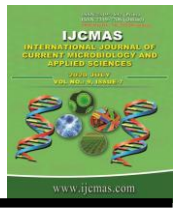
Int.J.Curr.Microbiol.App.Sci (2020) *9*(7): 346-354

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 7 (2020)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2020.907.037

In-Vitro Efficacy of Forms and Methods of *Adhatoda vasica* against Flacherie causing Bacteria of Silkworm, *Bombyx mori* L.

K. V. Anitharani*, R. N. Bhaskar and H. G. Anusha

Department of Sericulture, UAS GKVK, Bengaluru-560065 (Karnataka), India

**Corresponding author*

Keywords

Adhatodavasica, bacterial flacherie, inhibition zone.

Article Info

Accepted:
05 June 2020
Available Online:
10 July 2020

A B S T R A C T

The flacherie diseased silkworms may contain multiple species of bacteria *viz.*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Serratia marcescens* and *Bacillus* sp. The use of botanical formulations possessing antibacterial activity are preferred in silkworm rearing over the chemical agents which found to be effective on checking bacterial infection and enhance the growth of silkworm. Different forms and methods of application of *Adhatoda vasica* was found effective in the management of bacterial flacherie of silkworm. In the present study, three bacterial species (*Bacillus* sp., *Staphylococcus* sp. and *Streptococcus* sp.) were isolated from haemolymph of silkworm and used under *in-vitro* condition. The study revealed that, the aqueous, acetone and alcoholic extract of *A.vasica* at different concentrations (2, 4 and 6 %) were highly effective against three bacterial species. However, the maximum zone of inhibition was observed in alcoholic extract (8.00, 9.12 and 8.99, 9.62 mm)(7.42, 7.88 and 7.84, 8.63 mm) (8.25, 8.87 and 8.84, 9.21 mm) and minimum in aqueous extract(7.66, 8.46 and 8.25, 8.94 mm) (6.96 and 7.29., 7.35 and 7.79 mm) (6.46, 6.92 and 8.09, 8.46 mm) on 24 and 48 hours of incubationagainst10^{-5} and 10^{-7} spore dilution of *Bacillus*, *Staphylococcus* and *Streptococcus* species, respectively.

Introduction

Mulberry silkworm is being reared indoor, since they are highly susceptible to disease causing pathogens which lead to drastic reduction in cocoon production. Flacherieis a condition wherein the silkworms are infected by several species of bacteria *viz.*, *Streptococcus faecalis, Staphylococcus aureus, Serratia* sp. and *Bacillus* sp. (Sugun, 2000). Recently, many attempts have been made on the use of plant extracts to combat microbial infections as reported in ancient ayurvedic compendium "Charaka Samhita" and Sushrita Samhita" (Chopra, 1982; Chatterjee and Pakrashi, 1991). Action of plant on microorganisms pathogenic to other animals and insects has been reported (Ray and Majumdar, 1974).

This has commercially been exploited and utilized for management of pest and diseases of silkworm. Hence, an experiment was drawn in this line as to assess the efficacy of

application of *Adhatoda vasica* against bacterial pathogens causing flacherie in the mulberry silkworm, *Bombyx mori* L. The botanical extracted in aqueous, acetone and alcohol media were studied *in-vitro*.

Materials and Methods

Silkworms affected by flacherie disease were spotted, 1 ml of haemolymph was collected and mixed with 9 ml distilled water to make the stock suspension from which serial dilutions(10^{-5} and 10^{-7}) were prepared using 9 ml sterile water blanks (Siromani *et al.,* 1994; Nataraju *et al.,* 1999).

From the serial dilution prepared, 0.5 ml each was transferred to separate petridishes containing nutrient agar medium. The culture plates were incubated at 37°C and after 48 hours, the incubated bacterial culture was observed under microscope to know the type and nature of colony formed. Based on the shape, texture and colour, the colonies were selected and purified on nutrient agar plate. The purified bacterial isolates were identified as *Bacillus* sp., *Staphylococcus* sp.and *Streptococcus* sp. The pathogenicity of the individual bacterium was confirmed by the principle of Koch's postulates. These organisms were used for *in-vitro* studies (Figure- 1).

The botanical screened against *Bacillus* sp., *Streptococcus* sp. and *Staphylococcus* sp., bacteria isolated from silkworm haemolymph was leaf extract of *A.vasica* (common name: Adusoge, Family: Acanthaceae).

Preparation of herbal extracts

Herbal powder

The extract of *A.vasica* (Adusoge) was prepared as per procedure adopted by Krishnaprasad *et al.,* (1979). The leaves of *A.vasica* were collected from 'Sanjeevinivatika' (Herbal Garden) Division of Horticulture, University of Agricultural Sciences, Bengaluru. The required quantity of fresh leaves of *A.vasica* were harvested and surface sterilized with 70 % ethyl alcohol then washed with sterile distilled water and shade dried. The shade dried leaves were then powdered in electric blender at slow speed, sieved and kept stored in desiccators.

Aqueous plant extract

The required quantity of fresh leaves were harvested and surface sterilized with 70 % ethyl alcohol then washed with sterile distilled water. Later, they were taken in pestle and mortar separately and 10ml of sterile distilled water was added to 1 g of leaf for maceration. The extract was squeezed through double layered muslin cloth and used as stock solution. Further, the same was diluted by using sterile distilled water to achieve different concentrations (Karthikairaj *et al.,* 2013).

Acetone herbal extract

Ten grams of herbal powder of *A.vesica* was kept in a conical flask soaked with acetone for 6 hours under air tight condition. The content was then stirred for an hour in magnetic stirrer and filtered through a filter paper. The residual extract was collected in a flask and the solvent is allowed to evaporate at room temperature. The extract was then stored at 4 ˚C until use. The resultant residue was made up to required volume using double distilled water and used for the study (Karthikairaj *et al.,* 2013).

Alcoholic herbal extract

Ten grams of botanical powder was packed separately in a burette column keeping glass wool at the bottom and filter paper rings on

top. Alcoholic extract was collected from the leaf powder by pouring double distilled water and ethyl alcohol on top of the burette column. The collected extract was then concentrated in hot water bath and made up to 10 ml with distilled water and stored in the deep freezer. Required quantities of this extract was taken and diluted with distilled water to get the required concentrations (Karthikairaj *et al.,* 2013).

Placement of paper discs

Sterilized whatman No.1 filter paper discs of 5mm diameter dipped in botanical extracts for 1 minute and drained by the edges of petriplate were placed at the centre of the petriplate. Four replications were maintained for each treatment, further control (distilled water+bacteria and distilled water) was used for comparison. The bacteria inoculated plates were incubated for 48 hours at room temperature. The diameter of the inhibition zone by different forms of botanicals against each bacterium was measured (mm) and recorded on 24 and 48 hours of incubation.

T_1 – Aqueous extract
T_2 – Acetone extract
T_3 – Alcoholic extract
T_4 – Water

Results and Discussion

Zone of inhibition (mm) observed in different concentrations (2 %, 4 % and 6%) of *Adhatoda vasica* against *Bacillus* sp

The aqueous, acetone and alcoholic extracts of *A.vasica* at different concentrations (2, 4 and 6 %) revealed significant results on zone of inhibition of *Bacillus* sp. with the dilutions of 10^{-5} and 10^{-7}. The maximum zone of inhibition was recorded against T_3 =Alcohol +Spraying (8.00 mm and 9.12 mm) after 24 hours of incubation with bacterial dilution of $10^{-5}$and 10^{-7}.Further, after 48 hours of incubation, *Bacillus* sp. of both the dilutions (10^{-5} and 10^{-7}) exhibited the same trend of zone of inhibition *viz.,* maximum in T_3 (8.99 and 9.62 mm) and minimum of 7.66 and 8.46 mm., 8.25 and 8.94 mm (10^{-5} and 10^{-7})inT_1 on 24 and 48 hours respectively (Table 1 and Figure 2). These results follow the same trend as that of (Karthikairaj *et al.,* 2013) who reported maximum inhibition zone of (9.33, 11.27 and 12.13 mm) microbes involved in flacherie disease of silkworm for alcoholic plant extracts whereas comparatively less for their aqueous counterparts (8.83, 9.77 and 10.73 mm) at different concentrations of 50, 100 and 150 µl of *Andrographis paniculata* followed by *Momardica charentia* (7.83, 8.33, 9.43 and 6.77, 8.13 and 8.93 mm) and *Ocimum sanctum* (7.33, 7.67, 8.83 and 7.17, 7.33 and 8.30 mm).

Zone of inhibition (mm) observed in different concentrations (2 %, 4 % and 6 %) of *Adhatoda vasica* against *Staphylococcus* sp.

The data pertaining to zone of inhibition at different concentrations of *A.vasica* against *Staphylococcus* sp., was found significant among forms, methods and concentration of botanical. However, as the concentration of botanicals increased (2, 4 and 6 %) at 24 hours of incubation in artificial media, it revealed increased zone of inhibition for inoculation dose of 10^{-7}(5.35, 5.66 and 6.07 mm). The trend was same even at 10^{-5}dilution (4.97, 5.38 and 5.72 mm) recorded for 2, 4 and 6 per cent botanical formulation and found significant. The forms of botanicals used after 24 and 48 hours for 10^{-5} and 10^{-7} inoculation doses revealed significant results, however maximum of 7.42 and 7.88., 7.84 and 8.63 mm zone of inhibition was recorded for T_3 which was found comparatively more effective in inhibiting the growth of *Staphylococcus.*

Table.1 Zone of inhibition (mm) observed in different concentrations (2 %, 4 % and 6%) of *Adhatoda vasica* against *Bacillus*

Dilution	24 hours								48 hours							
	10^{-5}				10^{-7}				10^{-5}				10^{-7}			
Conc. of botanical / **Forms of botanical**	**2 %**	**4 %**	**6 %**	**Mean**	**2 %**	**4 %**	**6 %**	**Mean**	**2 %**	**4 %**	**6%**	**Mean**	**2 %**	**4 %**	**6 %**	**Mean**
T_1	6.87 (2.71)	7.37 (2.80)	8.75 (3.04)	7.66 (2.85)	7.50 (2.82)	8.37 (2.97)	9.50 (3.16)	8.46 (2.99)	7.37 (2.80)	8.12 (2.93)	9.25 (3.12)	8.25 (2.95)	8.00 (2.91)	8.87 (3.06)	9.95 (3.23)	8.94 (3.07)
T_2	7.75 (2.87)	7.87 (2.89)	7.87 (2.89)	7.83 (2.88)	7.75 (2.87)	8.62 (3.02)	9.12 (3.10)	8.50 (3.00)	8.25 (2.95)	8.67 (3.02)	10.00 (3.24)	8.97 (3.07)	8.50 (3.00)	9.50 (3.16)	10.50 (3.31)	9.50 (3.16)
T_3	7.12 (2.76)	8.00 (2.91)	8.87 (3.06)	8.00 (2.91)	8.75 (3.04)	9.12 (3.10)	9.50 (3.16)	9.12 (3.10)	7.62 (2.84)	9.00 (3.08)	10.35 (3.29)	8.99 (3.08)	8.75 (3.04)	9.50 (3.16)	10.62 (3.33)	9.62 (3.18)
T_4	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	5.44 (2.43)	5.81 (2.51)	6.37 (2.62)	5.87 (2.52)	6.00 (2.54)	6.53 (2.65)	7.03 (2.74)	6.52 (2.64)	5.81 (2.51)	6.45 (2.63)	7.40 (2.81)	6.55 (2.65)	6.31 (2.61)	6.97 (2.73)	7.77 (2.87)	7.02 (2.74)
F test	Dilutions		Forms		Concentrations				Dilutions		Forms		Concentrations			
	*		*		*				*		*		*			
S. Em ±	0.131 (0.022)		0.278 (0.046)		0.131 (0.022)				0.091 (0.015)		0.193 (0.031)		0.091 (0.015)			
CD 5%	0.362 (0.060)		0.768 (0.126)		0.362 (0.060)				0.251 (0.041)		0.533 (0.086)		0.251 (0.041)			

T_1 : Aqueous form + Spraying, T_2 : Acetone form + Spraying, T_3 : Alcohol form + Spraying, T_4 : Water; * - Significant

Table.2 Zone of inhibition (mm) observed in different concentrations (2 %, 4 % and 6%) of *Adhatoda vasica* against *Staphylococcus* sp.

Dilution	24 hours								48 hours							
	10^{-5}				10^{-7}				10^{-5}				10^{-7}			
Conc. of botanical / **Forms of botanical**	**2 %**	**4 %**	**6 %**	**Mean**	**2 %**	**4 %**	**6 %**	**Mean**	**2 %**	**4 %**	**6%**	**Mean**	**2 %**	**4 %**	**6 %**	**Mean**
T_1	6.62 (2.66)	6.88 (2.71)	7.38 (2.80)	6.96 (2.73)	6.88 (2.71)	7.25 (2.78)	7.75 (2.87)	7.29 (2.79)	6.88 (2.71)	7.38 (2.80)	7.8 (2.88)	7.35 (2.80)	7.25 (2.78)	7.63 (2.85)	8.5 (3.00)	7.79 (2.87)
T_2	6.50 (2.64)	7.13 (2.76)	7.50 (2.82)	7.04 (2.74)	7.13 (2.76)	7.50 (2.82)	8.13 (2.93)	7.59 (2.84)	7.13 (2.76)	7.50 (2.82)	8.25 (2.95)	7.63 (2.85)	7.53 (2.83)	8.50 (3.00)	8.75 (3.04)	8.26 (2.96)
T_3	6.75 (2.69)	7.50 (2.82)	8.00 (2.91)	7.42 (2.81)	7.38 (2.80)	7.88 (2.89)	8.38 (2.97)	7.88 (2.89)	7.13 (2.76)	7.63 (2.85)	8.75 (3.04)	7.84 (2.88)	8.13 (2.93)	8.13 (2.93)	9.62 (3.18)	8.63 (3.02)
T_4	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	4.97 (2.33)	5.38 (2.42)	5.72 (2.49)	5.36 (2.41)	5.35 (2.41)	5.66 (2.48)	6.07 (2.56)	5.69 (2.48)	5.29 (2.40)	5.63 (2.47)	6.20 (2.58)	5.70 (2.49)	5.73 (2.49)	6.06 (2.56)	6.72 (2.68)	6.17 (2.58)
F test	Dilutions		Forms		Concentrations				Dilutions		Forms		Concentrations			
	*		*		*				*		*		*			
S. Em ±	0.062 (0.011)		0.132 (0.024)		0.062 (0.011)				0.043 (0.007)		0.092 (0.016)		0.043 (0.007)			
CD 5%	0.172 (0.031)		0.365 (0.066)		0.172 (0.031)				0.120 (0.020)		0.254 (0.043)		0.120 (0.020)			

T_1 : Aqueous form + Spraying, T_2 : Acetone form + Spraying, T_3 : Alcohol form + Spraying, T_4 : Water; * - Significant

Table.3 Zone of inhibition (mm) observed in different concentrations (2 %, 4 % and 6%) of *Adhatoda vasica* against *Streptococcus* sp.

Dilution	24 hours								48 hours							
	10^{-5}				10^{-7}				10^{-5}				10^{-7}			
Conc. of botanical / **Forms of botanical**	**2 %**	**4 %**	**6 %**	**Mean**	**2 %**	**4 %**	**6 %**	**Mean**	**2 %**	**4 %**	**6%**	**Mean**	**2 %**	**4 %**	**6 %**	**Mean**
T_1	5.75 (2.50)	6.62 (2.66)	7.00 (2.73)	6.46 (2.63)	6.50 (2.64)	7.00 (2.73)	7.25 (2.78)	6.92 (2.73)	7.63 (2.85)	8.00 (2.91)	8.63 (3.02)	8.09 (2.93)	7.75 (2.87)	8.38 (2.98)	9.25 (3.12)	8.46 (2.99)
T_2	7.50 (2.82)	7.62 (2.84)	8.25 (2.95)	7.79 (2.87)	7.50 (2.82)	8.12 (2.93)	8.75 (3.04)	8.12 (2.93)	7.75 (2.87)	8.50 (3.00)	9.25 (3.12)	8.50 (3.00)	8.38 (2.97)	8.50 (3.00)	9.75 (3.20)	8.88 (3.06)
T_3	7.62 (2.84)	8.37 (2.97)	8.75 (3.04)	8.25 (2.95)	8.00 (2.91)	8.62 (3.01)	10.00 (3.24)	8.87 (3.06)	8.00 (2.91)	8.63 (3.02)	9.88 (3.22)	8.84 (3.05)	8.50 (3.00)	8.88 (3.06)	10.25 (3.27)	9.21 (3.11)
T_4	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	5.22 (2.39)	5.65 (2.48)	6.00 (2.54)	5.62 (2.47)	5.50 (2.44)	5.94 (2.53)	6.50 (2.64)	5.98 (2.54)	5.85 (2.51)	6.28 (2.60)	6.94 (2.73)	6.36 (2.61)	6.16 (2.58)	6.44 (2.63)	7.31 (2.79)	6.64 (2.67)
F test	Dilutions		Concentrations		Forms				Dilutions		Concentrations		Forms			
	*		*		*				*		*		*			
S. Em ±	0.072 (0.013)		0.152 (0.027)		0.072 (0.013)				0.066 (0.011)		0.141 (0.023)		0.066 (0.011)			
CD 5%	0.198 (0.035)		0.420 (0.073)		0.198 (0.035)				0.184 (0.030)		0.389 (0.064)		0.184 (0.030)			

T_1 : Aqueous form + Spraying, T_2 : Acetone form + Spraying, T_3 : Alcohol form + Spraying, T_4 : Water; * - Significant

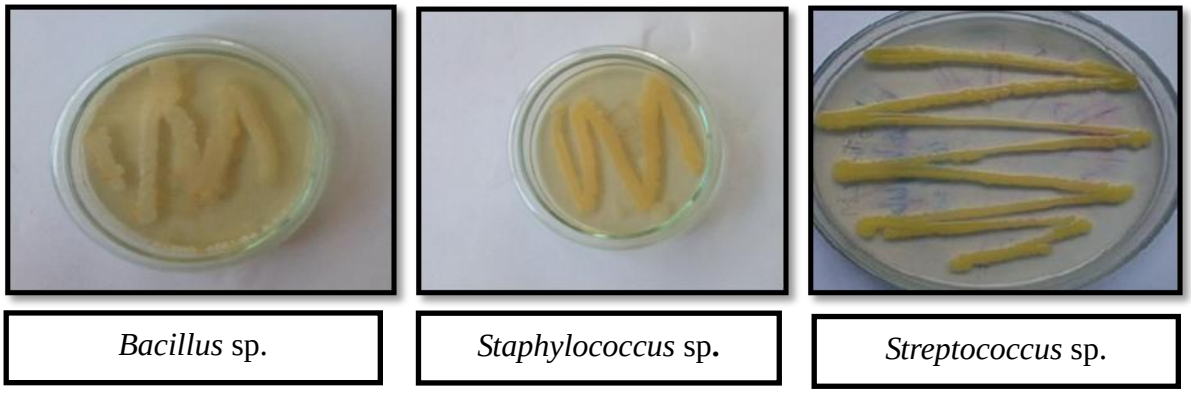


Fig.1 Bacterial species isolated from silkworm haemolymph

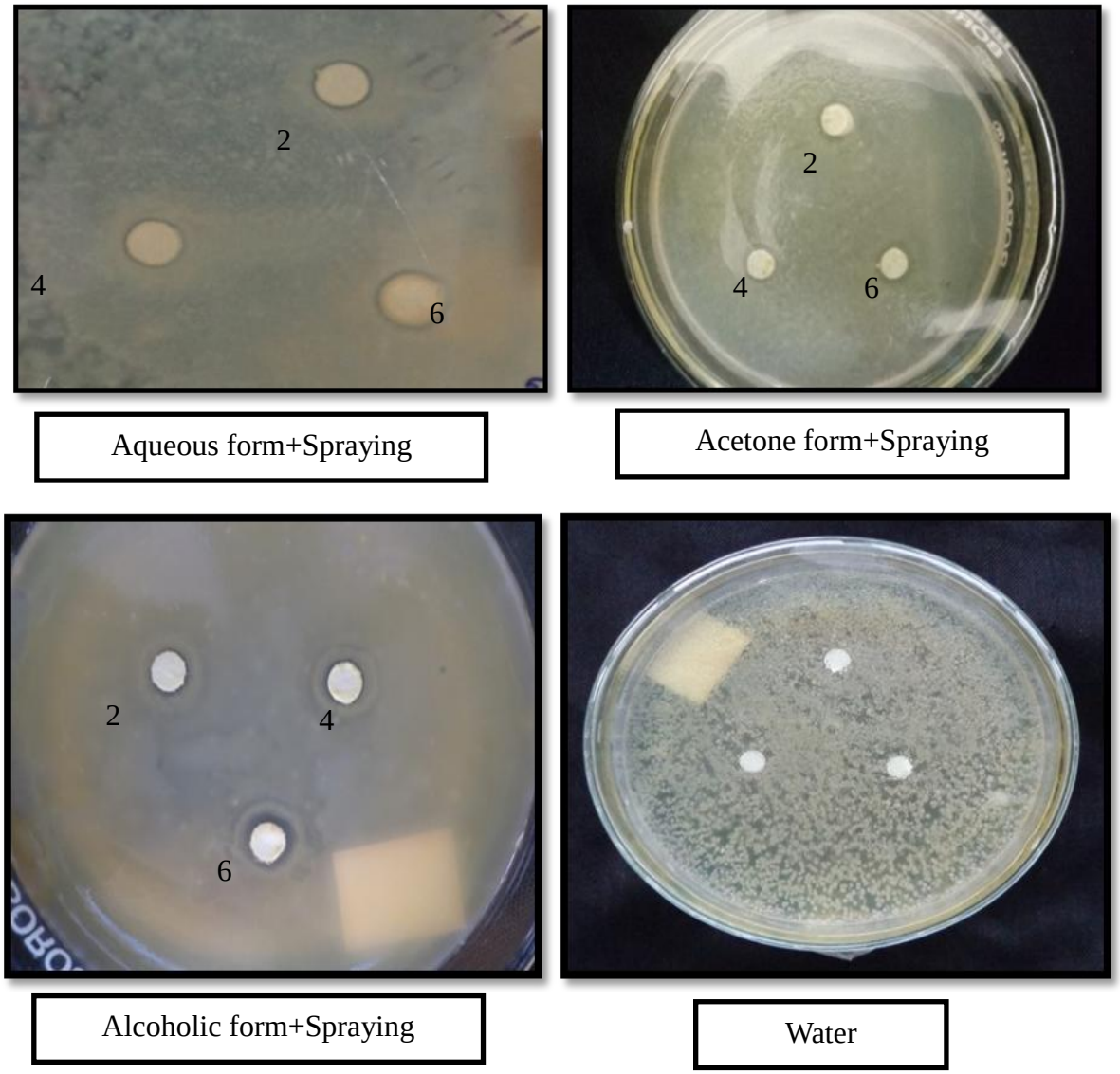


Fig.2 Inhibition zone of *Bacillus* sp. exhibited by *Adhatoda vasica*

The trend was observed similar with other liquid forms T_2 (Acetone form + spraying) (7.04 and 7.59., 7.63 and 8.26 mm) and T_1 (aqueous form + spraying) (6.96 and 7.29., 7.35 and 7.79 mm) and found significant in 10^{-5} and 10^{-7} dilution at 24 and 48 hours of incubation respectively compared to control batches (Table 2).

These results were found on par with the findings of (Hossain *et al.,* 2013), according to them, under *in-vitro* study the use of methanol, petroleum spirit, ethyl acetate and dichloromethane extracts of *Tinospora cordifolia* were found effective against *Bacillus subtilis* and *Escherichia coli*. The maximum zone of inhibition (11.33 and 8.33 mm) was recorded for *E. coli* in methanol and ethyl acetate, whereas zone of inhibition of 10.67 and 8.00 mm was observed for *B. subtilis* with petroleum spirit and dichloromethane extracts of *Tinospora cordifolia*. In addition, minimum zone (6.67 mm) was reported fordichloro-methane and ethyl acetate extracts used against *E. coli* and *B. subtilis.*

Zone of inhibition (mm) observed in different concentrations (2 %, 4 % and 6 %) of *Adhatoda vasica* against *Streptococcus* sp.

The application of botanical extracts of *A.vasica* at different concentrations against *Streptococcus* sp. revealed significant differences pertaining to forms and methods of botanical application, different concentrations of botanicals and different bacterial dilutions at 24 hours of incubation. Further, significant differences were noticed among the treatments *viz.,* T_1 (6.46 and 6.92 mm), T_2 (7.79 and 8.12 mm) and T_3 (8.25 and 8.87 mm) over water control T_4 (0.00 mm) after 24 hours of incubation. In addition, the significant difference was also noticed in between the per cent botanical concentration *viz.,* 2 (5.22 and 5.50 mm), 4 (5.65 and 5.50 mm) and 6 (6.00 and 6.50 mm) at both the bacterial dilutions of 10^{-5} and 10^{-7} respectively (Table 3).

The zone of inhibition after 48 hours of incubation recorded significant difference between liquid forms (T_1-8.09, T_2-8.50 and T_3-8.84 mm) and (T_1-8.46, T_2-8.88 and T_3-9.21 mm) and the results were on par with each other against 10^{-5} dilution whereas at 10^{-7} dilutions all the forms and methods resulted in significant differences in inhibiting growth of bacteria (Table 3).

It is further confirmed by (Samatha *et al.,* 2013) that, at 1:1 concentration, the aqueous extract of *Oroxylum indicum* showed greater inhibition of 11.7 *mm (Bacillus subtilis)*, 8.7 mm (*Staphylococcus aureus*), 6.6 mm(*Staphylococcus albus*) whereas at 1:2 concentration, maximum zone of inhibition(5.2 mm) was found in *Bacillus cereus* when compared to the control (6.3, 6.2, 4 and 3 mm).

The *in-vitro* effect of *Adhatoda vasica* at different concentrations, methods and forms of application were revealed, significant difference and found effective against all the three bacterial species. To assess the effectiveness of these botanical formulations against *Bacillus* sp., *Staphylococcus* sp. and *Streptococcus* sp., the zone of inhibition was assessed. Among three bacterial species, the maximum zone of inhibition was observed in alcoholic extract on 24 and 48 hours of incubation against *Bacillus* sp. at 10^{-5} and 10^{-7} spore dilution.

The trend was same in other bacterial species *viz., Staphylococcus* and *Streptococcus*. However the increased zone of inhibition was observed on 24 and 48 hours of incubation and on 72 hours onwards, the inhibition was found decreased due to the loss of

antibacterial components present in the botanical extracts might have decrease the property of botanical that leads to increase in all the three types of bacterial growth.

References

Chatterjee, A. and Pakrashi, S. C. 1991.The treatise on Indian medicinal plants. I, XII-XX publications and information directorate, New Delhi: Pp:14-15.

Chopra, R. N. 1982.Indigenous drugs of India, 2nd ed. Academic publishers, Calcutta.Pp: 72-74.

Karthikairaj, K., Prasannakumar, K. andIsaiarasu, L. 2013. Use of plant extracts for the control of flacherie disease in silkworm, *Bombyxmori* L. (Lepidoptera: Bombicidae). Int. J. Microbiol. Res., 4(2): 158-161

Krishna Prasad, K. S., Siddaramaiah, A. L. andLingaraju, S. 1979. Possible control of muscardine disease of silkworms by using a few plant extracts. Curr. Res.,8(5): 79-80.

Nataraju, B., Sivaprasad, V. andDatta, R. K. 1999. Studies on the cause of Thatteroga in silkworm, *Bombyxmori*L. Indian J. Seric.,38. Pp: 149-151.

Ray, P. G. and Majumdar, S. K.1974.Antimicrobial activity of some Indian plants. Economic Botany, 30: Pp: 317-320.

SamathaTalari, Sampath, A., Sujatha, K. and RamaSwamyNanna.2013.Antibacterial Activity of Stem Bark Extracts of *Oroxylumindicum*an Endangered Ethnomedicinal Forest Tree. J. Pharmacy and Biol. Sci.,7(1): 24-28.

ShamimHossain, TouhidaKhatun and Mehedi H. 2013.*In-vitro* bacterial effect of *Tinospoacordifolia* extracts against some selective bacterial pathogens. Int. J. Biosci., 3(7):156-161.

Sironmani, A. T., Meena, P. and Vanitha Rani, R. 1994. Isolation and characterization of pathogenic bacterial species in the silkworm, *Bombyxmori* L. Sericologia,34: 97-102.

Sugun, R. 2000.Status and management of Thatte disease of silkworm. Advances in Tropical Sericulture. Proc. Natl. Sem. Tropic. Seric.,UAS, GKVK, Bengaluru. Pp. 84-86.

How to cite this article:

Anitharani. K. V., R. N. Bhaskar and Anusha. H. G. 2020. *In-Vitro* Efficacy of Forms and Methods of *Adhatoda vasica* against Flacherie causing Bacteria of Silkworm, *Bombyx mori* L. *Int.J.Curr.Microbiol.App.Sci.* 9(07): 346-354. doi: https://doi.org/10.20546/ijcmas.2020.907.037