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Impact Of Medicinal Plant Extract Against Bacterial Pathogen Causing Septicaemia In The Mulberry Silkworm, Bombyx Mori L.

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Abstract:
Bombyx mori is domesticated for silk production and is reared in colonial forms. A code of conduct for rearing silkworm is practiced to ensure survival of silkworm and cocooning. One of the major constraints in silk production is the diseases in silkworm rearing. Bacterial infection is well managed by antibiotics, the ability of bacteria 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 the crude aqueous extracts of plants against silkworm bacterial pathogens. In the present study bacterial pathogens were collected from black thorax Septicemia diseased larvae of silkworm B. mori, the diseased larvae were crushed by using mortar and pestle. As the result the organisms identified were Klebsiella pneumonia, Bacillus subtilis, E.coli and Staphylococcus aureus. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. The zone of inhibition for Staphylococcus aureus was maximum (12.5±0.7) at 500µg/ml extract concentration. Medicinal plants are cheaper and effective natural antimicrobial agents with better potential, lesser side effects than antibiotics, good bioavailability and minimal toxicity.

Key words: Bombyx mori, Septicemia, Staphylococcus aureus, Bacillus subtilis, E.coli

1. Introduction:

The term “sericulture” is used to denote both the industry concerned with silkworm rearing and the science which provides the technical basis for the industries. Archeological and Bibliographical evidences show that the sericulture was practiced in china about 2500 BC. One of the major constraints in silk production is the diseases in silkworm rearing. Silkworm B. mori is domesticated for silk production and are reared in colonial forms. A code of conduct for rearing silkworm is practiced to ensure survival of silkworm and cocooning (Sakthivel, 2012).

The efficacy of antibiotics against bacterial pathogens of B. mori has been proved already by several authors (Manimegalai and Chandramohan, 2005). Medicinal and aromatic plants constitute a major source of natural organic compounds widely used in human health care. These plants produce many compounds as secondary metabolites that have no apparent metabolic, physiologic and structural role in the producer, but often have effects on other organisms. In many cases they are believed to function as biochemical defence (Jain et al., 2004). Abhijit et al. (2010) reported that plants contain in different types of medicines. Plants produced phytochemical constituents such as alkaloids, flavonoids, phenols, carbohydrates, steroids, tannins, and aminoacids etc.,

The plant Coccinia grandis belonging to the family Cucurbitaceae, commonly known as kovay in Tamil. Coccinia grandis commonly originated in Asia and Central Asia. Alternately arranged in leaves, large flowers, flowers are star shaped with white colour and the fruit is red colour. It is commonly known as Ivy gourd. It is an aggressive climbing vine that can spread quickly over trees, shrubs, fences and other supporters. Being a perennial plant, it can spread vegetatively or by seed. The leaves of grandis plant were approximately 5-10 cm in width and length. The leaves are bitter sweet and astringent. The Ivy gourd fruit belongs to the berry type, oval and hairless with thick and sticky skin. The raw fruit is green in colour which turns bright red on ripening. The mature fruit 25-60mm long and 15-35mm in diameter and contains several pale, flattened seeds 6-7mm long. The leaves and fruits used in edibles. The stem and root are used in skin diseases, asthma, bronchitis and used many diseases. The former plant Coccinia grandis has good antidiabietic activity with good digestive properties. The many bioactive compounds used in the anti inflammatory, antidiabeticand analgesic activity etc. Mirsanipa et al. (2014).

GC-MS take part an important responsibility in the investigation and detections of unidentified components existing in natural products. The main function of GC-MS is to measure the mass numbers of unknown compounds following ionizing the plant compounds and identification of unknown compounds spectrum by comparing with standard spectrum peaks of compound (Mohamad 2016). The present study was undertaken to find out the possibility of using the extracts of five medicinal plant Coccineagrandis for controlling the bacterialsepticaemia diseases in the mulberry silkworm, B.mori.

2. Materials and Methods:

2.1 Collection of plant materials

The plant materials (leaf, stem and fruit) of Coccinia grandis L. The plant materials were collected from Monday market of Kanyakumari District, Tamil Nadu. The plant materialswere washed thoroughly 2-3 times with running tap water and once sterile distilled water. After the plant parts were shade dried and coarsely powdered separately and stored in well closed containers for further laboratory use.

2.2 Extraction of the plant materials

These coarse powders (25g) were subjected to successive extraction in 250 ml of acetonsolvent by using Soxhletapparatus. The collected extracts were stored and then used for further analysis.

2.4 Qualitative phytochemical analysis

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Brain and Turner (1975) and Evans (1996).

2.4.1 Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

2.4.2 Detection of Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of flavonoids.

2.4.3 Detection of Phenols

Ferric chloride test: 10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.

2.4.4 Detection of Steroids

Two ml of acetic anhydride was added to five mg of the extracts, each with two ml of H2SO4. The colour was changed from violet to blue or green in some samples indicate that the presence of steroids.

2.4.5 Detection of Saponins

About 0.5mg of the extract was shaken with five ml of distilledwater. Formation of frothing shows that the presenceof saponins.

2.4.6 Detection of Tannins

A small quantity of extract was mixed with water andheated on a waterbath. The mixture was filtered and ferricchloride was added to the filtrate. A dark green colour wasformed. It indicates that the presence of tannins.

2.5 Isolation of bacteria

Bacterial pathogens were collected from black thorax Septicemia diseased larvae of silkworm B. mori, the diseased larvae were crushed by using mortar and pestle. The homogenate was then filtered with silica filter. The filtrate was centrifuged at 5000 rpm for 10 min.

The supernatant was discarded, and the pellet was used for bacterial culture after re-suspending in distilled water (Anija 2003).The microbes were isolated and sub cultured after 72 hrs.

The isolated colonies of bacteria were cultured in a nutrient broth for about 48 hrs for further experiments.Four bacterial colonies were obtained.

2.6 Agar well diffusion method

Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains were adjusted to 0.5 OD value according to McFarland standard, (Klebsiella pneumonia, Bacillus subtilis, E.coli and Staphylococcus aureus) Wells were cut and concentration of sample Coccineaglandis(500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control.

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis of the plant extract was made in a QP 2010 Plus SHIMADZU instrument under computer control at 70 eV. About 1 µL of the acetone extract was injected into the GC-MS using a microsyringe and scanning was done for 45 min, in which helium is used as the carrier gas.

As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever the compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by the computer.

The time from when the injection was made (initial time) to when elution occurred is referred to as the retention time (RT). When the instrument was run, the computer-generated graph from the signal called a chromatogram (Altameme et al.2015).

Table 1 Phytochemical analysis of Cocciniagrandis L.

S.No.	Name of the Sample	Phytochemical compound	Result
1.	**Coccinia grandis**	Tanins	-
2.		Steroids	+
3.		Flavonoid	+
4.		Protein	0.63
5.		Soponin	-
6.		Alkaloids	-

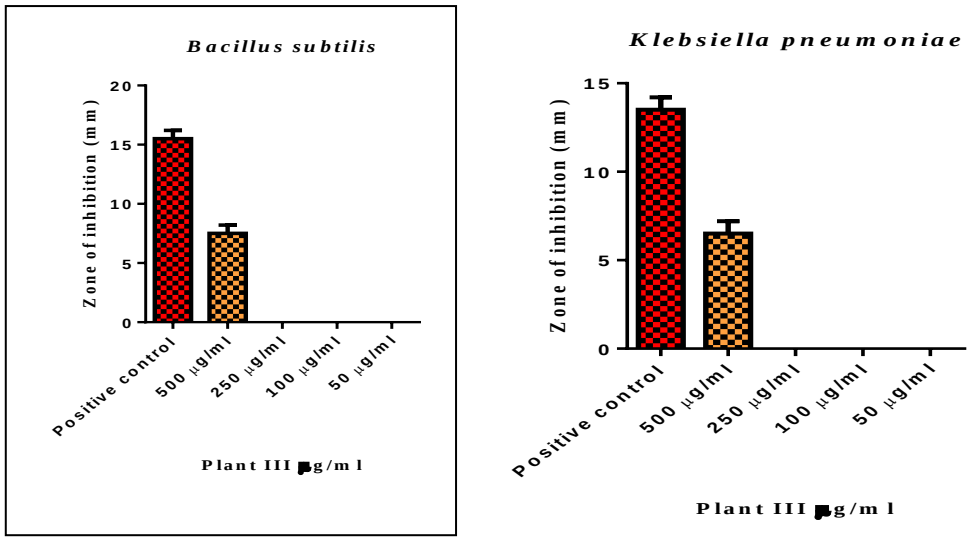


Fig: 1 Effect of sample **Coccinia grandis** against Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus and E. coli

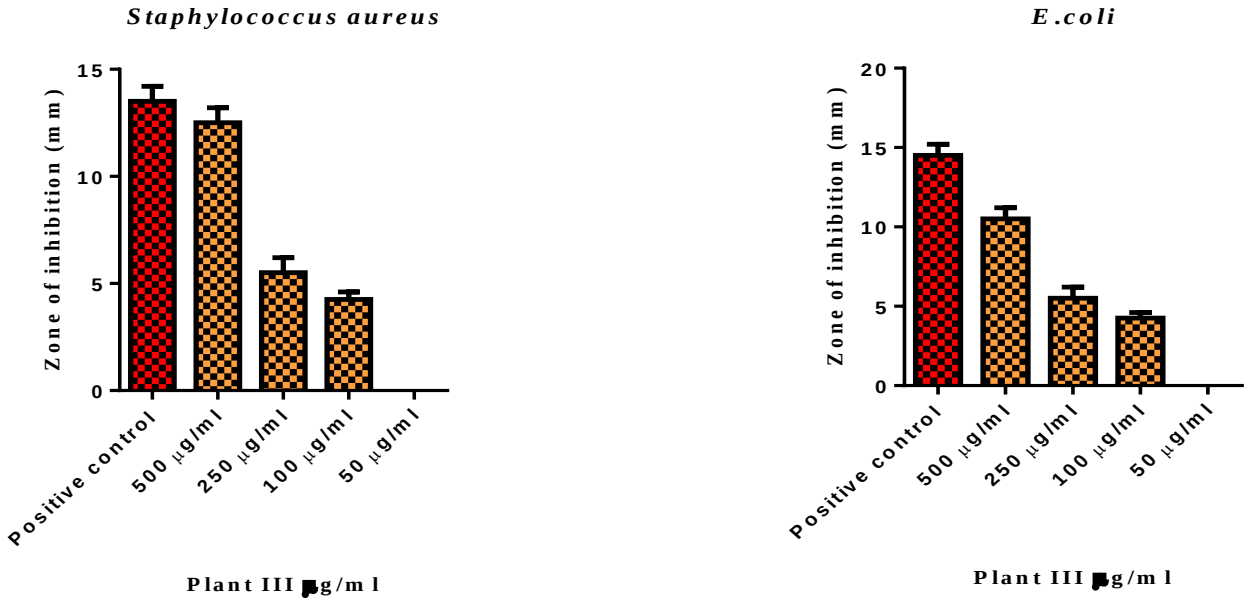
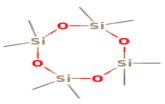
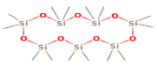
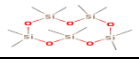
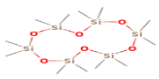
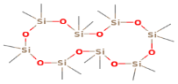
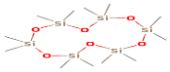
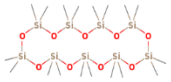
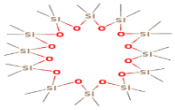
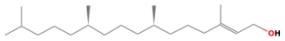


Table 2.zone of inhibition

S. No	Name of the test organism	Zone of inhibition (mm) SD ± Mean				
		500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	Klebsiellapneumoniae	6.5±0.7	0	0	0	13.5±0.7
2.	Bacillus subtilis	7.5±0.7	0	0	0	15.5±0.7
3.	Staphylococcus aureus	12.5±0.7	5.5±0.7	4.25±0.35	0	13.5±0.7
4.	E.coli	10.5±0.7	5.5±0.7	4.25±0.35	0	14.5±0.7

Table 3: Major components identified in the methanol extract of Coccineagrandis

SI. no	Retention time	Compound name	structure	Area (%)
1	6.429	Cyclotetrasiloxane, octamethyl-		5.64
2	17.561	Cycloheptasiloxane, tetradecamethyl-		**9.27**
3	10.015	Cyclopentasiloxane, decamethyl-		**12.7**

4	13.946	Cyclohexasiloxane, dodecamethyl-		**14.7**
5	20.81	Cyclooctasiloxane, hexadecamethyl-		6.13
6	23.6	Cyclononasiloxane, octadecamethyl-		5.04
7	22.08	Hexane, 3,3-dimethyl-		1.04
8	28.36	Cyclododecasiloxane, tetracosamethyl-		3.26
9	29.00	Phytol		1.04

3. Results:

In the present investigation, the pathogenicity study four bacterial speciesKlebsiella pneumonia, Bacillus subtilis, E.coli and Staphylococcus aureus were determined. This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as Tannins, Steroids, Flavanoid, Protein, Saponin and Alkaloids were present in the sample.The acetone extract of Coccineagrandis was assessed with various concentrations of the plant extract. Four bacterial strains were isolated and identified using biochemical tests were used for the present study. The plant extracts exhibited strong antibacterial effects against the tested bacteria with inhibition zones ranging from 6.5mm to 12.5 mm. The pathogens were Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus and E. coli was found to be more susceptible to the acetone leaf extract of C. glandis.The zone of inhibition for Staphylococcus aureuswas maximum (12.5±0.7) at 500µg/ml extract concentration.The zone of inhibition of E. coli(10.5±0.7) at 500 µg/ml of the acetone extract and the results indicated that the significant antibacterial activity,
The bioactive phytoconstituents present in the Acetone extract leaves of C. grandisidentified by GC-MS analysis. On comparison of the mass spectra of the constituent with the NIST library, the phytoconstituents were characterized and identified. The active principles with their retention time (RT), molecular formula, molecular weight and concentration (%) of the phytoconstituents present in C. grandisare presented in Table 3. The present study proved the C. grandis plant possess the good antibacterial and antioxidant property. This may be due to the presence of Cyclotetrasiloxane, octamethyl-, Cycloheptasiloxane, tetradecamethyl, cyclopentasiloxane decamethyl, cyclohexasiloxane dodecamethyl, cyclooctasiloxane hexadecamethyl, cyclonanosiloxane octadecamethyl, hexane 3-3-dimethyl-, cyclododecasiloxane tetracosamethyl, phytol.

4. Discussion

In the present investigation the pathogenic microbes isolated from the disease affected larvae, four species of bacteria were isolated. With increasing resistance of pathogens to antibiotics, medicinal plants are cheaper and effective natural antimicrobial agents with better potential, lesser side effects than antibiotics, good bioavailability, and minimal toxicity (Bhadury and Wright, 2004). Plant-derived natural products, such as alkaloids, flavonoids, phenol, and saponin, have served defence mechanism against invasion by many microorganisms, insects and other herbivores [Bonjar et al. 2004].
Use of 10% amla in aqueous solution and its intermittent spraying during rearing will improve larval survival by preventing the bacterial infection without affecting the silk production capability due to botanical treatment (Dilip Gore et al., 2014).In our study all the compounds of this plant are possessing antimicrobial activity (table 3) could have produced a number of active constituents responsible for many biological activities and its beneficial effects could be utilized to create a healthy environment (Sheela and Uthayakumari, 2013).
The present study reported that differential sensitivity of bacterial to herbal extract will reduce the pathogenicity of microbes in the silkworm B. mori L. are likely to throw much light on the possibility of using such extracts as a prophylactic measure during silkworm rearing to improve silk production and protecting the cocoon crops against the microbes.The medicinal plants contain large amounts of secondary metabolites act

as a biological activitieson physiological systems. It was also reported that the activities of some plant constituents with compound nature of alkaloids, flavonoids, palmitic acid (ethyl ester and heptadecanoic acid), unsaturated fatty acid and linolenic (Octadecenoic acid) as antimicrobial, antioxidant, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and immunomodulatory (Kumar et al.2010).

Conclusion:

The plant extract of C. Grandis possesses excellent antioxidant activity and antimicrobial activity against pathogens.The results from our present investigation demonstrated the presence of nine phytochemicals from the acetone extract of leaves of C. grandis. The presence of those phytochemicals may be responsible to exhibit different biological activities in the silkworm for the treatment septicaemia diseases.

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