JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

# EFFICACY OF *ALLIUM CEPA*, *ALLIUM SATIVUM* AND *SOLLANUM TRILOBATUM* AGAINST BACTERIAL FLACHERIE IN MULBERRY SILKWORM *BOMBYX MORI* L.

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**Abstract:** Plant extracts are the potent source, to minimize the flacherie disease as the bacteria develop resistance to conventional antibiotics. Thus the present work is designed to assess the efficacy of aqueous extracts botanicals like *Allium cepa, Allium sativum* and *Sollanum trilobatum*. *Bacillus subtilis* were isolated from flacherie diseased silkworm. Aqueous extracts A.cepa has significant activity against B.subtilis. and moderate activity was shown by *A.sativum* and least activity was shown by *S. trilobatum*. HPLC analysis was carried out with siginificantly active botanical *A. cepa*. The present study recommends, botanical extracts as the effective antibacterial agent utilize sericulture operations.

**Key words:** silkworm, botanicals, microbial activity, plant extract, *A.cepa*

## Introduction

Bacterial resistance is a growing problem world-wide (WHO, 2000 and Cohen, 2002). One of the problems in the development of resistance of haemotherapeutic agent was due to abuse of drugs (Al-Bakri and Afifi, 2007 and Neogi *et al*., 2007). Therefore there was a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Ekwency and Elegalam, 2005 ; Karthikairaj *et al.,* 2014 and Jespa and Brisca ,2021). When compared to others, botanicals are available at reduced cost, relative lower incidence and of reactions compared to modern conventional chemical pharmaceuticals (Planta *et al* ., 2000 and Karachi, 2006). The efficacy of aqueous extract of botanicals viz., *Psoralea corylifolia* L. and *Plectranthus amboinicus* L. against grasserie disease of *B. mori* have been reported (Manimegalai and Chandramohan, 2006 and Manimegalai, 2009).

*In vitro* evidence of the antimicrobial activity of fresh and freeze dried *Allium* extracts against many bacteria (Rees *et al.,* 1993) were studied. Onion extracts have both antifungal and antibacterial properties against *S. typhi, E. coli* and *S. aureus* (Noureddine *et al.,* 2005). An aqueous extract of garlic and onion were effective against gram positive and gram negative organisms and fungi was studied (Yani *et al.,* 2006 and Shan *et al.,* 2007). Antibacterial enhancement (synergistic effects) of *A. sativum* with *C. longa* against a number of infections for generations was reported (Neogi *et al.,* 2007). Garlic and other *Allium* vegetables shows antibacterial effect against *Aerobacter, Aeromonas, Bacillus, Citrella, Citrobacter, Clostridium, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Mycobacterium, Proteus, Pseudomonas, and Staphylococcus* was reported (Reuter *et al*., 1996). Antibacterial activity of an aqueous extract of dried garlic against *S.aureus* (Shakrazadeh and Ebadi, 2006) and against, *E.coli, S.typi* and *B.cereus* (Gomaa

and Hashish , 2003) were studied.45 extracts belonging to different groups of marine organisms including mangroves, seaweeds, sea grasses and cyanobacteria explored novel antibacterial and antifungal compounds against silkworm pathogens (Ravikumar *et al.*, 2009). Some plants like *A. indicum, B. diffusa, P. coryleifolia, T. terrestris* and *C nucifera* were studied for the suppression of grasserie disease in silkworm *B. mori* (Sivaprakasam *et al.*, 1999). *In vitro* and *In vivo* studies were conducted for the identification of effective botanicals for the management of bacterial flacherie of silkworm, *B. mori* against *B. thuringiensis* (Manimegalai and Chandramohan, 2005). *In vivo* studies conducted with botanicals revealed that the plant basil and asparagus were effective against *Staphylococcus sp*. and the plant amla and Boerhaevia *diffusa* L. were effective against *Bacillus sp*. in silkworm (Priyadharshini, 2006). Some medicinal plants like *Aadhathoda vasica L., Phyllanthus niruri L., P. coryleifolia L., Tribulus terrestris L.* and *Withania somnifera* showed an antiviral activity against silkworm pathogen BmNPV (Shubha *et al.*, 2010).

## MATERIALS AND METHODS

### Test bacteria – *B. subtilis* (Gram positive)

The pure culture of the test bacteria isolated from the fourth instar infected silkworm, *B.mori* (race: PM x CSR2) and were maintained in a nutrient agar slants. The cultures were maintained in refrigerator for use and regularly checked for contamination. Periodic transfers were made asceptically. Mortality rate of *B.mori* was recorded against *B.subtilis*.

### Extraction

For the aqueous extract preparation, stored in the refrigerator at 4oC until use. For the ethanolic and methanolic extract preparation, ten g of each plant were homogenized with morter and pestle and was extracted with 100 ml of 70% ethanol / methanol in conical flasks, sealed with foil and allowed to stand for 72h, they were filtered to obtain crude ethanolic extracts and stored at $4^{O}$C when not in use (Choudhury *et al.*, 2002). The antibacterial activity was assessed using the simple disc diffusion and well diffusion method.

### Screening for antibacterial activity

The antibacterial activities of plants were evaluated by agar disc diffusion method. Sterile filler paper disc (the Whatman No.1) were impregnated with different crude extracts and dried in a hot air oven at 60 $^{o}$C for 5 min. Then it was placed asceptically above the seeded agar and pressed a little to facilitate proper diffusion and incubated at 37 ± 1 $^{o}$ C for 24 h. The diameter of the inhibition was measured by using graduated.

### Methodology for HPLC analysis

For HPLC analysis, 1g of dried and powdered plant material was extracted with water (10ml) for 4 hours at room temperature. The plant extract was subjected to qualitative and quantitative analysis by using a 1090 liquid chromotograph with diode array detection, Hewlett-packard, Palo alto, USA, HPLC system, using column: 250 x 10mm. Sample amount taken was 125 µg. Stationary phase: Spherisocb ODDS-1, 5µn particle size. Mobile phase: Water - a cetonitrile (1:1 V/V) acidified with 0.00625% formic acid; ISO critic elution. At the flow rate of 1.5ml/min. Column over – 40OC. The results were detected in UV at 195 nm.

## RESULTS

### Percent mortality

Mortality rate of *B.mori* was measured through serial dilution technique. 50 % mortality was obtained at $1\times10^{-5}$µg / ml concentration of *B.subtilis* (Table 1).

Table 1. Percent mortality of fifth instar larvae of silkworm, *B.mori* at different hours,

| Concentration (µg /ml) | Hours of exposure | | | |
|---|---|---|---|---|
| | 24 h | 48h | 72h | 96h |
| 1×10-2 | 50 | 60 | 70 | 90 |
| 1×10-3 | 30 | 50 | 70 | 70 |
| 1×10-4 | 10 | 30 | 50 | 60 |
| 1×10-5 | - | 10 | 30 | 40 |
| 1×10-6 | - | - | 20 | 30 |
| 1×10-7 | - | - | 10 | 20 |
| 1×10-8 | - | - | 10 | 10 |

N=50

## Antimicrobial activity of plant extracts

The maximum antibacterial activity was observed in *A. cepa* aqueous extract with 22.3 mm diameter of inhibition zone (DIZ), and the lowest antibacterial activity was observed in ethanolic extract of *A. cepa* (8.0 mm)(DIZ) . *A. sativum aqueous* extract had maximum antibacterial property against B. *subtilis*. (DIZ 15.6 mm) and the lowest antibacterial activity was observed in methanol extract (8.2 mm). The extract prepared from leaves of S. *trilobatum* had low antimicrobial property when compared to the other two plant extracts. The highest antibacterial activity against B.subtilis was observed in aqueous extract (12.5 mm) and the least inhibition was shown in methanol extract (7.1 mm) (Table 2)

Table 2. Antimicrobial activity of plant extracts against silkworm pathogen *B.subtilis* ( 1 × 10-5 µg / ml )

| Treatments | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Extracts | | |
| | Aqueous | Methanol | Ethanol |
| *A.cepa* | 22.3 | 11.1 | 08.0 |
| *A.sativum* | 15.6 | 08.2 | 14.3 |
| *S.trilobatum* | 12.5 | 07.1 | 08.0 |

Note : The inhibition zones including disc ( 6mm )

## Antibacterial assay

Haemolymph of treated groups shows antibacterial activity. The highest zone of inhibition (23.3mm) was observed when the silkworm larvae was treated with *A. cepa* at 5% concentration. (Table 3). *A.sativum* shows zone of inhibition of antimicrobial activity 16.6mm at 10 % (Table 3) and *S. trilobatum* shows zone of inhibition at 1% was 13mm (Table 3).

Table 3. Antibacterial assay for silkworm haemolymph inoculated with (1×10-5 µg /ml ) *B. subtilis* and plant extracts.

| Treatments | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Concentration | | |
| | 1% | 5% | 10% |
| *A.cepa* | 15.9 | 23.3 | 16.1 |
| *A.sativum* | 14.8 | 12.3 | 16.6 |
| *S.trilobatum* | 13.2 | 08.2 | 07.9 |

Note : The inhibition zones including disc ( 6mm )

## Antimicrobial activity of haemolymph treated with *A.cepa.*

Application of *A. cepa* extracts on silkworm, have registered significant results on antimicrobial activity. Maximum inhibitory activity with 5 % was shown at 12 h of exposure (25.1 mm) and minimum at 1 hr of exposure (8.2 mm) at 1% (Table 4)

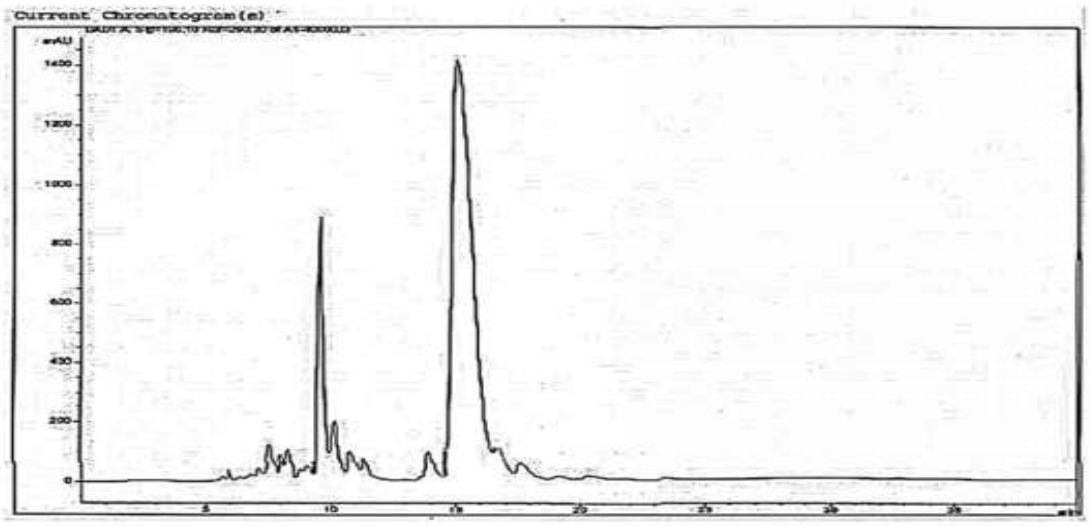
Table 1.4 Antimicrobial activity of haemolymph treated with *A.cepa.*

| Treatments concentration | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Exposure | | | | |
| | 1 hr | 2 h | 3 h | 6 h | 12 h |
| 1% | 8.2 | 10.1 | 10.3 | 11.2 | 16.0 |
| 5% | 8.8 | 11.6 | 18.3 | 20.3 | 25.1 |
| 10% | 8.3 | 9.3 | 16.0 | 16.7 | 16.2 |

Note: The inhibition zones including disc (6mm)

## HPLC analysis

HPLC analysis was used to determine Seleno group compounds, representing the major flavonoids in onion peel. Based on the peak value it was searched in the HPLC chromatogram analysing software-chemical database management software .The compounds obtained were Selenic acid, Selenate, Se-Methylselenocysteine,; Selenomethionine, and g-Glutamyl-Se-methyl selenocysteine,.




Semi preparative – high performance liquid chromatography
Equipment: 1090 liquid chromatograph with diode array detection, Hewlett- Packard, Palo Alto, USA.
Column: 250 x 10 mm. Sample amount: 125 µg.
Stationary phase: Spherisorb ODS-1, 5 µm particle size.
Mobile phase: Water – acetonitrile (1:1; v/v) acidified with 0.00625 % formicacid; isocratic elution.
Flow: 1.5 ml/min. Column oven: 40°C.
Fractionation: Manually. Detection: UV at 195 nm.

**fig-1 HPLC of *A. cepa* extract**

## DISCUSSION

The effects of plant extracts on bacteria, had been studied by a very large number of researchers in different parts of the world ( Chaithradhyuthi *et al.*, 2009., Shubha *et al.*, 2010., and Maribashetty *et al.*, 2010).In the present study antimicrobial activity of 3 plant extracts *viz., A.cepa, A.sativum* and *S.trilobatum* against *B.subtilis* were analysed. Maximum antibacterial activity was observed with *A.cepa* (22.3 mm). This was in corraberation with Garg and Jain (1998) and Mahesha *et al.*, (1999).

The antibacterial effect of essential oil from rhizomes of *Curcuma caesia*. Roxb. rich in curcumene, ionome and tumeron was demonstrated by Garg and Jain (1998) and Juven *et al.*, (1994) had demonstrated the possible mechanism of antimicrobial action of onion and garlic. *Panzaru et al.*, (2009) studied the antimicrobial activity of bulbs of *A. cepa* against *B.subtilis*. In the present study, the maximum antibacterial activity (22.8 mm zone of inhibition) against B.subtilis was resulted due to *A.cepa*. It was in agreement with Priyadharshini (2006) who attributed the inhibition zones of 7.0 mm and 9.0 mm by Amla at a dose of 20,000 and 30,000 ppm against *B.subtilis* while inhibition zones of 6.0 mm and 7.0 mm were observed due to Asparagus against *B.subtilis.* Manoharan (1996) had studied the effect of aqueous extract of *A.cepa* on grasserie in silkworm larvae. According to Bianchini and Vainio (2001), the most predominant sulphur containing compounds are the aminoacids, cysteine and methionine, the S-alk(en)yl substituted cysteine sulphoxides and the γ-glutamyl peptides.

In the present study, on HPLC analysis, the highly active compound present in *A. cepa* was Selenic acid, Se-methyl Selenocystene and Selenomethronine. This finding was in agreement with Steinegger *et al.*, (1999) and Bianchini and Vainio (2001). Block (1985) had reported that onions mainly contain S-propenyl cysteine sulfoxide, but also other sulfoxides, including S-propylcysteine sulfoxide and S-methylcysteine sulfoxide. Bruneton (1995) had reported that a fresh *A. cepa* bulb contains fructants with a low degree of polymerization and sulphur containing compounds. The presence of these compounds might explain the antimicrobial activity of this plant. The present study revealed the antimicrobial activity of three different extracts of A .*sativum* viz, aqueous, methanol and ethanol respectively, among that, aqueous extract shows maximum inhibition (15.6 mm) against silkworm pathogen *B. subtilis*. Choudhury *et al.*, (2002) evaluated ethyl acetate extract of *A. sativum* for antibacterial activity against silkworm pathogen *B. thuringiensis*. Dewitt et *al.*, (1979) reported that as a result of bactericidal activity of *A. sativum*, toxin production by the bacteria is prevented. Cavallito and Bailey (1994) had demonstrated that *A. sativum* juice diluted to one part in 1,25,000 inhibits the bacterial growth of *Staphylococcus, Streptocouus* and *Bacillus*.Chattopadhyay and Bhattacharyya (2007) revealed that preparation of fresh *A.sativum.*

## ACKNOWLEGMENT

Dr.G.S.Anantha Selvi is thankful to Dr. M.Ramani Bai, associate professor of Muslim Arts College , Thiruvithamcode, Kanyakumai District ,Tamilnadu, India , for her critical reading of the manuscript and helpful suggestions.

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