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Research Article

A comparative study of antibacterial activity of stem bark and leaves extracts of *Ficus mollis* (vahl)

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ABSTRACT

The present study was designated to evaluate the antibacterial activities of stem bark and leaves extracts of *Ficus mollis* Vahl. *Ficus mollis (*Vahl*)* belonging to the family Moraceae. Which is a monoecious evergreen large size tree. Branchlets fulvous with small several tomentosa. It is also used as folk medicine. The decoction of leaves of *Ficus mollis* used for ear ache. The antimicrobial activities of the extracts against 9 laboratory strains belong bacterial species were tested by using disc diffusion assay. Ciprofloxacin used as a standard drug to compare the all extracts of *Ficus mollis* vahl*.* All aerial parts of *Ficus mollis* vahl shows antibacterial activity against the gram positive and gram negative microorganisms. While comparing the leaves and stem bark extracts for anti-bacterial activity, the leaves extract having the better activity. Methanol extract showed stronger and broader spectrum of antimicrobial activity as compared to other extracts.

Keywords: *Ficus mollis;* Methanol; Anti-bacterial Activity; Petroleum Ether, Agar slats, FMB, FML, MEFMB, MEFML.

INTRODUCTION

Ficus mollis (Vahl) belonging to the family Moraceae. It is a monoecious evergreen large size tree. Branchlets fulvous with small several tomentosa. It is also used as folk medicine. The decoction of leaves of *F.mollis* mixed with leaves of *Madhuca indica* used for ear ache (K.Madhava chetty et al., 2008) (K.Venkataratnam, R.R.Venkata Raju, 2004). The Heb No (E324) of *Ficus mollis* bark is used to relieve in pain of limbs (Anitha Jain et al., 2005). The bark past of *Ficus mollis* is also used for the treatment of cuts and wounds in the form of ointments (R.Kottaimuthu, 2008).

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (J.L. Rios et al., 2005). In any natural product isolation program in which the end product is to be a drug or a lead compound, some type of bioassay screening or pharmacological evaluation must be necessarily used to guide the isolation towards the pure bioactive compound (Atta-ur Rahman et al., 2001). The biomass is collected, dried, and extracted in to a suitable solvent to give an extract, which is then screened for bioactivity. Bioassays (BA) could involve the use of in-vivo systems (e.g. clinical trials, whole animal experiments), ex-vivo systems (e.g. isolated tissues and organs) and *in-vitro* systems (e.g. cultured cells). Often BA are linked with the process of fractionation and isolation, known as bioassay-guided fractionation, in which chromatographic techniques are used to separate the extract into its individual components, the biological activity is checked at all stages until a pure active compound is obtained. Given below is a broad classification of Bioassays for phyto pharmacological screening.

The present study was to evaluate the anti-bacterial activity of the stem bark and leaves extract of *Ficus mollis* Vahl*.*

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MATERIALS AND METHODS

Plant Material

The stem bark and leaves of *Ficus mollis* (FMB, FML) were collected from the local area of Tirumala hills, Chittoor district, Andhra Pradesh, India during 2007 December. It is authenticated by Prof .K.Madhava Chetty, Botanist, Head of Botany Department, Sri Venkateswara University, Tirupati, Andhra Pradesh. A Voucher Specimen No is 870 (Eud-II/2006, 870) has been deposited for future reference in the Department of Botany, Venkateswara University, Tirupati, Andhra

Table 1: Minimum Inhibitory Concentration of Different Extracts of FMB against Various Organisms for Anti-Bacterial Activity

S.No.	Extracts	Concentration (µg/ml)	Name Of The Organisms								
			1	2	3	4	5	6	7	8	9
1.	Petroleum ether	133.33	+	+	+	+	+	+	+	+	+
		200	+	+	+	-	+	+	-	+	+
		333.33	-	-	-	-	-	-	-	-	-
2.	Chloroform	133.33	+	+	+	+	+	+	+	+	+
		200	-	+	+	+	+	+	-	-	+
		333.33	-	-	-	-	-	-	-	-	-
3.	Ethyl acetate	133.33	+	+	+	+	+	+	+	+	+
		200	+	-	-	-	-	-	-	+	+
		333.33	-	-	-	-	-	-	-	-	-
4.	Methanol	133.33	+	+	+	+	+	+	+	+	-
		200	+	+	-	-	-	-	-	-	-
		333.33	-	-	-	-	-	-	-	-	-

(+: Indicates Growth, –: Indicates No Growth)

1. Staphylococcus aureus, 2. Streptococcus grieseus, 3.Bacillus subtilis, 4.Escherichia coli, 5 Proteus vulgaris, 6. Pseudomonas aeruginosa, 7. Klebsiella pneumonia, 8. Candida albicans, 9. Salmonella typhi

Table 2: Minimum Inhibitory Concentration of Different Extracts of FML against Various Organisms for Anti-Bacterial Activity

S.No.	Extracts	Concentration (µg/ml)	Name Of The Organisms								
			1	2	3	4	5	6	7	8	9
1.	Petroleum ether	133.33	+	+	+	+	+	+	+	+	+
		200	+	+	+	+	+	+	-	+	+
		333.33	-	-	-	-	-	-	-	-	-
2.	Chloroform	133.33	+	+	+	+	+	+	+	+	+
		200	+	+	+	+	+	+	-	-	+
		333.33	-	-	-	-	-	-	-	-	-
3.	Ethyl acetate	133.33	+	+	+	+	+	+	+	+	+
		200	-	+	+	+	+	+	+	+	-
		333.33	_	-	-	-	-	-	-	-	-
4.	Methanol	133.33	+	+	+	+	+	+	+	+	-
		200	+	+	+	-	-	-	+	-	-
		333.33	-	-	-	-	-	-	-	-	-

Table 3: Zone of inhibition of different Extracts of FMB against Various Organisms for Anti-Bacterial Activity

Extracts	Zone Of Inhibition (mm)								
	1	2	3	4	5	6	7	8	9
Petroleum ether	8	10	9	8	11	10	8	7	7
Chloroform	15	9	10	13	15	15	10	11	11
Ethylacetate	17	14	12	20	15	15	16	15	10
Methanol	19	13	12	21	14	16	16	15	12
Ciprofloxacin	25	17	15	28	21	22	18	18	16

Pradesh,India. The plant scientific classification was shown in the table No: 01 (Subramanian. S.S, Nair AGR, 1972)(Ficher, 1928) .

Micro-Organisms and Standard Drugs Used

For the present study, the micro-organisms used includes, *Gram positive bacteria like Staphylococcus aureus,* Streptococcus griseus and Bacillus subtilis. *Gram negative bacteria like Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans and Proteus vulgaris.* These microorganisms were collected from the National Collection of Industrial Microorganisms, Pune. India. The organisms were maintained on Mueller Hinton agar slopes and stored at 4˚C. We were selected Ciprofloxacin as reference antibiotic. These antibiotics were collected from the National Chemical Laboratory, Pune, India. (Satish Gupta, 2002)

Table 4: Zone of inhibition of different Extracts of FML against various organisms for Anti-Bacterial Activity

Extracts	Zone Of Inhibition (mm)								
	1	2	3	4	5	6	7	8	9
Petroleum ether	13	10	9	12	13	14	10	13	11
Chloroform	15	9	10	13	15	15	10	11	11
Ethylacetate	19	13	15	20	16	15	15	16	10
Methanol	20	14	14	23	15	16	17	16	12
Ciprofloxacin	24	18	19	30	20	19	19	18	13

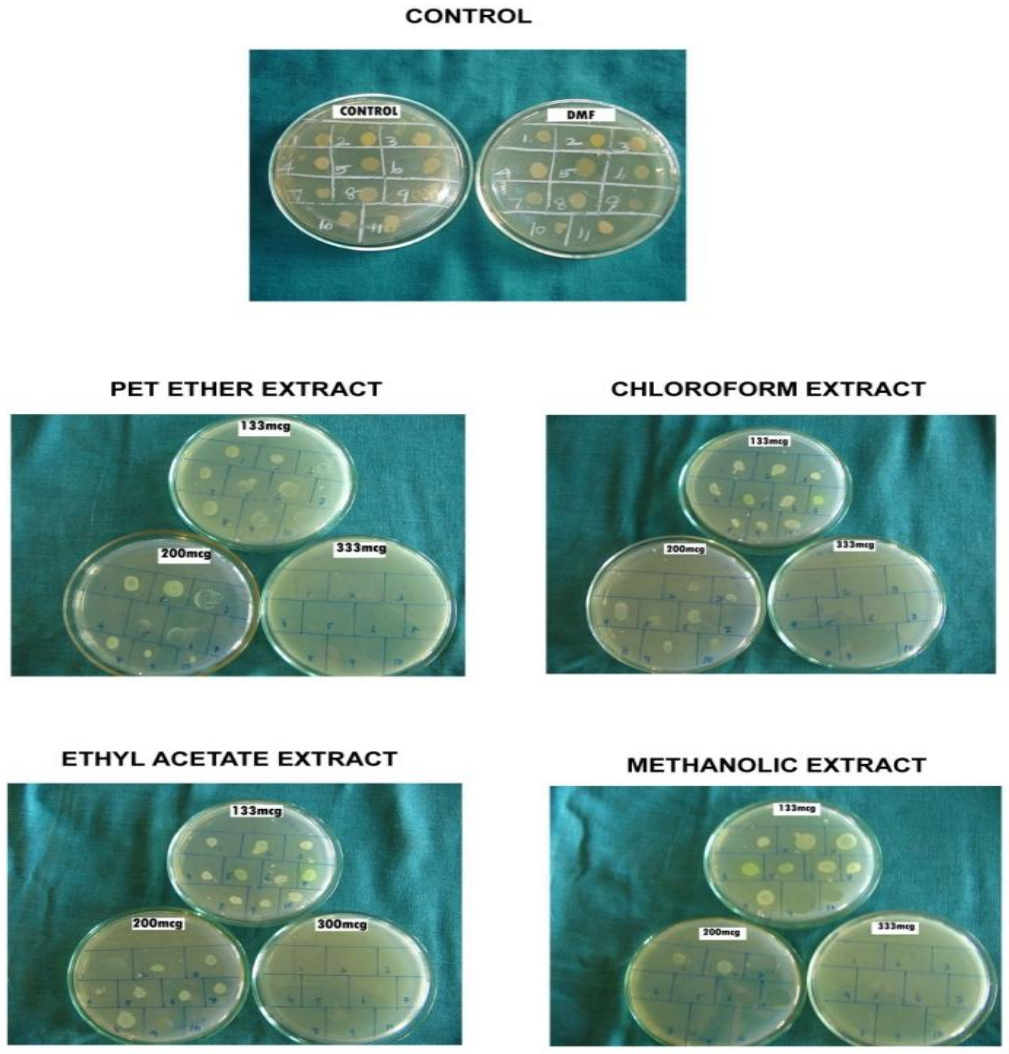

Figure 1: Minimum inhibitory concentration of various extracts of stem bark of *Ficus mollis* vahl for anti-bacterial activity

Preparation of Extracts

The freshly collected stem bark and leaves were cut into small pieces by using cutter mill, Shade dried and coarsely powdered and then the plant material was packed in Soxhlet apparatus and successively extracted with Petroleum ether, Ethylacetate, Chloroform and Methanol then concentrated by using Rotavapor Buchi R-114. After each extraction the powdered materials were dried in hot air oven at below 50^{o}C. The dried powder again subjected for soxhlet extraction with Petroleum ether, Ethylacetate, Chloroform and Methanolic as a solvent systems. The extract was then stored in desiccators for further use. All the extracts were dissolved in 6% Dimethyl formamide (DMF).

Minimum Inhibitory Concentration

Inoculum Preparation

These obtained stock cultures are sub-cultured separately. The sub-culturing has been carried out under aseptic conditions. Mueller Hinton agar medium has been used for the sub-culturing the microorganisms. From the stock culture, the test organisms were selected and streaked onto the fresh Mueller Hinton agar slants, incubated at 37^{0}c for 24hrs. The growth content of each slant was scrapped into 10 ml of sterile distilled

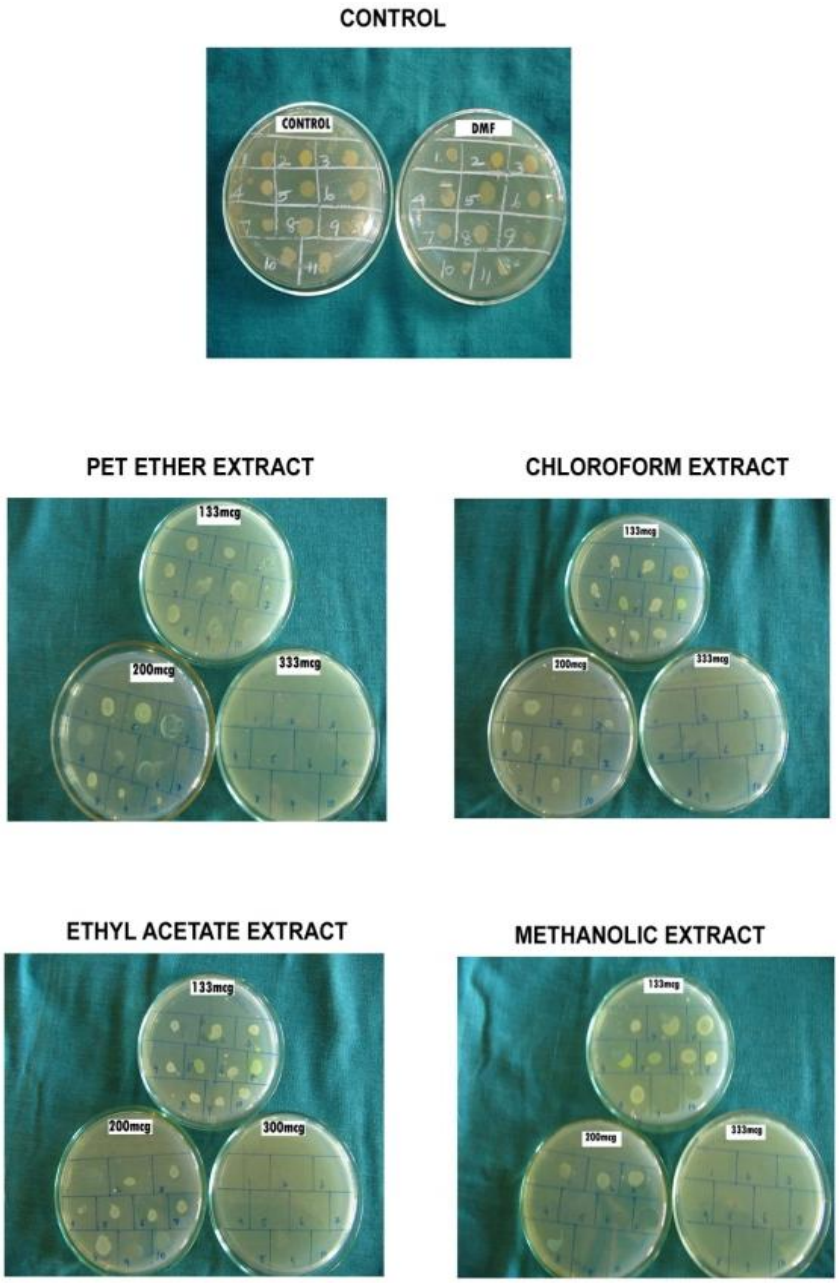

Figure 2: Minimum inhibitory concentration of various extracts of leaves of *Ficus mollis* vahl for anti-bacterial activity

water and a uniform suspension was prepared and cell count was adjusted to 10^4/ml. It was used as inoculums. All the tests were performed and their activities were expressed as the mean of zone of inhibition diameter produced by the test organisms. From the prepared bacterial suspension 2% of inoculums per total volume of Mueller Hinton agar medium in a Petri dish were used for microbiological assay (Collee, J.G et al., 1989)

Preparation of Agar Plates

The extracts were incorporated into Mueller Hinton agar such that the final concentration of extracts is being 2000 μg/15ml, 3000 μg/15ml and 5000μg/15ml in the plates according to the Table nos. 1 and 2. The plates were prepared using agar and different extracts of various dilutions, allowed to solidify and dry. Then a loopful of the cultures was inoculated at the labeled spots. The plates were then incubated at 37°C for 24 hours. The results were read by the presence or absence of growth of the organism.

Antibiotic Disc Diffusion Method

Preparation of Standard Antibiotic Solution (Agarwal, K.C, 1974)

Standard preparation is an authentic sample of appropriate antibiotic for which the potency has been precisely determined by reference to the appropriate international standard. The potency of the standard preparation may be expressed in international units (I.U) or in μg/ml of the pure antibiotic. To perform microbiological assay by cup plate method, the standard

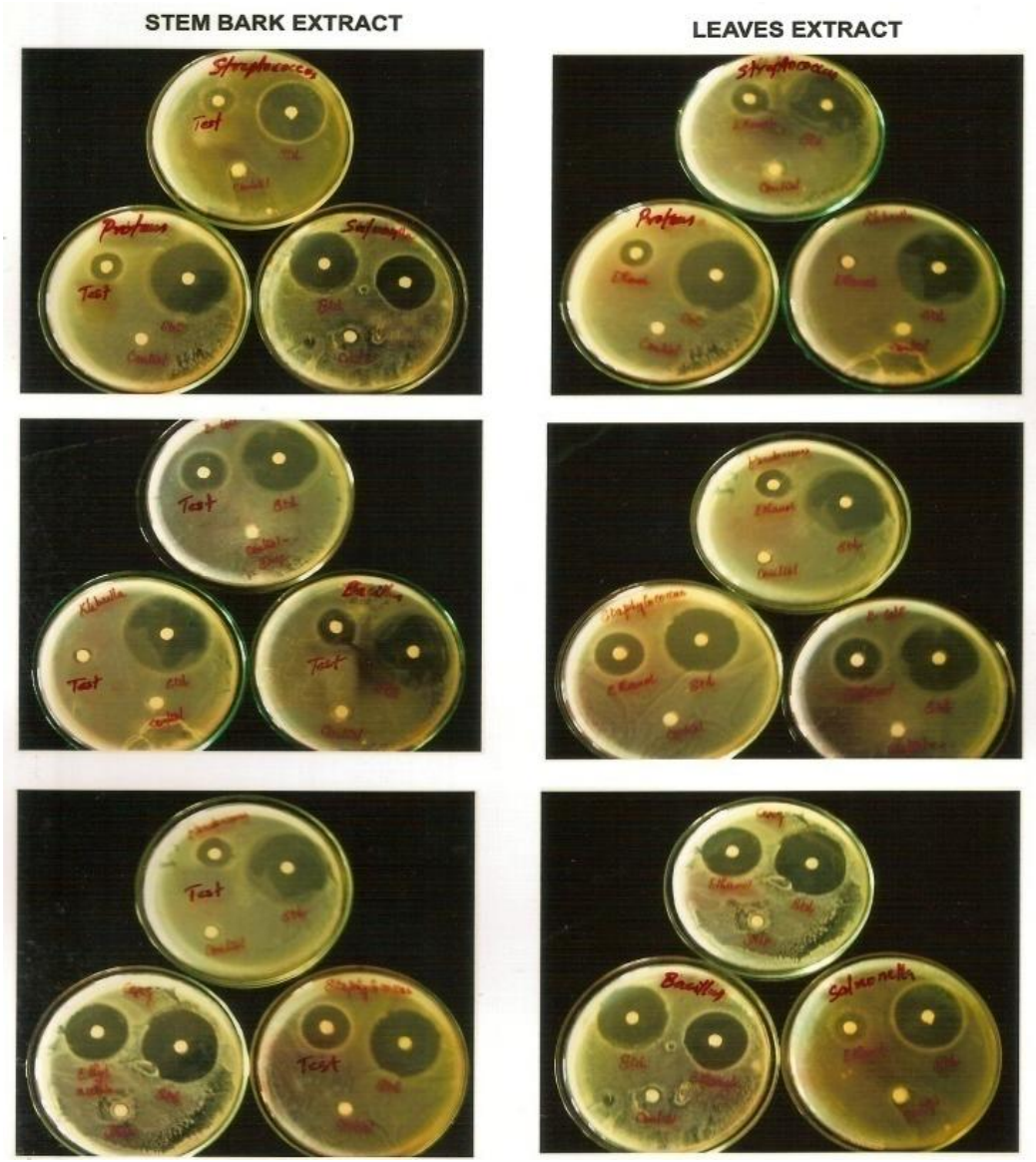

Figure 3: Minimum inhibitory concentration of various extracts of stem bark and leaves of *Ficus mollis* vahl for anti-bacterial activity

antibiotic should be taken in appropriate concentrations. The cup (Disc) of Petri plate should contain 10 µg/disc of antibiotic sample. Where the quantity per disc is 50µl. As per the minimum sensitivity of the digital balance to achieve the required concentration, serial dilution technique has been applied.

Preparation of Discs

The discs of 6mm diameter were prepared from whatmann filter paper number 1 and sterilized in hot air over at 160°C for one hour. The Discs were then impregnated with the minimum inhibitory concentration of the extracts [petroleum ether, Chloroform, Ethylacetate and methanol], standard antibiotic [Ciprofloaxacin] (V.Harinadha babu et al., 2007) and the solvent Dimethyl formamide [DMF]. Antibiotic discs were prepared such that each disc contains 5µg. From the peptone water, seeding was done on nutrient agar medium with the help of a sterile swab. Care was taken for the even distribution of culture all over the plate. The seeded plates were allowed to dry and then the extracts, anti-biotic and dimethyl formamide discs were placed on the seeded medium plates, kept at 4°C for 30 minutes to allow the prediffusion of the antibiotic, extracts and DMF. The plates were then incubated at 37°C for 24 hours. The results were read by measuring the zone of inhibition around the discs. (R.Mythreyi, 2009)

RESULTS AND DISCUSION

The minimum inhibitory concentration studies of various extracts of FMB and FML were performed on various gram negative and gram positive bacteria. MIC on different organisms was tabulated in Table no.1 and 2 for FMB and FML which ranges from 200 µg/ml to 333.33 µg/ml. The antibacterial activity performed by Disc diffusion method were seen in all the extracts of stem bark and leaves of *Ficus mollis* vahl on both

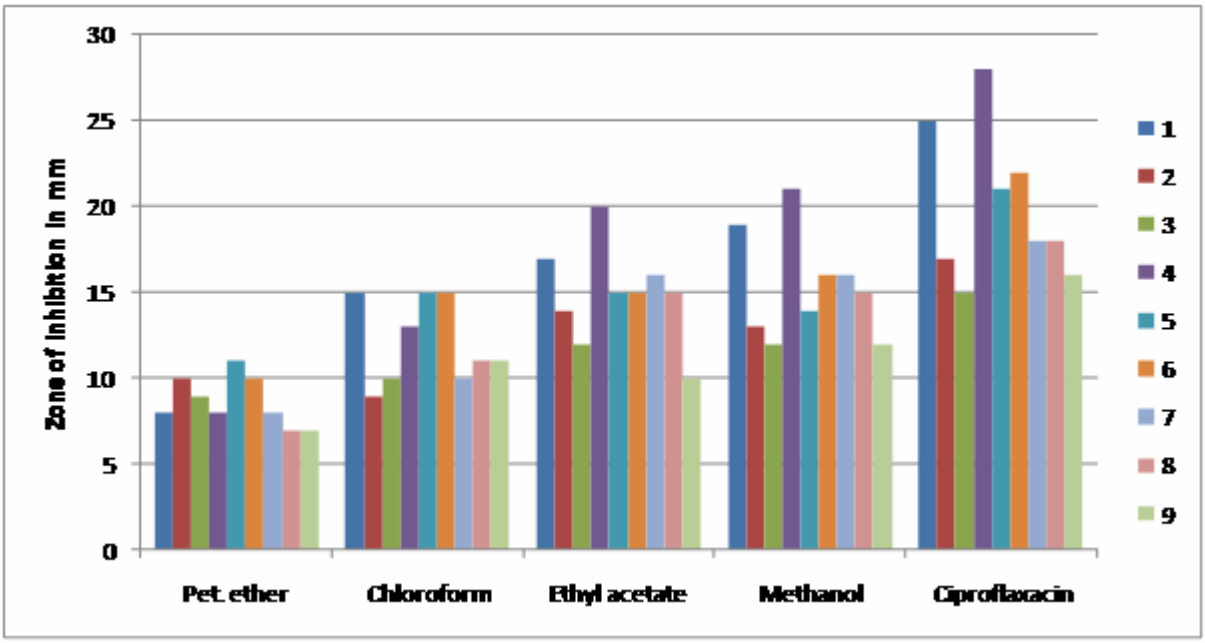

Figure 4: Zone of inhibition of different extracts of FMB

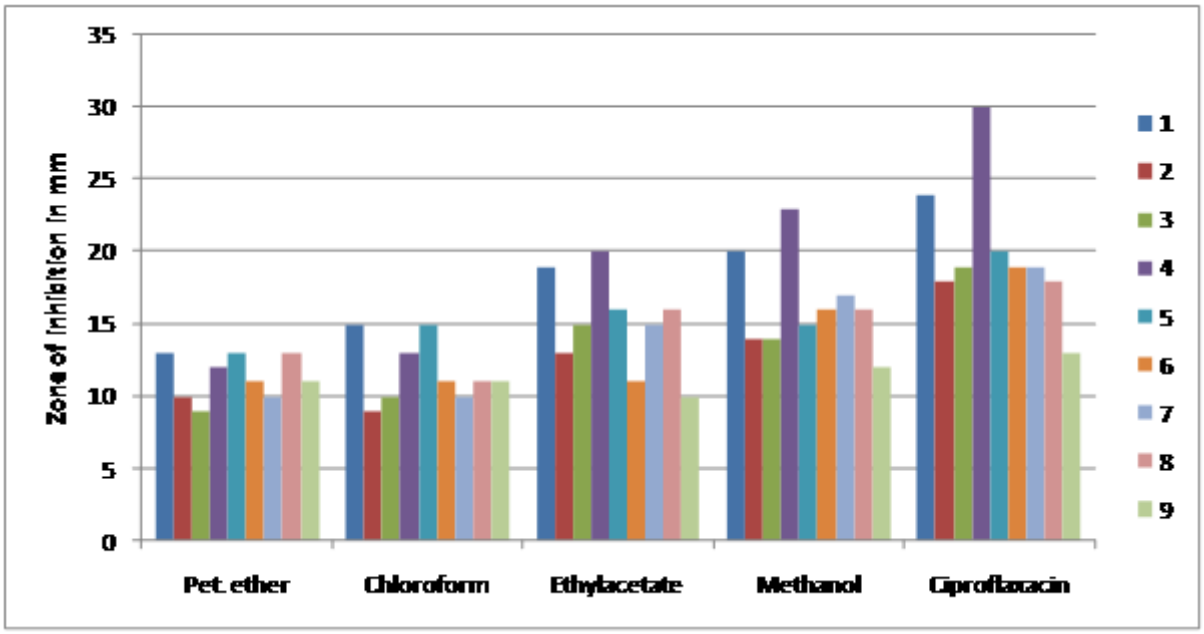

Figure 5: Zone of inhibition of different extracts of FML

strains of +ve and gram –ve bacteria. Methanolic extracts of FMB and FML were shown better results compared with other solvents. This may be due the presence of potent secondary metabolites in the plant specimen. When compared between stem bark and leaves of *Ficus mollis*. Leaves were shown slightly more potent as per the results shown in Table no. 3 and 4. The diameter of zone of inhibition of each extracts was recorded with the help of zone measuring scale (Hi-Media).

CONCLUSION

Stem bark and leaves extracts of *Ficus mollis* were shown good antibacterial activity on all the organisms performed i.e gram +ve and gram –ve bacteria. But Methanol extracts were shown better results when compared with other solvents. This is may be due to high polarity of methanol it can extract more phyto-constituents qualitatively and quantitatively. Further work has to be done to isolate the phytochemical responsible for the antibacterial activity.

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