INVESTIGATIONS ON MULBERRY AND NON MULBERRY SILKWORM PUPAL OIL

SUMMARY
of
THESIS

SUBMITTED IN FULFILMENT FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy
IN
APPLIED ANIMAL SCIENCES

Submitted By

PARAM DEV
M.Sc.

Under the supervision of

Dr. VENKATESH KUMAR R.
Associate Professor

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

DEPARTMENT OF APPLIED ANIMAL SCIENCES
SCHOOL FOR BIOSCIENCES AND BIOTECHNOLOGY
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226 025 (U.P.), INDIA

Enrolment No: 212/11 **Year 2018**

Summary

The present thesis entitled "**INVESTIGATIONS ON MULBERRY AND NON MULBERRY SILKWORM PUPAL OIL**" describes the physico-chemical, antimicrobial, antioxidant and antidiabetic properties of mulberry and non mulberry silkworm pupal oil. These mulberry and non mulberry silkworm pupal oil were investigated for their antimicrobial, antioxidant and antidiabetic activities. The results of the gas chromatography and mass spectroscopy study showed alpha linolenic acid (omega-3) fatty acids. This compound showed moderate to weak antimicrobial, antioxidant and antidiabetic activity. This thesis contains four chapters which are concisely discussed below.

Insects have always played a prominent role as supplier of food in the history of human civilization, generally in renowned countries. Insects are commonly a rich source of fat, as well as being a good source of vitamins and minerals. The consumption of insects has been familiar in Japan, Thailand, Africa, Latin America, Australia, Mexico and other parts of the developing world where they signify a cheap source of good quality protein. In them, the silkworm is one of the best known useful insects in the world. It is an amazingly significant animal model for researchers next to Drosophila, belonging to the order Lepidoptera of the phylum Arthropoda. Once the silk is spun at the larval stage, immediately they are converted into cocoon shell within.

Pupae are obtained after the extraction procedure of silk thread and are not used commercially. They are also used as edible insect in India except in the northeastern states. Pupae are used as animal feed, organic fertilizer, food material, and traditional

medicine in a few Asian countries, namely Korea, China, Thailand, Japan, and India. They are the optional food product and people began its commercialization in different food and biomedical industry to fulfill the nutritional requirements of hidden hunger and treatment of various diseases. pupae are also a good source of vitamin B_2 intake, which can be important to prevent the serious effects of vitamin B_2 deficiency. Pupae contain protein, fat, carbohydrate, minerals K, Ca, Mg, Na, Cu, Fe, and Zn. vitamins and essential fatty acids that are secure and crucial to the human body. Seri-compost could be used to obtain higher yields of French bean. The pupae are economically essential in the field of Pharmacology, Agriculture and Biodiesel production. The results of the experiment clearly indicated that the application of SLPW (Silkworm litter-pupal waste) and Vermicompost recorded significantly higher leaf yield (32,098.5 kg) and NPK content (3.11%, 0.39%, and 2.48 %) respectively.

The pupae powder contains moisture 7.6%, crude protein, 71.9%, lipid 34.4%, fat 20% and ash 4%. Pupae can significantly increase the hemoglobin and serum protein in rats, producing protective effects on the liver in carbon tetrachloride-induced rat hepatic injury. pupae also protect against Alzheimer's disease. The fermented silkworm powder has a protective effect on alcohol-induced hepatotoxicity in a rat model. In China, silkworm pupae have been consumed as food since very earliest time., silkworm pupae have been listed as "novel food resources managed as common food" by Ministry of Health PR China.

Aim: Investigations of antimicrobial, antioxidant and antidiabetic properties of mulberry and non mulberry silkworm pupae oils

Mulberry silkworm pupae:

In order to get mulberry silkworm pupae oil, silkworm *Bombyx mori* the rearing was conducted according to the protocol.

Whereas cocoons were directly procured from following places to extract oil from non mulberry silkworms namely tasar, eri and muga.

1. Research extension center (Tasar Central Silk Board, Ministry of Textiles, Govt. of India Bundelkhand University, Jhansi.
2. Pradesic Co-operative Sericulture Federation Limited Uttar Pradesh (Office of Assistant Director Sericulture Sonbhadra) Tasar silk Koya Market Dudhi Sonbhadra.
3. Banvasi Seva Asram, Govindpur vaya Tura Sonbhadra Uttar Pradesh.
4. Director, Central Muga Eri Research and Training Institute, Central Silk Board, Ministry of Textiles, Govt. Of India, P.O.-–Lahdoigarh Jorhat, Assam (India) PIN -785700.

Preparation of silkworm dry pupae powder for the extraction of pupae oil

In the presentstudy, pupae were cleaned and grinded into powder form with help of mortar and pestle. Further pupal powder of silkworms was extracted by using solvents namely, n -hexane, Petroleum ether or Di-methyl ether.

Extraction of pupae oil by maceration method:

About 10 gm of dried silkworm pupae powder was transferred to a reagent bottle and 30 ml of N-hexane was mixed. Care was taken that pupa powder was completely submerged in the solvent. The reagent bottle was tightly capped and sealed with glycerin to prevent it from being evaporated.; The sample was kept for about a week time till the texture the pupae turns little yellowish in colour. The texture itself indicates that the sample in ready for extraction.

The yield of oil obtained from Mulberry and Non-Mulberry silkworm pupae powder.

Maceration method was carried out for the extraction of mulberry and non mulberry pupae oils. About 100gm of mulberry silkworm pupal powder was taken and 150ml n-hexane was added. Further, both the mixtures of solvent and pupae powder maintains separate layers because of different specific gravity. Soon the color of the solvent changes to yellowish color. Soon the solvent was filtered with the help of filter paper and placed in a petri dish. The lid of the petri dish was opened for a while in order to remove the moisture from the oil.

Extraction of non-mulberry silkworm pupal oil by using petroleum ether as solvent

The oil of Tasar, Eri and Muga were extracted from their pupae by Maceration method. 100gm of pupal powder of each variety were added separately into 150ml of petroleum ether solvent. Further, both the mixtures of each variety were for a period of one week. Again, it was filtered with filter paper and pupal oil were collected to find out the yield.

Antifungal activity was tested by the disc diffusion method (**Meena *et al.*, 1994**). In disc diffusion method the fungal suspensions were prepared to identify the growth of fungal strains on potato dextrose agar (PDA) media, and Whatman sterile filter paper (6 mm diameter) was placed into the wells of potato dextrose agar plate. Mulberry and non-mulberry silkworm pupae oils were kept in the filter disc in different concentration, i.e. 25%, 50%, 75%, and 100% in order to check for contamination. Different concentrations were made by using sterile glycerin which is a non-polar solvent. All the plates were incubated at 28^0C for 48 hours. After incubation, the antifungal activity was evaluated by measuring the growth of bacterial strains. The zones of inhibition were measured by digital vernier calliper.

Antibacterial activity was again tested by the disc diffusion method (**Meena *et al.*, 1994**). In this method the bacterial suspensions were prepared to identify the growth of bacterial strains on nutrient agar media, and Whatman sterile filter paper (6 mm diameter) was placed into the wells of the nutrient agar plate. Mulberry and non-mulberry silkworm pupae oils were kept in the filter disc in different concentration, i.e. 20%, 40%, 60%, 80% and 100% in the same manner, with one control.

The MIC test was done by using (**Fuente *et al.*, 2006**) method. The media to MIC test were used as Muller Hinton Broth (MHB). The bacterial suspensions were prepared to identify the growth of bacterial strains *Staphylococcus sciuri* strain CD_{97} in MHB media. The tests were conducted in forty test tubes: Following method used to the screening of antibacterial activity by MIC method.

In all four group five test tubes were arranged in which one test tube for control, four test tubes were with bacterial suspension in which oils of **mulberry** silkworm pupal oils added at a different concentration of 12.5, 25, 50, and 100 µl/ml respectively. Another five-test tube was arranged in which one test tube was for control, four test tubes were for bacterial suspension with DMSO (Dimethyl Sulfoxide) in which antibiotic streptomycin added at the different concentration of 12.5, 25, 50, 100 µl/ml respectively.

Silkworm pupae oils did not affect the isolated mulberry leaves fungus. While the bacterial species had shown significant zone of inhibition in case of mulberry silkworm pupae oils at 100%, 12.13 ± 0.318 mm and at 100%, 14.125 ±0.315 mm at 24 and 72 hours respectively when compared to other pupae oils. The MIC of mulberry and non mulberry silkworm pupae oils showed very less significant results.

The oils were evaluated for its fatty acid composition by gas liquid chromatography using methyl estrification method. The oils thus obtained through different extraction procedure were evaluated of its density, viscosity, acid value, iodine value, ester value, saponification value and unsaponificable matter.

The fatty acid components present in silkworm pupae oils were analyzed by gas chromatography mass spectroscopy (gas chromatography and mass spectroscopy). Five compounds were identified from the silkworm pupae oil, including alpha linolenic acid, Linoleic acid, oleic acid, palmitic acid, and stearic acid.

Density and viscosity of mulberry, tasar, eri and muga silkworm pupal oil are 0.742±0.0068, 0.884±0.01, 0.676±0.002 and 0.957±0.017 and 31.66±0.378, 35.2±0.36, 32.56±0.768 and 36.29±0.45 respectively. The acid and iodine value of mulberry, tasar, eri and muga are 12.41±0.505, 12.21±0.235, 13.15±0.309 and 14.05±0.149 and 130.55±0.579, 134.05±0.201, 136.21±0.1006 and 132.99±0.137 respectively. Ester and saponification value of mulberry, tasar, eri and muga silkworm pupal oil are 193.25±0.975, 191.11±0.394, 193.17±0.395 and 191.94±1.04 and 205.66±0.577, 203.33±0.577, 206.33±0.577and 206±1.0 respectively. The unsaponifiable matter of mulberry, tasar, eri and muga silkworm pupae oils are 0.164±0.0005, 0.843±0.001, 0.125±0.0015 and 0.883±0.1011 respectively.

The antioxidant property of oil will be evaluated by using following methods:

(a) DPPH assay

(b) FRAP assay

(c) Hydrogen peroxide assay

(d) Superoxide assay

(e) Reducing assay

The decrease in absorbance of **DPPH** radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which result in the scavenging of the radical by the hydrogen donation.

A concentration dependent ferric reducing antioxidant power (**FRAP**) of mulberry tasar, eri and muga silkworm pupal oils was observed with the highest activity of absorbance were 0.197±0.017, 1.446±0.054, 0.440±0.030 and 0.478±0.043µmol Fe (II)/g) at 50% concentration respectively

Assessment of hydrogen peroxide assay of mulberry tasar, eri and muga silkworm pupal oils were significant ($p<0.05$) at 10µl/ml, 20µl/ml, 30µl/ml and 40µl/ml and 50 µl/ml concentration respectively.

Superoxide radicals are known to be very harmful to the cellular components. Superoxide free radicals were formed by alkaline DMSO which reacts with NBT to produce coloured diformazan. Mulberry and non-mulberry silkworm pupal oil scavenges superoxide radical and thus inhibits formazan formation.

In reducing power assay, the presence of reducing agents causes the conversion of Fe_3^+ ferricyanide complex to the ferrous form that may be followed at 700 nm due to the formation of Perl's Prussian blue $Fe_4[Fe(CN)_6]_3$. Increasing absorbance at 700 nm indicates an increase in reducing ability (**Joshi *et al.,* 2010**). The antioxidants present in the mulberry, tasar, eri and muga silkworm pupal oils due to their reduction of Ferric (Fe_3^+**)** ferricyanide complex to the ferrous (Fe_2^+) form, and thus proved the reducing

A group of rats were rendered diabetic by single intravenous injection of 50 mg/kg of STZ (Streptozotocin) dissolved in 0.1 M citrate buffer (pH 4.5). Diabetes was confirmed in STZ treated rats by measuring the fasting blood glucose concentration 96 hours after the injection of STZ. The rats with blood glucose level (BGL) >200 mg/dL (milligrams per deciliter) were considered to be diabetic and were used in the experiment.

Group normal 1: Rats were fed on standard diet mentioned as normal control (negative control group). Standard diet was prepared according to standard methods. It contained 14% casein, 2% cellulose, 5% salt mixture, 1% vitamin mixture, 0.25% choline, 0.3% Dl-methionine, 8% corn oil and remaining percentage was starch.

Group 2: Rats were fed on fructose rich diet + 8% mulberry silkworm pupal oil

Group 3: Rats were fed on fructose rich diet + 16% mulberry silkworm pupal oil

Group 4: Rats were fed on fructose rich diet + 24% mulberry silkworm pupal oil

Group 5: Rats were fed on fructose rich diet + 32% mulberry silkworm pupal oil

For in-vitro assessment the albino rats weighing between 180-250 grams were kept in animal house individually in stainless steel cages under controlled condition at constant temperature (22°C) and lighting 12 h. light-dark cycle) and given free access to food and water at all time.

The blood glucose levels of control of rat were as 144.333±1.527, 147.666±1.527, 151.000±1.000 and 156.666±1.5275. After induction of STZ (Streptozotocin) diabetic the blood sugar level increases as 343.333±18.823, 282.666±45.346, 241.000±12.165

and 211.000±22.538. The different concentration as 8mg/ml, 16mg/ml, 24mg/ml and 32mg/ml mulberry and non-mulberry (tasar, eri muga) silkworm pupal oil given orally to diabetic rats.

The increasing concentration of mulberry, tasar, eri and muga silkworm pupal oil lowering the blood glucose level as on 16mg/ml the blood sugar level were as 173.666±14.843, 188.000±2.000, 207.000±16.462 and 185.666±11.590 respectively.

In organ bath that the absorption of mulberry and non-mulberry (tasar, eri, muga) silkworm pupae oils in ileum (small intestine) of rat initially at 0min were 0.518±0.005, 0.338±0.005, 0.019±0.0001 and 0.165±0.006 respectively. The absorption of oil was increases with time interval increases as 15min, 30min, 60min, 120min, 240min and finally at 480min the absorption of mulberry and non-mulberry (tasar, eri, muga) silkworm pupal oil were 3.328±0.006, 2.179±0.010, 1.470±0.051 and 1.624±0.057 respectively.

Conclusion:

The omega-3 compound as alpha-linolenic acid, which is essential for the human body is present in mulberry and non-mulberry silkworm pupae oils. The alpha-linolenic compound breaks into EPA and DHA during metabolic activity. It showed that the EPA has antimicrobial property. So, mulberry and non-mulberry silkworm pupae oils were shown antibacterial activity on *Staphylococcus sciuri* strain CD_{97}, while same pupae oil did not show any effect on the *Phyllactinia corylea* fungal strain. Analysis of mulberry and non mulberry pupae oils properties showed fatty acids, such as alpha linolenic acid, in mulberry, tasar, eri and muga were 0.69%, 32.08%, 20.95% and 10.91% respectively. Investigated antioxidant activity of the mulberry and non mulberry silkworm pupae oils could be used as natural antioxidants on food products due to presence of omega-3 fatty acids in pupae oils. The mulberry and non-mulberry (tasar, eri and muga) silkworm pupal oils possess antidiabetic effect and has significantly hypolipidamics activity due to presence of omega-3 fatty acids in pupae oils.