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In-vitro evaluation of antibacterial efficacy of certain medicinal plants against bacterial isolates associated with late larval flacherie disease of Silkworm, *Bombyx mori* L.

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Abstract
The *In-vitro* evaluation acetone extract of medicinal plants showed significant effect on inhibition zone. Among the nine medicinal plants extracts, *Ocimum tenuiflorum* recorded maximum zone of inhibition against *Bacillus* sp., *Asparagus officinalis* against *Staphylococcus* sp. and *Phyllanthus emblica* against *Streptococcus* sp., respectively in the concentrations 2, 4 and 6 per cent on 24 and 48 hours of observation for the 10^{-4} and 10^{-6} dilutions compared to control.

Keywords: Flacherie, bacterial isolates, medicinal plant extracts and zone of inhibition

Introduction
Domestication of silkworms for the production of silk over hundreds of years made them to susceptible to number of diseases. The important diseases affecting silkworm are flacherie, grasserie, muscardine and pebrine. Among these four diseases, the flacherie is most devastating disease of silkworm accounting for the cocoon crop loss to the tune of 33.88 per cent (Tayal and Chauhan, 2017) [12]. The primary cause of Flacherie disease in silkworms is due to the physiological weakness of the organism combined with the invasion of pathogenic or non-pathogenic microbes. Flacherie may be caused either by microbial or amicrobial agents. Microbial flacherie may be caused by bacteria and viruses. The bacterial agents that induce flacherie are, *Bacillus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Bacillus thuringiensis*, *Serratia marcescens etc*., (Chaitra *et al.*, 1975). A wide variety of chemical bed disinfectants and antibiotics are used for the management of flacherie, but the ability of microbes to acquire resistance to drugs makes it ineffective within a short duration and hence attempts are being made for the use of plant compounds especially crude aqueous extracts of seven medicinal plants against silkworm bacterial pathogens (Priyadarshini *et al.*, 2008) [9]. The present work has been undertaken to study the anti-bacterial efficacy of acetone extract of medicinal plants *viz.*, *Curcuma longa* (Turmeric), *Tinospora cordifolia* (Amruthaballi), *Tridax procumbens* (Coat buttons), *Phyllanthus niruri* (Kirunelli), *Phyllanthus emblica* (Amla), *Punica granatum* (Pomegranate), *Aloe vera* (Aloe vera), *Ocimum tenuiflorum* (Tulasi) and *Asparagus officinalis* (Asparagus), against the bacterial isolates of silkworm flacherie disease *viz.*, *Bacillus* sp. *Staphylcoccus* sp. and *Streptococcus* sp.

Material and Methods
Preparation of plant extracts
The extracts from different plants were prepared as per the procedure adopted by Karthikairaj *et al*., (2014) [4]. The above mentioned plant parts were collected from 'Sanjeevini vatika' (Herbal garden), Department of Horticulture, UAS, GKVK, Bengaluru and Botanical garden UAS, GKVK, Bengaluru. The collected plant samples were washed in running tap water, rinsed with sterile distilled water and shade dried. The shade dried plant samples were then powdered in electric blender at slow speed, sieved and kept stored in desiccators. Ten grams of fine powder was soaked with 100 ml of acetone solution for 6 hours under air tight condition. The content is then stirred for an hour using magnet stirrer and filtered through a filter paper. The residual extract was collected in a flask and the solvent was allowed to evaporate at room temperature. The extracts was then stored at 4° C till further use. The resultant residue was

then made up to required volume (2, 4 and 6%) using double distilled water and used for the study.

Treatment Details

T_1 – Turmeric (*Curcuma longa*)
T_2 – Amruthaballi (*Tinospora cardifolia)*
T_3 – Coat buttons (*Tridax* procumbens)
T_4 – Kirunelli (*Phyllanthus niruri*)
T_5 – Amla (*Phyllanthus emblica*)
T_6 – Pomegranate (*Punica granatum*)
T_7 – Aloe vera (*Aloe vera*)
T_8 – Tulasi (*Ocimum tenuiflorum*)
T_9 – Asparagus (*Asparagus officinalis*)
T_{10} – Distilled water control.

Isolation of pathogens

Mulberry silkworms exhibiting specific symptoms of late larval flacherie were collected and surface sterilized. The midgut juice was collected by blocking oral and anal openings of the larvae. Further, midgut was blocked by ligating and dissected to collect the alimentary canal. The alimentary canal was surface disinfected and washed with sterile distilled water macerated and filtered through double layered muslin cloth and stock suspension was prepared from which serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were prepared using 9 ml sterile water blanks. In the same way haemolymph was also collected by cutting the front pair of prolegs and mixed with sterile distilled water and filtered through filter paper to obtain the stock suspension from which serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were prepared using 9 ml sterile water blanks. (Nataraju *et al.*, 1999; Siromani *et al.*, 1994; Patil, 1990 & Chitra *et al.*, 1973) [7, 8, 1]. Midgut juice and haemolymph each of 0.5 ml dilution was prepared and each of which were transferred to separate petridishes containing nutrient agar medium and spread thoroughly. Later the culture plates were incubated at 37 ^{0}C for three days. The colonies developed on the culture plates were picked, purified by using streak plate method. Pathogenicity of the individual bacterial isolates conforming to the principle of koches' postulates in causing the disease was identified.

Anti-microbial test

Sensitivity to plant extracts were tested for selected pathogens isolated from flacherie affected silkworms. The air dried nutrient agar plates were taken and 0.1 ml of test organisms were swabbed. Sterilized Whatman No. 1 filter paper discs impregnated in plant extracts at different concentration (2, 4 and 6%) were placed in the plate, discs impregnated in distilled water is used as control. Plates are incubated at room temperature for 2 days. After incubation the zone of inhibition was measured.

Measurement of inhibition zone of bacteria

Sterilized Whatman No. 1 filter paper discs of 5 mm diameter were dipped in botanical extracts for 1 minute and drained by the edges of petriplate then placed at the centre of the petriplate. Three replications were maintained for each treatment along with distilled water control was used for comparison. The same plates were incubated for 48 hours at room temperature. The diameter of the inhibition zone of the bacteria by various botanicals was measured (mm). The effective concentration of the plant extracts which inhibited the bacterial growth effectively were used for *in-vivo* study.

Results and Discussion

***In-vitro* effect of plant extracts on inhibition zone (mm) at 2, 4 & 6 per cent concentrations against *Bacillus* sp.**

The effect of nine plant extracts at 2, 4 and 6 concentrations on inhibition zone against the *Bacillus* sp. for spore dilutions of 10^{-4} and 10^{-6} was found significant on 24 hours and 48 hours of observation. The maximum zone of inhibition on 24 hours (7.22 and 7.72 mm) and 48 hours (7.99 and 8.55 mm) of incubation period for both 10^{-4} and 10^{-6} dilutions was recorded in T_8 (*Ocimum tenuiflorum*) followed by T_9 (*Asparagus officinalis*) (6.99, 7.44 mm on 24 hr and 7.55, 7.94 mm on 48 h, respectively in 10^{-4} and 10^{-6} dilutions). However, minimum zone of inhibition among the plant extracts was observed in T_6 (*Punica granatum*) (5.88, 6.27 on 24 h., 6.05, 6.49 mm on 48 h, respectively) in 10^{-4} and 10^{-6} bacterial dilutions. Among 2, 4 and 6 concentrations of plant extracts used, 6 per cent showed maximum inhibition zone (6.13, 6.61., 6.43 and 7.09 mm) and minimum inhibition zone (5.74, 5.94., 6.11 and 6.38 mm) was recorded at 2 per cent concentration for 10^{-4} and 10^{-6} *Bacillus* sp. dilutions on 24 and 48 hours of incubation period, respectively (Table 1., Plate 1). Similar findings were reported from Manjunath *et al.* (2009a) [6] who reported that among the various botanicals used, maximum zone of inhibition was recorded in *Aegle marmelos* (11.08 mm) and minimum in *Solanum nigrum* (6.45 mm) against *Bacillus* sp. Harish Babu *et al.* (2011) also reported among the different concentrations (25, 50, 70 and 100%) of *Aloe vera gel* extract used, maximum inhibition was observed in 100 and 75 per cent with inhibition zone of 8.80 and 5.70 mm, respectively when compared to that of control and sterilized batches.

***In-vitro* effect of plant extracts on inhibition zone (mm) at 2, 4 & 6 per cent concentrations against *Staphylococcus* sp.**

The statistical data on effect of nine different plant extracts on inhibition zone against *Staphylococcus* sp. found significant on 24 and 48 hours of observation recorded. The maximum zone of inhibition was recorded in T_9 (*Asparagus officinalis*) (7.49, 8.16 mm on 24 h and 8.21, 9.44 mm on 48 h, for the bacterial dilutions of 10^{-4} and 10^{-6}, respectively) followed by T_2 (*Tinospora cordifolia*) (6.99, 7.55., 7.66 and 8.33 mm), T_8 (*Ocimum tenuiflorum*) (7.10, 7.50., 7.55 and 8.16 mm), T_5 (*Phyllanthus emblica*) (6.94, 7.38., 7.66 and 7.94 mm) and T_1 (*Curcuma longa*) (6.60, 6.94., 6.83 and 7.44 mm). Whereas, the minimum inhibition zone on 48 hours incubation period was observed in T_7 (*Aloe vera*) (6.27 and 6.55 mm) followed by T_6 (*Punica granatum*) (6.44 and 6.77 mm) for the bacterial dilutions of 10^{-4} and 10^{-6}, respectively. Among the 2, 4 and 6 per cent concentrations plant extracts used, maximum (6.33, 6.83., 6.61 and 7.24 mm) and minimum (5.82, 6.06., 6.04 and 6.54 mm) inhibition zone was recorded at 6 and 2 per cent in 10^{-4} and 10^{-6} on both 24 and 48 hours of incubation, respectively (Table 2., Plate 2).

The above findings are in conformity with results of Karthikairaj *et al.* (2014) [4] who reported that the alcoholic extracts of *Leucas aspera* produced maximum zone of inhibition (241.5 mm^2 area) than the extracts of *Ocimum sanctum* and *Acalypha indica* against *Staphylococcus* sp. Selvamohan *et al.* (2012) [10] reported that the methanolic extract of *Phyllanthus niruri* showed maximum antibacterial activity against *Staphylococcus* sp.

***In-vitro* effect of plant extracts on inhibition zone (mm) at 2, 4 & 6 per cent concentrations against *Streptococcus* sp.**

The acetone extract of plant materials against *Streptococcus* sp. was found to be effective by inhibiting the growth of bacteria. Among the plant extracts used, the maximum zone of inhibition (7.99, 8.60 mm on 24 h, and 9.22, 10.16 mm on 48 h, of incubation) was recorded in T_5 (*Phyallanthus emblica*) for the bacterial dilutions of 10^{-4} and 10^{-6} followed by T_2 (*Tinospora cordifolia*) (7.50, 8.16., 8.55 and 9.22 mm) and T_9 (*Asparagus officinalis*) (7.33, 7.66., 8.10 and 8.49 mm) which are statistically on par with each other. Whereas, the

minimum inhibition zone was recorded in T_3 (*Tridax procumbence*) (6.38, 6.58., 6.44 and 7.05 mm) followed by T_4 (*Phyllantus niruri*) (6.33, 6.66., 6.61 and 7.16 mm). Among 2, 4 and 6 per cent concentrations of plant extracts used, maximum zone of inhibition 6.54, 6.93 mm on 24 hours incubationand 7.11, 7.58 mm on 48 hours of incubation was recorded in 6 per cent concentration for the bacterial dilutions of 10^{-4} and 10^{-6}, respectively. While, minimum inhibition zone of 5.83, 6.21 mm on 24 hours of incubation and 6.29, 6.89 mm on 48 hours of incubation was noticed in 2 per cent concentration for 10^{-4} and 10^{-6} dilutions of *Streptococcus* sp., (Table 3., Plate 3).

Table 1: *In-vitro* effect of plant extracts on zone of inhibition (mm) at 2, 4 and 6 per cent concentrations against *Bacillus* sp.

	Zone of inhibition (mm)															
Duration	24 hours								48 hours							
Dilutions	10^{-4}				10^{-6}				10^{-4}				10^{-6}			
Concentrations / **Treatments**	**2%**	**4%**	**6%**	**Mean**	**2%**	**4%**	**6%**	**Mean**	**2%**	**4%**	**6%**	**Mean**	**2%**	**4%**	**6%**	**Mean**
T_1	6.00 (2.55)	6.16 (2.58)	6.33 (2.61)	6.16 (2.58)	6.66 (2.67)	7.00 (2.73)	7.33 (2.79)	6.99 (2.73)	6.33 (2.61)	6.5 (2.64)	6.83 (2.70)	6.55 (2.65)	7.00 (2.73)	7.16 (2.76)	7.83 (2.88)	7.33 (2.79)
T_2	6.50 (2.65)	6.83 (2.71)	7.00 (2.74)	6.77 (2.70)	6.83 (2.70)	7.16 (2.76)	7.5.0 (2.82)	7.16 (2.76)	6.83 (2.70)	7.00 (2.73)	7.16 (2.76)	6.99 (2.73)	7.16 (2.76)	7.50 (2.82)	8.50 (3.00)	7.72 (2.86)
T_3	6.33 (2.61)	6.50 (2.65)	6.50 (2.65)	6.44 (2.63)	6.33 (2.61)	6.66 (2.66)	6.83 (2.70)	6.60 (2.66)	6.66 (2.67)	6.83 (2.70)	6.66 (2.67)	6.71 (2.68)	6.66 (2.67)	7.00 (2.73)	7.16 (2.76)	6.94 (2.72)
T_4	6.16 (2.59)	6.66 (2.68)	6.66 (2.68)	6.49 (2.64)	6.50 (2.64)	6.66 (2.67)	7.16 (2.76)	6.77 (2.69)	6.83 (2.70)	6.83 (2.70)	7.00 (2.73)	6.88 (2.71)	6.83 (2.70)	7.33 (2.79)	7.50 (2.82)	7.22 (2.77)
T_5	6.66 (2.68)	6.83 (2.71)	7.33 (2.80)	6.94 (2.73)	6.66 (2.67)	7.16 (2.76)	7.66 (2.85)	7.16 (2.76)	7.16 (2.76)	7.33 (2.79)	7.50 (2.82)	7.33 (2.79)	7.50 (2.82)	8.00 (2.91)	8.66 (3.02)	8.05 (2.92)
T_6	5.66 (2.48)	5.83 (2.52)	6.16 (2.58)	5.88 (2.53)	5.83 (2.51)	6.16 (2.58)	6.83 (2.70)	6.27 (2.60)	5.66 (2.48)	6.16 (2.58)	6.33 (2.61)	6.05 (2.55)	6.50 (2.64)	6.16 (2.58)	6.83 (2.70)	6.49 (2.64)
T_7	6.33 (2.61)	6.66 (2.68)	6.66 (2.68)	6.55 (2.66)	6.16 (2.58)	6.33 (2.61)	6.66 (2.67)	6.38 (2.62)	6.50 (2.64)	6.33 (2.61)	6.83 (2.70)	6.55 (2.65)	6.33 (2.61)	6.33 (2.61)	7.00 (2.73)	6.55 (2.65)
T_8	7.00 (2.73)	7.16 (2.77)	7.50 (2.83)	7.22 (2.78)	7.33 (2.79)	7.50 (2.82)	8.33 (2.97)	7.72 (2.86)	7.83 (2.88)	8.00 (2.91)	8.16 (2.94)	7.99 (2.65)	8.16 (2.94)	8.33 (2.97)	9.16 (3.10)	8.55 (3.00)
T_9	6.83 (2.70)	7.00 (2.73)	7.16 (2.77)	6.99 (2.74)	7.16 (2.76)	7.33 (2.79)	7.83 (2.88)	7.44 (2.81)	7.33 (2.79)	7.50 (2.82)	7.83 (2.88)	7.55 (2.91)	7.66 (2.85)	7.83 (2.88)	8.33 (2.97)	7.94 (2.90)
T_{10}	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Mean	5.74 (2.50)	5.96 (2.54)	6.13 (2.57)		5.94 (2.53)	6.19 (2.79)	6.61 (2.66)		6.11 (2.57)	6.24 (2.59)	6.43 (2.63)		6.38 (2.62)	6.56 (2.65)	7.09 (2.75)	
F–test	Treatments		Dilutions		Concentrations				Treatments		Dilutions		Concentrations			
	*		*		*				*		*		*			
S.Em ±	0.095 (0.771)		0.045 (0.738)		0.055 (0.744)				0.111 (0.781)		0.053 (0.743)		0.064 (0.750)			
CD at 5%	0.266 (0.875)		0.125 (0.790)		0.154 (0.808)				0.313 (0.901)		0.147 (0.804)		0.180 (0.824)			

* - Significant at 5% level. Values in brackets of parentheses are square root transferred.

Table 2: *In-vitro* effect of plant extracts on zone of inhibition (mm) at 2, 4 and 6 per cent concentrations against *Staphylococcus* sp.

	Zone of inhibition (mm)															
Duration	24 hours								48 hours							
Dilutions	10^{-4}				10^{-6}				10^{-4}				10^{-6}			
Concentrations / **Treatments**	**2%**	**4%**	**6%**	**Mean**	**2%**	**4%**	**6%**	**Mean**	**2%**	**4%**	**6%**	**Mean**	**2%**	**4%**	**6%**	**Mean**
T_1	6.33 (2.61)	6.66 (2.67)	6.83 (2.70)	6.60 (2.66)	6.50 (2.64)	7.00 (2.73)	7.33 (2.79)	6.94 (2.72)	6.50 (2.64)	6.83 (2.70)	7.16 (2.76)	6.83 (2.70)	7.00 (2.73)	7.50 (2.82)	7.83 (2.88)	7.44 (2.81)
T_2	6.66 (2.67)	6.83 (2.70)	7.50 (2.82)	6.99 (2.73)	7.16 (2.76)	7.33 (2.79)	8.16 (2.94)	7.55 (2.83)	7.33 (2.79)	7.66 (2.85)	8.00 (2.91)	7.66 (2.85)	8.00 (2.91)	8.33 (2.97)	8.66 (3.02)	8.33 (2.97)
T_3	6.16 (2.58)	6.33 (2.61)	6.50 (2.64)	6.33 (2.61)	6.66 (2.67)	6.83 (2.70)	7.00 (2.73)	6.83 (2.70)	6.33 (2.61)	6.50 (2.64)	6.66 (2.67)	6.49 (2.64)	7.16 (2.76)	7.16 (2.76)	7.33 (2.79)	7.21 (2.77)
T_4	6.33 (2.61)	6.50 (2.64)	6.83 (2.70)	6.55 (2.65)	6.33 (2.61)	6.66 (2.67)	7.16 (2.76)	6.71 (2.68)	6.66 (2.67)	6.66 (2.67)	7.00 (2.73)	6.77 (2.69)	6.33 (2.61)	6.83 (2.70)	7.00 (2.73)	6.72 (2.68)
T_5	6.66 (2.67)	7.00 (2.73)	7.16 (2.76)	6.94 (2.72)	7.00 (2.73)	7.33 (2.79)	7.83 (2.88)	7.38 (2.80)	6.83 (2.70)	7.16 (2.76)	7.66 (2.85)	7.21 (2.77)	7.66 (2.85)	7.83 (2.88)	8.33 (2.97)	7.94 (2.90)
T_6	5.66 (2.48)	6.00 (2.54)	6.33 (2.61)	5.99 (2.54)	6.00 (2.54)	6.66 (2.67)	6.83 (2.70)	6.49 (2.64)	6.16 (2.58)	6.50 (2.64)	6.66 (2.67)	6.44 (2.63)	6.50 (2.64)	6.83 (2.70)	7.00 (2.73)	6.77 (2.69)
T_7	6.66 (2.67)	6.50 (2.64)	6.66 (2.67)	6.60 (2.66)	6.50 (2.64)	6.83 (2.70)	7.00 (2.73)	6.77 (2.69)	5.66 (2.48)	6.83 (2.70)	6.33 (2.61)	6.27 (2.60)	6.50 (2.64)	6.33 (2.61)	6.83 (2.70)	6.55 (2.65)
T_8	6.83 (2.70)	7.16 (2.76)	7.33 (2.790	7.10 (2.75)	7.00 (2.73)	7.50 (2.82)	8.00 (2.91)	7.50 (2.82)	7.33 (2.79)	7.50 (2.82)	7.83 (2.88)	7.55 (2.83)	7.83 (2.88)	8.16 (2.94)	8.50 (3.00)	8.16 (2.94)
T_9	7.00 (2.73)	7.33 (2.79)	8.16 (2.94)	7.49 (2.82)	7.50 (2.82)	8.00 (2.91)	9.00 (3.08)	8.16 (2.94)	7.66 (2.85)	8.16 (2.94)	8.83 (3.05)	8.21 (2.95)	8.50 (3.00)	8.83 (3.05)	11.00 (3.39)	9.44 (3.15)
T_{10}	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Mean	5.82 (2.51)	6.03 (2.55)	6.33 (2.61)		6.06 (2.56)	6.41 (2.62)	6.83 (2.70)		6.04 (2.55)	6.38 (2.62)	6.61 (2.66)		6.54 (2.65)	6.78 (2.69)	7.24 (2.78)	
F–test	Treatments		Dilutions		Concentrations				Treatments		Dilutions		Concentrations			
	*		*		*				*		*		*			
S.Em ±	0.096 (0.772)		0.045 (0.738)		0.055 (0.744)				0.102 (0.775)		0.048 (0.740)		0.059 (0.747)			
CD at 5%	0.269 (0.876)		0.127 (0.764)		0.155 (0.809)				0.287 (0.887)		0.135 (0.7960		0.166(0.816)			

* - Significant at 5% level. Values in brackets of parentheses are square root transferred.

Table 3: *In-vitro* effect of plant extracts on zone of inhibition (mm) at 2, 4 and 6 per cent concentrations against *Streptococcus* sp.

	Zone of inhibition (mm)															
Duration	**24 hours**								**48 hours**							
Dilutions	**10^{-4}**				**10^{-6}**				**10^{-4}**				**10^{-6}**			
Concentrations / **Treatments**	**2%**	**4%**	**6%**	**Mean**	**2%**	**4%**	**6%**	**Mean**	**2%**	**4%**	**6%**	**Mean**	**2%**	**4%**	**6%**	**Mean**
T_1	6.50 (2.64)	6.83 (2.70)	7.00 (2.73)	6.77 (2.69)	7.00 (2.73)	7.63 (2.85)	7.83 (2.88)	7.48 (2.82)	7.16 (2.76)	7.50 (2.82)	7.66 (2.85)	7.44 (2.81)	7.66 (2.85)	8.00 (2.91)	8.16 (2.94)	7.94 (2.90)
T_2	7.00 (2.73)	7.50 (2.82)	8.00 (2.91)	7.50 (2.82)	7.50 (2.82)	8.33 (2.97)	8.66 (3.02)	8.16 (2.94)	7.83 (2.88)	8.83 (3.05)	9.00 (3.08)	8.55 (3.00)	8.33 (2.97)	9.55 (3.16)	9.83 (3.21)	9.22 (3.11)
T_3	6.00 (2.54)	6.50 (2.64)	6.66 (2.67)	6.38 (2.62)	6.30 (2.60)	6.63 (2.67)	6.83 (2.70)	6.58 (2.66)	5.83 (2.51)	6.66 (2.67)	6.83 (2.70)	6.44 (2.63)	7.00 (2.73)	7.16 (2.76)	7.00 (2.73)	7.05 (2.74)
T_4	6.16 (2.58)	6.33 (2.61)	6.50 (2.64)	6.33 (2.61)	6.50 (2.64)	7.16 (2.76)	6.33 (2.61)	6.66 (2.67)	6.50 (2.64)	6.50 (2.64)	6.83 (2.70)	6.61 (2.66)	7.33 (2.79)	7.00 (2.73)	7.16 (2.76)	7.16 (2.76)
T_5	7.16 (2.76)	8.00 (2.91)	8.83 (3.05)	7.99 (2.91)	7.83 (2.88)	8.83 (3.05)	9.16 (3.10)	8.60 (3.01)	8.00 (2.91)	9.50 (3.16)	10.16 (2.64)	9.22 (3.11)	8.83 (3.05)	10.66 (3.34)	11.00 (3.39)	10.16 (3.26)
T_6	5.66 (2.48)	6.00 (2.54)	6.50 (2.64)	6.05 (2.55)	6.16 (2.58)	6.66 (2.67)	7.33 (2.79)	6.71 (2.68)	6.16 (2.58)	6.50 (2.64)	6.66 (2.67)	6.44 (2.63)	6.50 (2.64)	7.00 (2.73)	7.66 (2.85)	7.05 (2.74)
T_7	6.33 (2.61)	6.50 (2.54)	6.83 (2.70)	6.55 (2.65)	6.66 (2.67)	7.00 (2.73)	7.66 (2.85)	7.10 (2.75)	6.83 (2.70)	7.00 (2.73)	7.33 (2.79)	7.05 (2.74)	7.16 (2.76)	7.33 (2.79)	7.83 (2.88)	7.44 (2.81)
T_8	6.66 (2.67)	7.00 (2.64)	7.33 (2.79)	6.99 (2.73)	7.00 (2.73)	7.33 (2.79)	7.83 (2.88)	7.38 (2.80)	7.16 (2.76)	7.83 (2.88)	8.00 (2.91)	7.66 (2.85)	8.00 (2.91)	8.16 (2.94)	8.33 (2.97)	8.16 (2.94)
T_9	6.83 (2.70)	7.33 (2.79)	7.83 (2.88)	7.33 (2.79)	7.16 (2.76)	7.83 (2.88)	8.00 (2.91)	7.66 (2.85)	7.50 (2.82)	8.16 (2.94)	8.66 (3.02)	8.10 (2.93)	8.16 (2.94)	8.50 (3.00)	8.83 (3.05)	8.49 (2.99)
T_{10}	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.700	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Mean	5.83 (2.51)	6.19 (2.58)	6.54 (2.65)		6.21 (2.59)	6.74 (2.69)	6.93 (2.72)		6.29 (2.60)	6.84 (2.70)	7.11 (2.75)		6.89 (2.71)	7.33 (2.79)	7.58 (2.84)	
F–test	Treatments		Dilutions		Concentrations		Treatments		Dilutions		Concentrations					
	*		*		*		*		*		*					
S.Em ±	0.101 (0.775)		0.048 (0.740)		0.058 (0.746)		0.127 (0.791)		0.060 (0.748)		0.073 (0.756)					
CD at 5%	0.283 (0.884)		0.133 (0.795)		0.163 (0.814)		0.355 (0.924)		0.167 (0.816)		0.205 (0.839)					

* - Significant at 5% level. Values in brackets of parentheses are square root transferred.

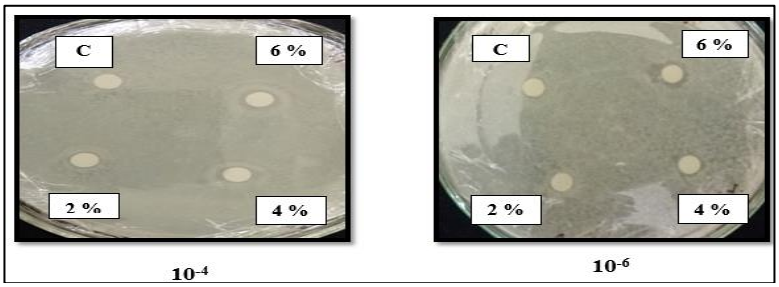

Plate 1: *In-vitro* effect of *Ocimum tenuiflorum* plant extract at 2, 4 and 6 per cent concentrations and control (distilled water) on inhibition zone against *Bacillus* sp., for 48 hours of incubation period.

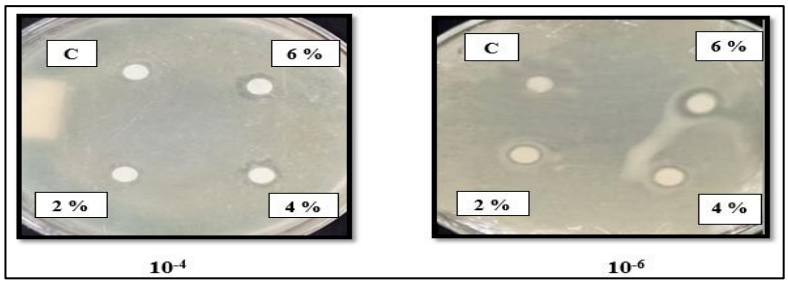

Plate 2: *In-vitro* effect of *Asparagus officinalis* plant extract at 2, 4 and 6 per cent concentrations and control (distilled water) on inhibition zone against *Staphylococcus* sp., for 48 hours of incubation period.

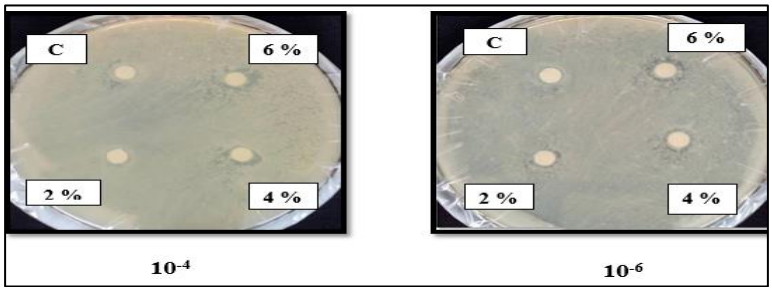

Plate 3: *In-vitro* effect of *Phyllanthus emblica* plant extract at 2, 4 and 6 per cent concentrations and control (distilled water) on inhibition zone against *Streptococcus* sp., for 48 hours of incubation period.

The present results are in agreement with the findings of Manjunath (2007) [5], who reported that the application of botanical extracts to test the inhibition zone against *Streptococcus* sp., the maximum inhibition zone was found in *Withania somnifera* (8.05 and 8.57 mm) and minimum of 5.97 and 6.50 mm in case of *Ocimum sanctum* on first and second day of 1:1 and 1:3 proportion of botanical extracts used.

Conclusion

The *in-vitro* evolution results of nine different plant extracts clearly showed that, these plant extracts possess antibacterial activity against the pathogenic bacteria used for the study. The increasing concentration of plant extracts also showed increased inhibition zone it may be due to increased quantity of antibacterial constituent with increasing concentrations. Among the concentrations used, 6 per cent found effective by inhibiting the growth of all the three bacterial species.

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