+	+	-
6	Triterpinoid	+	-	+	-
7	Glycoside	-	+	+	+
8	Steroid	-	+	+	+
9	Coumarin	-	-	+	+
10	Volatile oil	-	-	-	-

3.2 Antimicrobial Activity

The antimicrobial activity was examined by agar well diffusion method. The Ethyl acetate extract from *Eupatorium triplinerve* leaf exhibited potent antimicrobial activity towards all the microbes. The zone of inhibition values are presented in (Table 4).

Staphylococus aureus was found to be more highest activity towards the ethyl acetate extract from the leaf with maximum inhibitory zone (19.4mm) followed by Hexane (11.3mm), Chloroform(11.8mm) and Methanol(17.3mm). *Streptococus pyogens* was found to be more higher activity towards the ethyl acetate extract from the leaf with maximum inhibitory zone (17.8mm) followed by Hexane (12.5mm), Chloroform (12.8mm) and Methanol (15.0mm).*Pseudomonas aeruginosa* was found to be more higher activity towards the ethyl acetate extract from the leaf with maximum inhibitory zone (14.8mm) followed by Hexane (11.2mm), Chloroform (11.5mm) and Methanol (13.5mm). *Klebsiella pneumonia* was found to be more higher activity towards the ethyl acetate extract from the leaf with maximum inhibitory zone (14.5mm) followed by Hexane (12.0mm), Chloroform (13.0mm) and Methanol (13.5mm). *Escherichia coli* was found to be more higher activity towards the ethyl acetate extract from the leaf with maximum inhibitory zone (12.8mm) followed by Hexane (10.2mm), Chloroform (11.0mm) and Methanol (12.0mm). *Aspergillus niger* was found to be more higher activity towards the ethyl acetate extract from the leaf with maximum inhibitory zone (19.8mm) followed by Hexane (13.5mm), Chloroform (14.0mm) and Methanol(17.5mm). *Aspergillus flavus* was found to be more higher activity towards the ethyl acetate extract from the leaf with maximum inhibitory zone(18.8mm) followed by Hexane (15.5mm), Chloroform (16.3mm) and Methanol (17.3mm). *Aspergillus fumigatus* was found to be more higher activity towards the ethyl acetate extract from the leaf with maximum inhibitory zone (21.5mm) followed by Hexane (12.7mm), Chloroform (14.3mm) and Methanol (18.4mm). The result obtained shown that all the extracts showed very significant antimicrobial activity against the tested organisms. The ethyl acetate root extract of gram positive bacteria was highest activity of *Staphylococcus aereus* (20.8mm), gram negative bacteria was highest activity of *Klebsiella pneumonia* (14.5mm) and fungal pathogen highest activity of *Aspergillus fumigates* (21mm).(Table:5). The ethyl acetate stem extract of gram positive bacteria was highest activity of *Staphylococcus aereus* (19mm), gram negative bacteria was highest activity of *Klebsiella pneumonia* (14.2mm) and fungal pathogen highest activity of *Aspergillus fumigatus* (20.3mm).

4. Discussion

Eupatorium triplinerve leaf extract has a significant antimicrobial activity against broad spectra of microorganisms. The antibacterial activity from the extract against Staphylococcus *aureus, Streptococcus pyogens, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Aspergillus niger, Aspergillus flavus,* and *Aspergillus fumigatus* were reported for the first time. The microbial studies of the extract showed the most promising antimicrobial properties indicating the potential for the discovery of novel drugs from plant. Extracts containing phenol and triterpenes (Chloroform, Ethyl acetate and Methanol) were shown to be more efficient in the antibacterial efficiency than the other extracts. *Staphylococcus aureus* was found to be the highest activity towards the ethyl acetate extract from the leaf with maximum inhibitory zone (19.4mm). Ethyl acetate extract was shown to be as potent as *Ciproflaxin* (zone of inhibition-22.5 for *Pseudomonas aeruginosa).* The order for the antibacterial efficiency is Hexane, Chloroform, Ethyl acetate, and Methanol. The results clearly shown that Alkaloids, Flavonoids, Saponin, Tannin, Phenol, Triterpinoid, Glycoside, Steroids, Coumarin, and Volatile oil. Which was abundantly founding in Hexane, Chloroform, Ethyl acetate.

and Methanol extracts were responsible for the antimicrobial activity of *Eupatorium triplinerve* Vahl.

The present study through light on the antibacterial activity of *Eupatorium triplinerve* leaves this study offers a valuable source for the discovery of alternative to the present antibacterial drugs. The study also concludes that *Eupatorium triplinerve* leaf, stem and root contain a number of pharmaceutically important Phytochemical like Alkaloids, Flavonoids, Saponin, Tannin, Phenol, Triterpinoid, Glycoside, Steroids, Coumarin, and Volatile oil. A further study of the extracts is in progress to isolate, characterize and elucidate the structure of the bioactive compounds present which were responsible for potent antimicrobial activity.

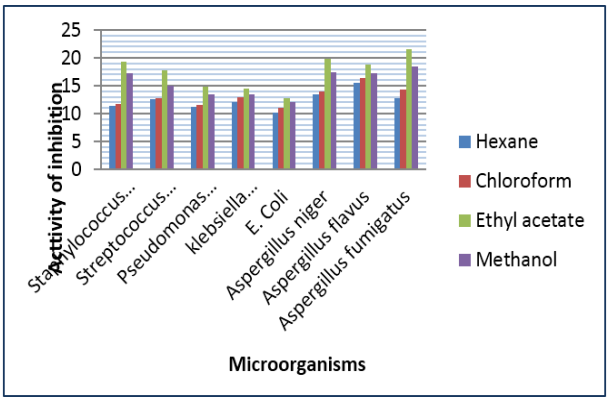

Fig 1: Antimicrobial activity of different extract of leaf of *Eupatorium triplinerve* Vahl

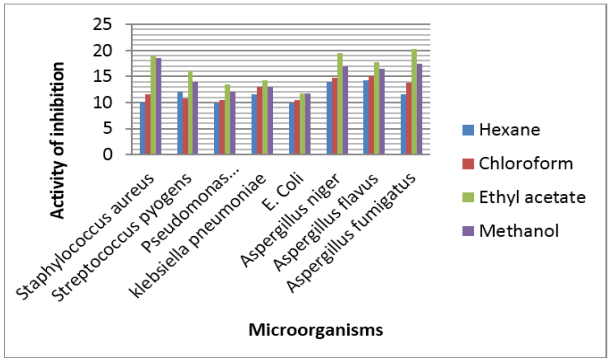

Fig 2: Antimicrobial activity of different extract of stem of *Eupatorium triplinerve* Vahl

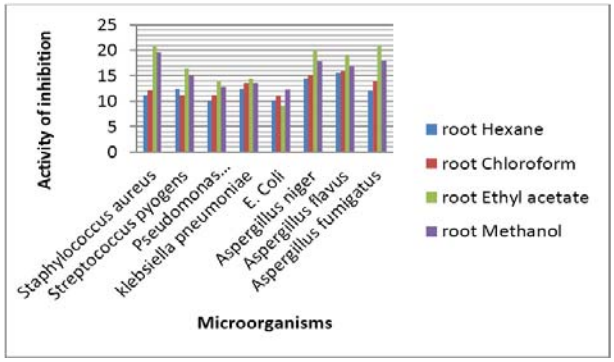

Fig 3: Antimicrobial activity of different extract of root of *Eupatorium triplinerve* Vahl

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Table 4: Antimicrobial activity of different extract of leaves of *Eupatorium triplinerve* Vahl (250, 500, 1000 µg / disc)

S. No	Microorganism	Hexane				Chloroform				Ethyl acetate				Methanol			
		Control	250	500	1000	Control	250	500	1000	Control	250	500	1000	Control	250	500	1000
1	*Staphylococcus aureus*	25.8± 0.655	7.2± 503	9.5± 0.556	11.3± 0.404	21.5± 0.602	7.5± 0.611	8.0± 0.300	11.8± 0.611	22.0± 0.301	14.5± 0.450	17.8± 0.529	19.4± 0.450	22.5± 0.351	12.8± 0.305	15.5± 0.450	17.3± 0.251
2	*Streptococcus pyogens*	20.8± 0.529	7.3± 0.404	10.8± 0.351	12.5± 0.305	21.3± 0.251	8.5± 0.361	10.0± 0.251	12.8± 0.305	21.5± 0.450	12.4± 0.400	15.3± 0.300	17.8± 0.416	20.4± 0.4041	11.5± 0.404	13.8± 0.400	15.0± 0.251
3	*Pseudomonas aeruginosa*	21.8± 0.529	7.3± 0.300	9.5± 0.351	11.2± 0.200	22.0± 0.408	7.5± 0.404	9.2± 0.210	11.5± 0.404	22.5± 0.404	10.8± 0.450	12.3± 0.404	14.8± 0.416	21.6± 0.400	9.6± 0.420	11.8± 0.360	13.5± 0.500
4	*klebsiella pneumoniae*	22.5± 0.551	7.8± 0.402	9± 0.351	12± 0.405	22.2± 0.407	9.5± 0.603	11. ± 0.402	13± 0.302	21.3± 0.403	10± 0.358	12± 0.554	14.5± 0.408	21.5± 0.502	9.8± 0.402	11± 0.502	13.5± 0.602
5	*E. Coli*	23± 0.403	7.0± 0.254	8.5± 0.453	10.2± 0.404	23.2± 0.254	7.5± 0.403	9± 0.412	11± 0.358	21.5± 0.402	7.8± 0.302	10.5± 0.401	12.8± 0.362	22.1± 0.258	8.5± 0.203	10.8± 0.254	12± 0.523
6	*Aspergillus niger*	21.5± 0.408	8.8± 0.416	10.5± 0.404	13.5± 0.282	21.8± 0.424	9.5± 0.404	11.8± 0.360	14.0± 0.450	22.5± 0.450	14.5± 0.458	16.6± 0.500	19.8± 0.404	24.3± 0.450	13.4± 0.282	15.8± 0.438	17.5± 0.351
7	*Aspergillus flavus*	23.8± 0.404	10.5± 0.400	13.8± 0.503	15.5± 0.351	22.5± 0.404	11.6± 0.503	14.5± 0.404	16.3± 0.416	23.5± 0.556	13.6± 0.501	15.2± 0.503	18.8± 0.450	24.0± 0.458	12.1± 0.550	15.0± 0.416	17.3± 0.412
8	*Aspergillus fumigatus*	24.5± 0.450	8.8± 0.503	10.2± 0.351	12.7± 0.416	23.8± 0.611	9.8± 0.503	12.5± 0.401	14.3± 0.400	21.3± 0.468	16.2± 0.550	19.8± 0.458	21.5± 0.300	24.8± 0.416	13.5± 0.453	16.0± 0.500	18.4± 0.450

**mean±=Standarded deviation Control: Ciproflaxin5µg/ml, Ketocazale 10µg/ml

Table: 5 Antimicrobial activity of different extract of root of *Eupatorium triplinerve* Vahl (250, 500, 1000 µg / d

S. No	Microorganism	Hexane			Chloroform			Ethyl acetate			Methanol			Control
		250	500	1000	250	500	1000	250	500	1000	250	500	1000	
1	*Staphylococcus aureus*	7.5±0.50	9.2±0.50	11.3±0.52	8.8±0.52	10.5±0.50	12.1±0.52	15.7±0.51	18.3±0.52	20.8±0.52	15.5±0.50	17.2±0.41	19.5±0.40	25.8±0.65
2	*Streptococcus pyogens*	8.8±0.52	10.8±0.65	12.5±0.32	7.6. ±0.50	9.5±0.52	11.2±0.52	12.3±0.40	13.8±0.41	16.5±0.50	10.8±0.52	13±0.50	15±0.50	20.8±0.52
3	*Pseudomonas aeruginosa*	7±0.50	8.5±0.50	10±0.50	7.5±0.50	9.0±0.51	11.2±0.40	11±0.41	12.5±0.41	14±0.40	9.6±0.50	11.2±0.41	12.8±0.52	21.8±0.52
4	*klebsiella pneumoniae*	8.8±0.52	10±0.50	12.5±0.50	9.5±50	11.5±0.50	13.5±0.52	10±0.50	11.5±0.50	14.5±0.41	8.5±0.50	11.2±0.42	13.5±0.53	22.5±0.55
5	*E. Coli*	7±0.50	8.8±0.63	10.2±0.41	7.5±0.50	9±0.51	11±0.40	-	7.5±0.50	9±0.40	7±0.50	9.5±0.50	12.3±0.40	23±0.40
6	*Aspergillus niger*	11±0.51	12.8±0.52	14.5±0.50	11.5±0.50	13.5±0.53	15±0.42	16.5±0.40	18.2±0.41	20±0.50	13.5±0.53	15.2±0.41	17.8±0.52	21.5±0.40
7	*Aspergillus flavus*	11.2±0.52	13.1±0.32	15.5±0.51	12.2±0.52	14.5±0.52	16±0.51	15.5±0.41	17.0±0.40	19.1±0.26	13.8±0.41	15.3±0.40	17±0.40	23.8±0.40
8	*Aspergillus fumigatus*	7.5±0.50	10.3±0.51	12±0.42	10.3±0.51	12±0.47	14±0.41	16.3±0.40	18±0.45	21±0.51	13.2±0.41	16.5±0.50	18±0.45	24.5±0.45

**mean±=Standarded deviation Control: Ciproflaxin5µg/ml, Ketocazale 10µg/ml,

Table 6: Antimicrobial activity of different extract of stem of *Eupatorium triplinerve* Vahl (250, 500, 1000 µg / disc)

S. No	Microorganism	Hexane			Chloroform			Ethyl acetate			Methanol			Control
		250	500	1000	250	500	1000	250	500	1000	250	500	1000	
1	*Staphylococcus aureus*	-	8 ± 0.50	10±0.45	7.0±0.50	9.8±0.63	11.5±0.50	14.2±0.52	17±0.47	19±0.50	13.8±0.52	16±0.50	18.5±0.50	21.5± 0.60
2	*Streptococcus pyogens*	7.5±0.50	9.3±0.51	12±0.40	_	8.2±0.53	10.8±0.65	11.8±0.52	13±0.52	16±0.67	10.3±0.51	12.8±0.91	14±0.40	21.3± 0.25
3	*Pseudomonas aeruginosa*		7.0±0.50	9.8±0.63	7.0±0.50	8.3±0.51	10.5±0.50	10.8±0.65	12.1±0.52	13.5±0.50	8.5±0.50	10.8±0.65	12±0.47	22.0± 0.40
4	*klebsiella pneumoniae*	7.5±0.50	9.2±0.51	11.5±0.50	8.7±0.51	11.8±0.52	13±0.52	9.5±0.50	11.3±0.51	14.2±0.52	7.3±0.51	10.8±0.65	13±0.52	22.2±0.40
5	*E. Coli*	-	7.5±0.50	9.8±0.63	-	8.1±0.52	10.5±0.50	7.2±0.52	9.5±0.50	11.8±0.53	7.1±0.52	9±0.50	11.8±0.53	23.2±0.25
6	*Aspergillus niger*	10.8±0.65	12±o.39	14±0.55	11.3±0.51	13±0.52	14.8±0.52	15.6±0.50	17±0.50	19.5±0.50	13.8±0.52	15.3±0.51	17±0.50	21.8± 0.42
7	*Aspergillus flavus*	10.8±0.65	12.5±0.50	14.3±0.51	11.5±0.50	14.2±0.52	15±0.50	14±0.50	16.2±0.52	17.8±0.52	11.5±0.50	13.8±0.52	16.5±0.50	22.5± 0.40
8	*Aspergillus fumigatus*	7.8±o.62	9.2±0.52	11.5±0.50	9.3±0.51	11.0±0.47	13.8±0.52	14.8±0.52	17.6±0.50	20.3±0.51	13.8±0.52	15.2±0.52	17.5±0.50	23.8± 0.61

**mean±=Standarded deviation Control: Ciproflaxin5µg/ml, Ketocazale 10µg/ml