



AN ANALYSIS ON THE LARVICIDAL ACTIVITY OF BANANA PEEL EXTRACT AND ITS SILVER NANOPARTICLES AGAINST CULEX SPECIES ENDEMIC IN ADYAR, TAMIL NADU, INDIA

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ABSTRACT

Mosquito borne diseases are found to be prevalent in more than 100 countries across the world. To obstruct vector borne diseases and provide a better environment to public, mosquito moderation becomes obligatory. The *Culex* species can transmit a number of illnesses that can present serious health problems to human beings including the spreading of the West Nile virus, Filariasis, and Encephalitis. Plants are rich source of alternative agents despite insecticides and chemical agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target insects and are eco-friendly. Thus, current study is focused on the larvicidal activity of plant derived compounds and its secondary metabolites on *Culex* species endemic at Adyar, Chennai, Tamil Nadu, India, targeting larval destruction at one of its growth stage. The peels of Banana, which are considered as an agricultural waste has gained attention as a natural source of antioxidant and phytochemical content which are rich in compounds such as terpenoids, tannins, alkaloids, saponins, steroids, phenols, fixed oils and fats with free radical scavenging activity. Silver nanoparticles are considered to be potential agents for various biological applications including antimicrobial. Hence its application as a mosquito larvicidal agent has been investigated, wherein the larvicidal activity of silver nanoparticles of Banana Peel extract (SNBPE) against 4th instar larvae of *Culex spp* performed in this study clearly indicated that they could provide an excellent larval control of *culex spp*.

Key words: Vector borne disease, larvicidal activity, SNBPE, Silver Nanoparticles

Introduction

Mosquitoes are small, midge-flies that constitute the Culicidae. Females of most species are ecto-parasites, whose tube-like mouthparts pierce the host's skin to consume blood for replica. Mosquito's canister spreads more diseases than the other group of arthropods and affects countless people throughout the globe. There are approximately 2,700 species of mosquito within the world; the three most important genus are the Aedes, Anopheles, and Culex, as these styles of mosquitoes are accountable for transmitting various disease that are hazardous to mankind. Culex quinquefasciatus, one among the foremost dreadful infectious mosquito plays an important role in transmitting numerous human diseases like west Nile, Japanese Encephalitis and Filariasis (Hubalek and Halouzka 1999). The Culex mosquitoes prefer stagnating water to get its eggs; however, unlike the Anopheles, it doesn't necessarily go for plant and wild life surroundings. Instead, it often breeds within the outdoor objects on our property, like barrels, cans, garden pots, used tires, moreover as other places where standing water can collect. Banana is an green groceries produced by several forms of large herbaceous flowering plants within the liliopsid genus. The fruit is variable in size, colour, and firmness, but is usually elongated and curved, with soft flesh rich in starch which can be green, yellow, red, purple, or brown when ripe. The world's largest producers of banana in 2016 was India and China, which together accounted for 28% of total production. Banana and Plantain peels are major agricultural wastes which are used as medicine, animal feeds, blacking of leathers, soap making (Arawande and komolafe 2010). banana skin, an underutilized source of antioxidants for foods and functional foods against cancer and heart condition (Someya et al.2002). The depart the fruit contains various antioxidant compounds, like gallocatechin (Someya et al.2002) and dopamine (Kanazawa and Sakakibara 2000). Nanotechnology provides a platform for the event of eco-friendly and green synthesis of nanoparticles with the assistance of biological sources like plants and microorganisms. Nanoparticles exhibit completely new or improved properties supported specific characteristics, like size, distribution and morphology. Silver nanoparticles are non-toxic to humans and simplest against bacteria, virus, and other eukaryotic microorganisms at low concentrations and with none side effects (Jeong et al 2005). Since the assembly of nanoparticle methods involve the utilization of hazardous chemicals, low material conversions, high energy

requirements, difficult and wasteful purifications. Therefore, there's a growing have to develop environmentally friendly processes for nanoparticles synthesis without using toxic chemicals (Shanmugaveldivu et al.2014). Green nanoparticle synthesis has been using environmentally acceptable plant extract and ecofriendly reducing and capping agent. The peels of a range of fruits have gained attention as a natural source of antioxidants and phytochemical content which are rich in compounds with radical scavenging activity. Most of the synthetic chemicals used for mosquito control are expensive and destructive to the environment and also toxic to humans, animals and other non-target organisms. a number of the insecticides act as carcinogenic agents (Piyarat et al.,1974; Kalyanasundaram and Das, 1985). the majority tropical regions of the globe are experiencing the resurgence and reoccurrence of 1 of the world's deadly disease like malaria, filariasis, dengue and chikungunya in world.

Traditionally plants and their derivatives were accustomed kill mosquitoes and agricultural pests, these plants wont to control insects contained insecticidal phytochemicals produced by plants to safeguard themselves against herbivorous insects.(Shaalán et al.,2005; Preeti Sharma et al.,2009).

MATERIALS AND METHODS

Preparation of banana skin extract:

Banana (Musa) peels were collected, washed and boiled in water for half-hour at 90°C. The peels (100g) were crushed in 100ml of H₂O and therefore the extract was filtered through a cheese cloth to get rid of insoluble fractions and macromolecules. This filtrate was treated with equal volume of chilled acetone and therefore the resultant precipitate was centrifuged at 1000 rpm for five minutes. This precipitate was resuspended in water and stored in refrigerator for 4°C for further studies. This extract was used as reducing additionally as stabilising agent.

Antibacterial activity of peel extract:

The test organisms that were used for the study B, a gram positive bacteria and escherichia, a gram negative bacteria. These organisms were procured from Kings Institute of medicine, Guindy, Chennai- 600 032.

Cultural media and inoculum:

One loop-full of microorganism were inoculated into 100 ml of Nutrient Broth (Himedia) and incubated for twenty-four h at 37° C for bacterial culture . These cell suspensions are then diluted with the sterile Nutrient Broth to supply initial cell counts of about 10⁴ CFU/ml. Streptomycin (1mg/ml) was used as standard medicinal drug.

Antibiotic sensitivity test using Kirby-Bauer disc diffusion method:

Filter paper discs (6.0mm)charged with the concentrations of the drugs were used. 25 ml of MHA was prepared, autoclaved and aseptically poured into Petri dishes and allowed to solidify. Sterilized swabs were dipped in overnight cultures and spread evenly over the medium. The antibiotic discs were placed over the medium in each of the plates containing the swabbed cultures and incubated overnight at 37°C. After 24 hours of incubation the diameter of the zones were measured and recorded.

Agar well diffusion method:

The bacterial culture was added aseptically to the agar medium at 45°C, mixed well and poured immediately onto sterile petri dishes. After solidification, wells of about 6mm in diameter were cut into agar and 5, 10, 20, 30, 60, 120, 240 and 400 µg of the BPE was placed within the wells. The plates were incubated at 37°C and observations were made after 18 h. The zones appearing around the disc were a measure of the susceptibility to the aqueous extract at a particular concentration.

Minimum growth inhibitory concentration (MIC) by Micro dilution method:

E.coli and B. subtilis cultures were diluted with saline to get concentrations starting from 10⁶ cells) and different concentrations of bacterial cultures were incubated at 37°C for 24h. Bacterial turbidity was measured at 600nm×lower to higher concentrations. The test tubes containing 3ml of LB broth, 0.1ml of bacterial suspension (1 to work out the bacterial growth inhibition. BPE (100µg/ml) was used as reference antibiotic and tubes containing only the expansion medium and organism were used as control. The concentration of viable cells at 10mg/ml, 20 mg/ml and 30 mg/ml of B. subtilis were also confirmed by means of pour plate method and therefore

the values measured at 600nm was expressed as CFU/ml. .

Synthesis of silver nano particles :

(AgNO₃) solution was prepared fresh. Double distilled water was used throughout the experiments. The aqueous extract was challenged with silver nitrate solution (1mM) under controlled parameters and ambient conditions. The bio-reduction was monitored by periodic sampling of aliquots of the components and measuring the UV –Vis spectra of the solution

Agar well diffusion method:

The bacterial culture was added aseptically to the agar medium at 45°C, mixed well and poured immediately onto sterile petridishes. After solidification, wells of about 6mm in diameter were taken in agar and 5, 10, 20, 30, 60, 120, 240 and 400 µg of the SNBPE was placed within the wells. The plates were incubated at 37°C and observations were made after 18 h. The zones appearing round the disc were a measure of the susceptibility to the aqueous extract at a selected concentration.

QUALITATIVE PHYTOCHEMICAL SCREENING:

the various qualitative chemical tests were performed for establishing the phyto constituents present within the Banana Peel extract and its silver nanoparticles. the subsequent qualitative analyses were administered to screen the phytochemical constituents present within the drug

1.1 Mayer's test for Alkaloids (1997): To 5ml of filtrate, 0.1ml of Mayer's reagent was added by sides of tube. A white cream precipitate indicated presence of alkaloid.

1.2 Dragendroff's reagent: 10g of Bi(NO₃)₃.5H₂O was dissolved in 20ml of HNO₃ and a couple of 72g of iodide in 50ml of H₂O. The contents were carefully mixed to permit the formation of crystals. The supernatant was decanted off and made up to 100ml with H₂O. To 0.5ml of the extract 2ml of HCl was added and to the current solution, 1ml of Dragendroff's reagent was added. An orange produced immediately indicates the presence of alkaloids (Sofowora, 1993).

2.1 Shinoda's test for flavonoids : In a tubing containing 0.5ml of extract, 5-10 drops of diluted HCL and tiny piece of ZnCl or magnesium were added and therefore the solution was boiled for few minutes. Appearance of reddish brown colour indicated the presence of flavonoids.

3.1 Ferric chloride test for Phenolic compounds (Mace, 1983):

The 5ml of extract and few drops of fifty neutral ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

4.TERPENOIDS

5ml of extract and mixed with 2ml of chloroform and 2ml of concentrated vitriol carefully added to make a layer. A reddish brown colouration of interface indicated the presence of terpenoids.

5. CARBOHYDRATES AND GLYCOSIDES (Ramakrishnan et al., 1994) 5.1 Molisch's test:

To 2ml of extract and a couple of drops of 1% alcoholic solution of α -naphthol were added. The mixture was shaken well and 1ml of concentrated H_2SO_4 was added slowly along the perimeters of the tube and allowed to stand.

6.PROTEINS AND AMINO ACIDS (Ruthmann, 1970)

6.1 Biuret test (Gahan, 1984)

2ml of extract was mixed with one drop of 2% $CuSO_4$ solution. To this, 1ml of ethanol 95% was added, Followed by excess of Potassium hydroxide pellets. Pink colour in the ethanolic layer indicated the presence of proteins.

7.FIXED OILS AND FATS (Kokate,1999)

7.1 Spot test: a little quantity of extract was pressed between two papers. discolouration on the paper indicated the presence of fatty oil.

7.2 Saponification: some drops of 0.5 N alcoholic caustic potash solution was added to a tiny low quantity of extract together with a drop of phenolphthalein. The mixture was heated on a water bath for two hrs. Formation of soap or neutralization of alkali indicated the presence of fixed oils and fats.

8.1 Legal's test for Glycoside

To the extract, 1ml of pyridine and some drops of sodium nitroprusside solution was added so it had been made alkaline with caustic soda solution. Appearance of pink to red colour showed the presence of glycosides.

9. TEST FOR PHYTOSTEROLS:

9.1 Libermann-Buchard Test: The extract 50mg was dissolved in 2ml of anhydride to the present 1 or 2 drops of concentrated sulfuric acid were added slowly along the edges of the tube. An array of colour changes showed the presence of phytosterols (Sofowora, 1993).

10. TEST FOR TRITERPENOID:

10.1 Noller's test: 5 mg of the extract in a very dry test tube was treated with a bit of tin foil and 0.5mg of thionyl chloride. It was heated gently. Pink colour indicated the presence of triterpenoids (Sofowora, 1993).

10.2 Test for acid: 10 mg of the extract is treated with aqueous bicarbonate. Effervescence showed the presence of acid, which was because of liberation of greenhouse emission.

10.3 Test for Quinones: 10 mg of the extract in alcohol was treated with vitriol or aqueous caustic soda. Any colouration indicated the presence of quinonoid compound

10.4 Test for Coumarin: 10 mg of the extract in alcohol was treated with alcoholic sodium hydroxide. Yellow colour indicates the presence of coumarin.

11. TEST FOR FURANOID

11.1 Ehrlich's test:

5 mg of the extract in alcohol was treated with a pinch of paradimethylamino benzaldehyde. Red colour indicated the presence of furanoid.

High Performance thin layer chromatography (HPTLC):

Chromatographic Conditions:

Stationary phase: HPTLC Plates silica gel 60 F24,10x10cm. The plates are prewashed with methanol and then dried at 120°C for 20mins. Otherwise the solvent may interfere with the detection under UV (254nm).

Mobile phase: Toluene: Ethyl alcohol: Diethylamine (7:2:1)

Procedure:

20µl of BPE and SNBPE was applied as 8mm bands, minimum 2mm apart, 8mm from lower fringe of plate. The plates were developed in CAMAG twin trough chamber saturated for 20min (filterpaper), 5ml developing solvent per trough, developing distance 60mm from lower fringe of plate. The plate was then dried with a blower (cold air) for 5min. The HPTLC chromatogram was went to observe the results obtained.

Selection of mosquito species:

The mosquito species selected for the current study was culex quinquefasciatus. This species was selected due to its prevalence in Adyar and huge population exposure to the species and disease.

Mosquito culture:

All tests were administered against Sewage water reared vector mosquitoes at the Ist instar larval stage collected near the college canteen viz., Cx quinquefasciatus freed from exposure to insecticides and pathogens . Larvae was fedon larval food (powdered dog biscuit and yeast within the ratio 3:1) .

Larval susceptibility test

The larval susceptibility tests were carried consistent with standard Procedures (WHO, 2005). The extract solutions of various concentrations were prepared and larvae of culex species were placed in each test solution to watch the larvicidal property as per the subsequent procedure. Groups of 15 larvae were placed in glass beakers containing 100ml of the Banana peel extract solution. Control experiments without extract were in parallel. The larvae in each solution were then left for twenty-four hour of exposure, and therefore the mortality was recorded when a control mortality ranged.

Mosquito larvicidal bioassay:

Standard methods for testing biologically synthesized Nanoparticle toxicity and the susceptibility nanoparticle toxicity and the susceptibility of mosquito larvae to insecticides was performed as stipulated by WHO. The larvicidal bioassay were performed at a space temperature of $27 \pm 1^\circ \text{C}$. Randomly, twenty five (25) 4th instar larvae were placed in to 200ml of Chamber at 27°C with a photoperiod 16 : 8 –h light / dark cycle. The effectiveness of silver nanoparticles as mosquito larvicides resolve from all the twenty five 4th instar larvae with exposure to time periods . The larvae were separated in to 5 small beaker containing 25ml distilled water and the larvae were then exposed to each of the concentration of the extracts in a final volume of 200ml distilled water taken in 500ml beaker. The nano particle solution were diluted using double distilled water as a solvent according to the desired concentrations (5.0, 4.0 , 2.0,1.0, 0.5 mg / L) At each tested concentration , four trials were made and each trial consists of five replicates and a control (aqueous peel extracts) were tested for anti – larval effects . The larval mortalities were assessed to determine the acute toxicities on 4th instar larvae of culex quinifaciens at intervals of 24 hours of exposure . The number of dead larvae were counted from the 24th hour of exposure, and the percentage of mortality was reported from the average of five replicates. The larval mortality data were corrected for control mortality by the formula of Abbott .

Gas Chromatography- Mass Spectrum Analysis (GC-MS):

GC-MS technique was used in this study to make a distinction of the phytochemical components present in the aqueous extract of banana peel. GCMS analysis of this extract was performed using GC SHIMADZU QP2010 Ultra and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with DbB5ms capillary column. Detection of GC-MS, was used electron ionization power system by ionization energy of 0.70 KV. Helium gas (99.999%) was used as the carrier gas at a purge flow rate of 3ml/min and an injection volume of 2 μ l was employed (split ratio: 20). The pressure 106.1kPa, total flow 40.7 ml/min, Injector temperature 250°C ; Ion-source temperature 200°C . The oven temperature was programmed from 50°C (isothermal for 2 min.), with an increase of 280°C for 10 min. The time of running was 28 min. The percentage of area the peak of each compound account by comparing its average peak area to the whole areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver.2.5319.

Results and Discussion

Plants are rich source of different agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target insects and are eco-friendly. Thus, current study is concentrated on larvicidal activity of plant derived compounds and its secondary metabolites on mosquito targeting larval destruction at one in every of its growth stage. Plant based pesticides are less toxic, delay the event of resistance thanks to its new structure and are easily biodegradable. The fruit and vegetable wastes (e.g. peels, seeds) are the non-product flows of raw materials whose economic values are but the value of collection and recovery for reuse; and so discarded as wastes. These wastes can be considered valuable by-products if there have been appropriate technical means and if the worth of the next products were to exceed the price of reprocessing (Scheiber et al. 2001). banana skin could be a part of banana fruit that also has the antibacterial activity against microorganisms but has not been studied extensively. banana skin which is an outer shell of banana even have been studied for the treatment of mosquito bites, gastrointestinal disorders, warts, and nipple fissures caused by *Bacillus subtilis*. skins were found to contain terpenoids, tannins, alkaloids, saponins steroid, phenols, fixed oils and fats. The peels of a spread of fruits have gained attention as a natural source of antioxidants and phytochemical content which are rich in compounds with radical scavenging activity. Banana peel, an underutilized source of phenolic compounds is taken into account as a decent source of antioxidants for foods and functional foods against cancer and heart condition. The depart the fruit contains various antioxidant compounds like gallic acid and dopamine. Agar well diffusion method using aqueous banana skin extract, for *Bacillus* & *E. Coli* on MHA media was observed. Distinct zones of growth inhibition was seen at 20, 30 and 50 µl/ml with 1mm, 3mm and 9mm zone, which indicates that skin extract was effective against *E. coli* and no zone was observed at the identical concentration with *B. Subtilis*. There are several studies showing the antimicrobial activity of banana skin. Ighodaro evaluated antibacterial activity of skin extract (*M. paradisiaca*) against human pathogenic bacteria and located that skin extract showed inhibition against, *Bacillus subtilis*, *Escherichia coli*, and *Proteus mirabilis*. Chabuck et al studied antimicrobial activity on clinical isolates of two Gram-positive (*Bacillus subtilis*), four Gram-negative (*Enterobacter aerogenes*, *Klebsiella pneumoniae*, *E. coli*, and *Moraxella catarrhalis*), and one yeast (*Candida albicans*). within the present in vitro study, we focused on determining the antibacterial activity of

aqueous extract of peel and its silver nanoparticles against standard strains of Gram-negative organism like *E.coli* and Gram positive organism like *B.subtilis*. this study was allotted to validate the larvicidal potential of peel extract and a comparative analysis of its silver nanoparticles to test out the efficacy of the extract against fourth instar larvae *Culex* sp. The banana skin selected supported the ethnobotanical literatures. Qualitative analysis of BPE and SNBPE depicts the presence of phytochemical constituents. From the above results, BPE was found to exhibit a high amount of phytochemical components showing the presence of carbohydrates, Saponin, Tannin, Cardiac glycosides, Quinone, Coumarins, Flavanones and Amino acids. This phytochemical screening for BPE and SNBPE indicates presence of several constituents whereby further analysis can be done to derive quantitative resolution of the components so as to isolate the active principle present within the extract. Plant based products doesn't have any hazardous effect on ecosystem.

The larvicidal activity of silver nanoparticles against 4th instar larvae of *Culex* spp is present within the figure. the info obtained from this Study clearly indicate that silvernanoparticle could provide a superb larval control of *Culex* spp. Around 12 out of 15 larvae underwent mortality with SNBPE comparatively high with BPE at an occasional concentration of about 5mg/l. The mechanism behind larval mortality may be the flexibility of Nanoparticles to penetrate through the larval membrane. The silver nanoparticles within the intracellular space can bind to sulphur Containing proteins or to phosphorus containing compounds like DNA resulting in the denaturation of some organelles and enzymes. Subsequently the decrease in membrane permeability and disturbance in proton motive force causes loss of cellular function and at last necrobiosis. the utilization of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of metal nanoparticles offers several benefits like eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they are doing not use toxic chemicals within the synthetic protocols.

Literature report reveals that reducing sugars and terpenoids are suggested to play a key role within the reduction of silver ions and also the formation of corresponding nanoparticles, while ketones and / or aldehydes bind to emerging spherical nanoparticles to make large nanotriangles and hexagons. However, biochemical pathways that may be chargeable for the assembly of metal nanoparticles from plants are yet to be elucidated. Mechanisms of toxicity are still poorly understood although it seems clear that in some cases,

nanoscale specific properties may cause bio-uptake and toxicity over and above that's been caused by the dissolved Silver ion. the precise mechanism of the formation of those nanoparticles in these biological media is unknown. it's to be presumed that biosynthetic products or reduced cofactors play a very important role within the reduction of respective salts to nanoparticles. It seems quite probable that the phenols play a vital part within the reduction of silver ions to Silver nanoparticles because the postulation of antioxidant action of phenol compounds is preowned. Further Spectral analysis revealed peaks at 250nm and 440nm. Those peaks are indicative of triterpenoids and alkaloids and also the HPTLC pattern also reveals the presence of such compounds which needs to be further analysed and identified to seek out the precise constituents present in SNBPE compared with BPE taken as standard.

The GC-MS spectrum confirmed the presence of assorted components with different retention times as illustrated in figure. The spectrometer analyzes the compounds eluted at different times to spot the character and structure of the compounds. the massive compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which might be identified from the info library. the current study helps to predict the formula and structure of 18 biomolecules. Further investigation may result in the isolation of bio-active compounds and their structural elucidation and screening of pharmacological activity which can be helpful for further drug development.

Conclusion

The agro-residues can't be considered the wastes but become an extra valuable resource to elevate existing natural materials. Recycling, reprocessing and eventual utilization of food processing residues offer prospective of returning these by-products to beneficial uses instead of their discharge to the environment which may cause detrimental environmental effects (Anil Kumar Anal et al., 2014). the employment of natural product chemistry as well as nanotechnology that reduces mosquito populations at the larval stage can provide many associated benefits to vector control. Since silver nanoparticles are considered to be potential agents for various biological applications including antimicrobial, its application as a mosquito larvicidal agent was investigated. Hence identification of the bioactive principle involved and their mode of action and Field trials are necessary to recommend SNBPE -an effective formulation as an anti – mosquito product au fait programs.

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RESULTS: Figures and tables



Fig:1 Banana peels were collected

Preparation of Banana peel extract:

Fig:2 Banana peel Extract was prepared

**Antibiotic sensitivity test using disc diffusion method**

Fig:3 *Bacillus subtilis* is showing sensitive to the above given Antibiotics.

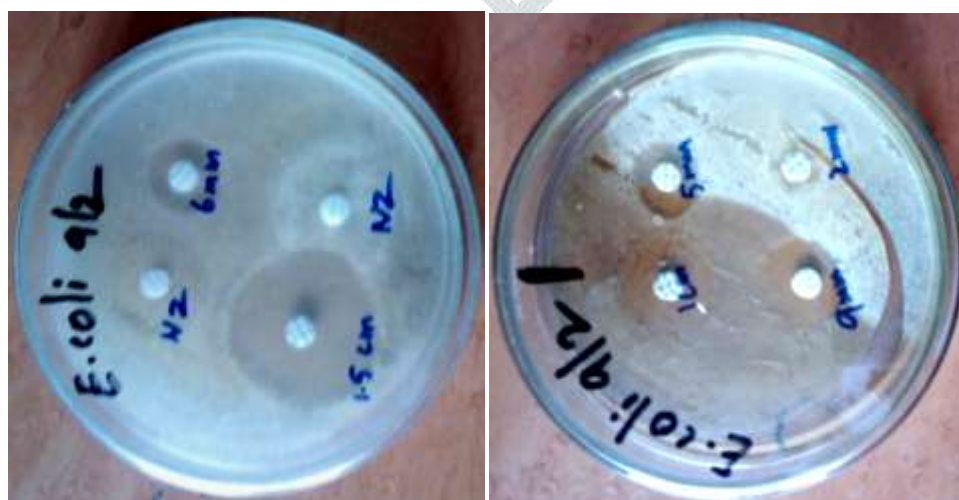


Fig:4 *E. coli* is showing sensitive to the above given antibiotics

Table 1 : Antibiotic Sensitive Test

Roll No.	Antibiotic (mg)	Zone of Inhibition (mm) E.Coli	Zone of Inhibition (mm) B. subtilis
1	Nalidixic acid – N30	1cm	1.4cm
2	Kanamycin-K30	No zone	1cm
3	Erythromycin – E15	No Zone	1.5cm
4	Neomycin-N30	6mm	1.5cm
5	Ciprofloxacin –C5	1.5cm	1cm
6	Chloramphenicol-C30	2mm	9mm
7	Vancomycin-Va10	5mm	1cm
8	Oflaxacin-Of2	9mm	9mm

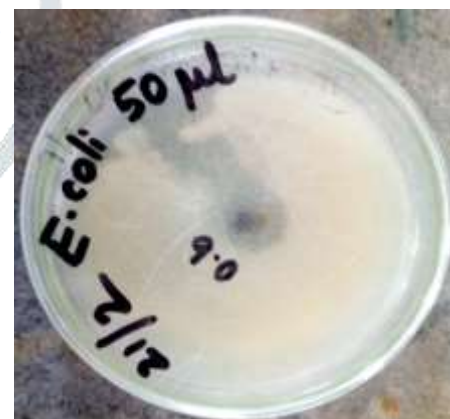
Agar well diffusion method:

Fig 5:Zone formation in Agar well Diffusion method in Different Concentrations of BPE by *E.coli* & *B.subtilis*

Agar well diffusion method



Fig: 6 Zone formation is observed at 20 & 30 µl concentration for *E.coli*

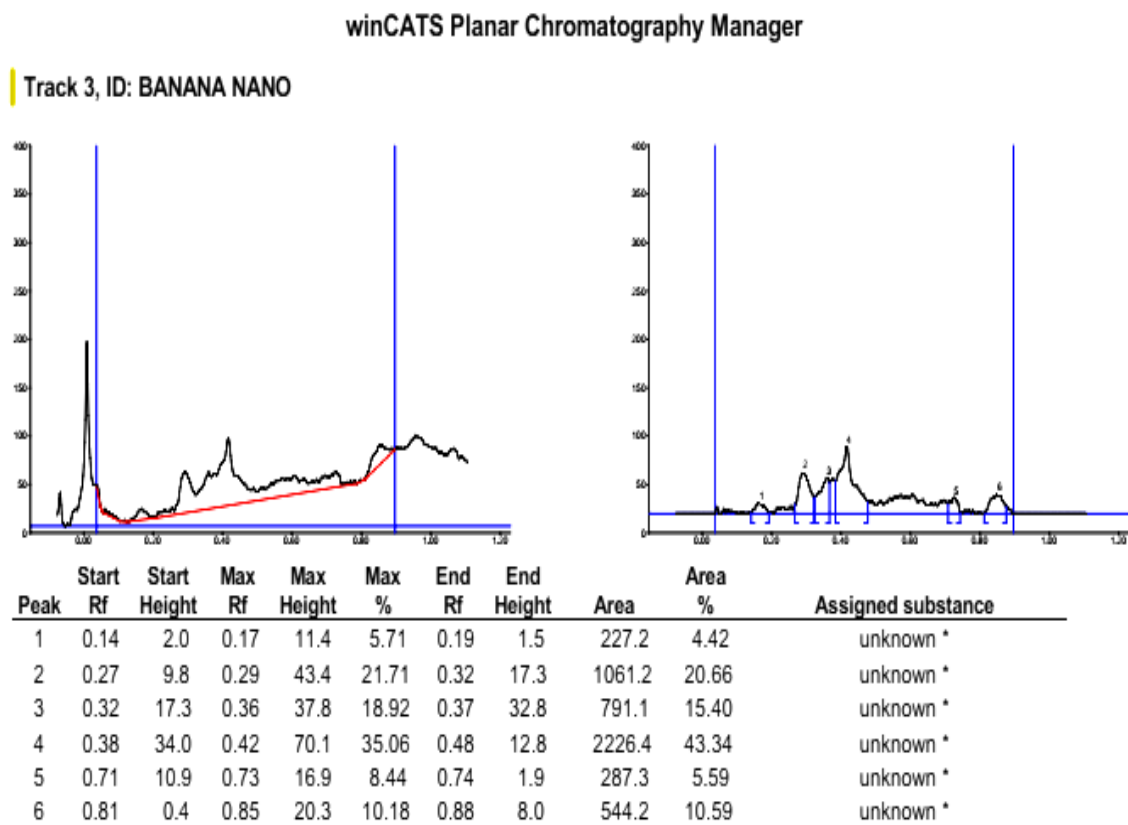


Table 2 : PHYTOCHEMICAL ANALYSIS;

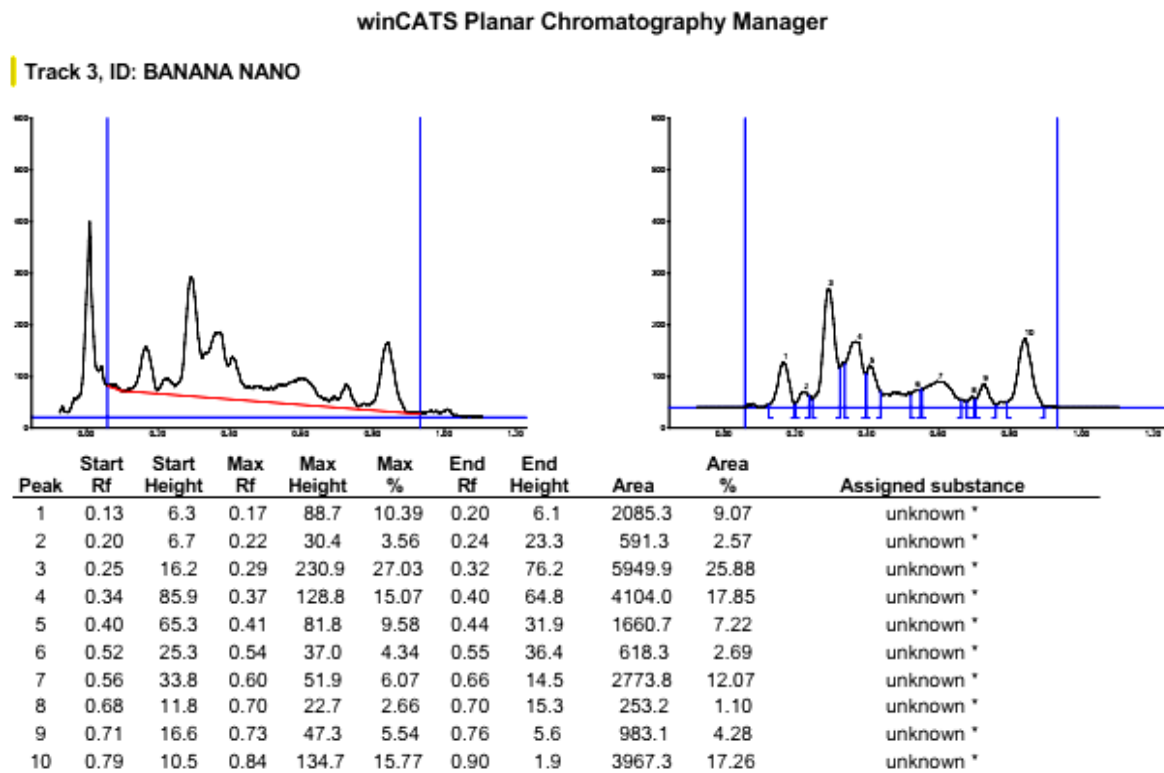
S.No	Name of the test	Component	Inference for BPE	Inference for SNBPE
1	Mayer’s test	Alkaloids	+	+
2	Flavonoids test	Alkaline	–	+
3	Molisch’s test	Carbohydrates	+	–
4	Borntrager’s test	Glycosides	+	–
5	Millons test	Protein	+	+
6	Libermann-Buchard Test	Phytosterols	+	+
7	Noller’s test	Triterpenoids	+	–

8	Test - Acid, Quinones, Coumarins	Acids, Quinones / Coumarins	+	+
9	Ehrlich’s test	Furanoid	+	–

Fig :7 High Performance thin layer chromatography



(HPTLC): HPTLC ANALYSIS of BPE & SNBPE at 254 nm



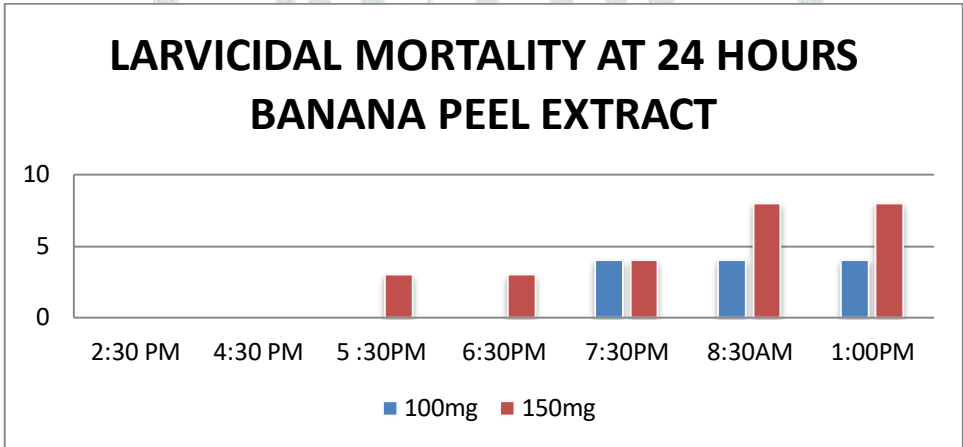
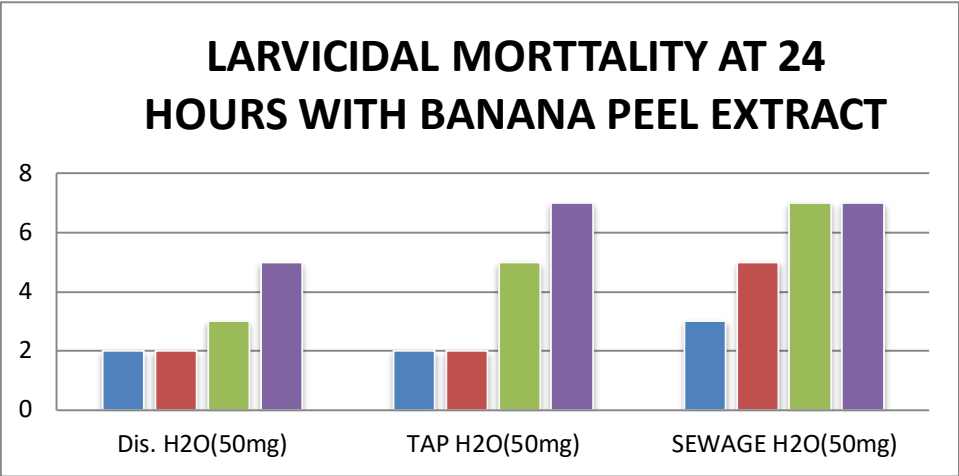
HPTLC ANALYSIS of BPE & SNBPE at 366nm

Mosquito culture:

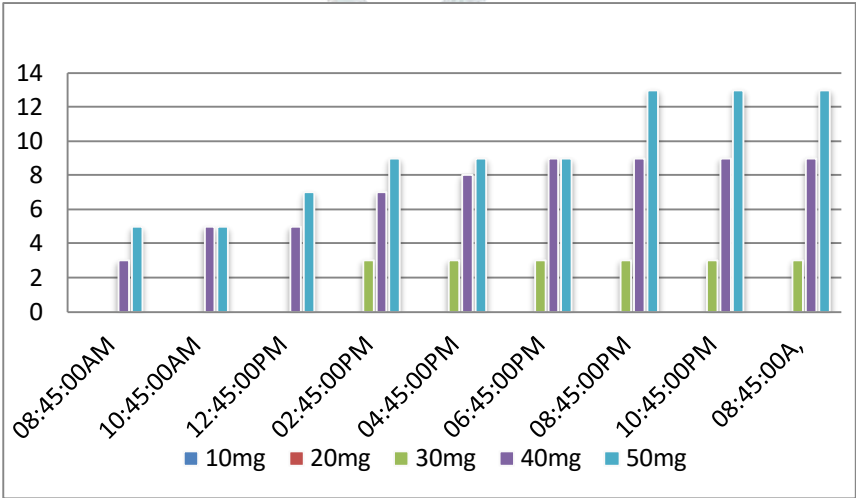


Fig:8 1st instar larval stage was collected and grown by using the Larval Food till IVth instar larval stage to carry out the larvicidal bioassay

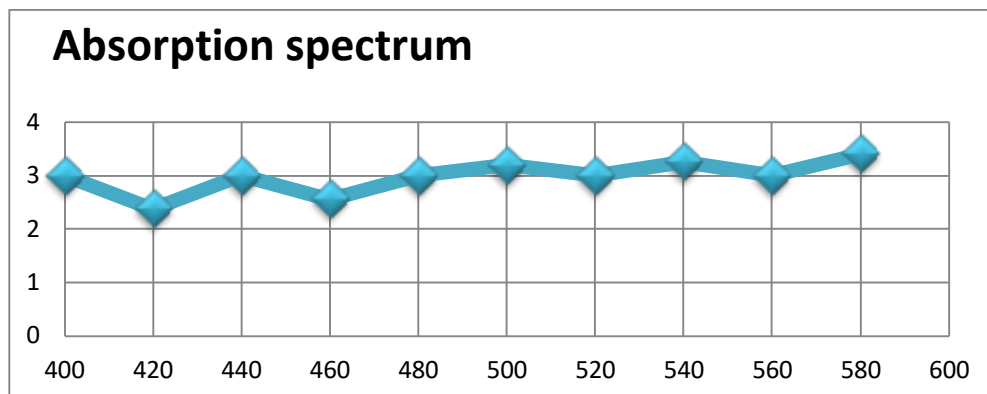
Larval susceptibility tests:



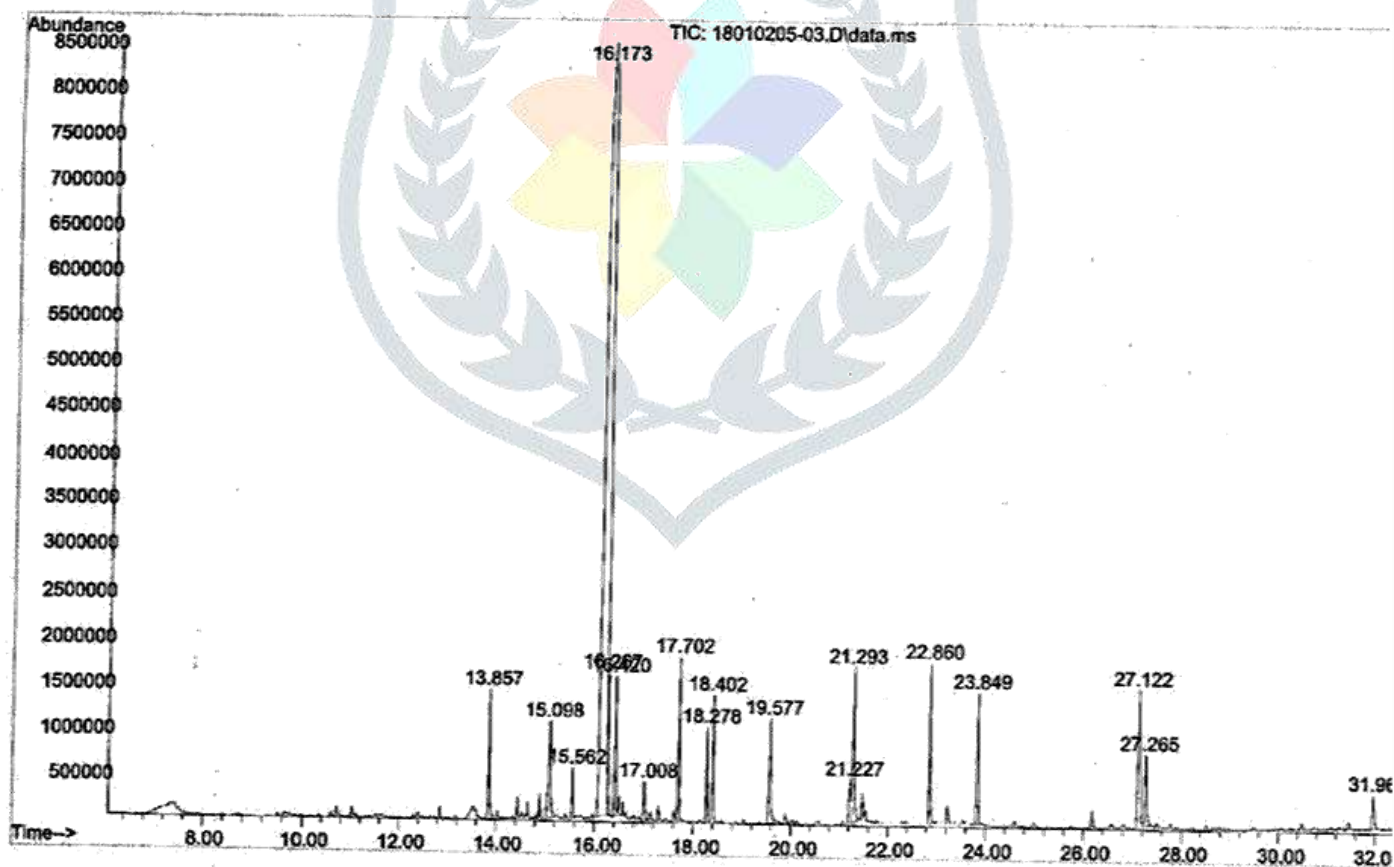
Mosquito larvicidal bioassay for SNBPE:



Larvicidal Mortality at 24Hrs with (Silver Nanoparticle) Banana Peel Extra



Gas Chromatography- Mass Spectrum Analysis (GC-MS):



Sample : 18010205-03
 Misc : AG
 ALS Vial : 1 Sample Multiplier: 1

Search Libraries: C:\Database\NIST11.L

Minimum Quality:

Unknown Spectrum: Apex

Integration Events: ChemStation Integrator - autoint1.e

PK#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	13.858	2.20	C:\Database\NIST11.L cis-.beta.-Farnesene cis-.beta.-Farnesene (E)-.beta.-Farnesene	64354 64350 64344	028973 97-9 028973-97-9 018794-84-8	89 72 50
2	15.096	2.69	C:\Database\NIST11.L 4-Chromanol 1H-Pyrazole, 3-methyl-5-(trifluoro methyl)- Imidazole, 2-methyl-4-trifluoromet hyl-	24387 24102 24100	001481-93-2 010010-93-2 033468-67-6	43 38 38
3	15.563	0.81	C:\Database\NIST11.L Diethyl Phthalate Diethyl Phthalate Diethyl Phthalate	78782 78784 78785	000084-66-2 000084-66-2 000084-66-2	98 98 96
4	16.173	65.18	C:\Database\NIST11.L (2,6-Dimethylphenyl)borate Benzoic acid, 2,4-dimethyl- Tranylcypromine	24354 24448 14765	100379-00-8 000611-01-8 000155-09-9	68 49 35
5	16.268	1.48	C:\Database\NIST11.L 5-Acetamido-2-methylphenyl acetate 3-Buten-2-one, 4-(2,2,6-trimethyl- 7-oxabicyclo[4.1.0]hept-1-yl)- l-Valine, N-(3-fluorobenzoyl)-, me thyl ester	66693 67773 104584	1000373-28-5 023267-57-4 1000299-67-6	43 35 32
6	16.420	2.52	C:\Database\NIST11.L Hydrazinecarboxamide, 2-(1-phenyle thylidene)- Mesitylacetic acid 4-Ethylbenzamide	43509 43734 23584	002492-30-0 004408-60-0 033695-58-8	27 22 22
7	17.006	0.78	C:\Database\NIST11.L Phenol, 4-butyl- Benzenemethanol, .alpha.-(1-methyl ethyl)-, (R)- o-Toluidine	23696 23873 5158	001638-22-8 014898-86-3 000095-53-4	47 43 38
8	17.701	2.51	C:\Database\NIST11.L Benzenamine, 2-nitro-5-(1-piperazi nyl)- 2-Propenoic acid, 3-phenyl-, methy l ester 2-Propenoic acid, 3-phenyl-, methy l ester	78567 32019 32021	096103-52-5 000103-26-4 000103-26-4	38 27 27
9	18.277	1.47	C:\Database\NIST11.L Farnesol, acetate 2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (E)-	114184 47025	1000352-67-2 000105-86-2	96 72

			2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	26832	000106-25-2	64
10	18.401	2.06	C:\Database\NIST11.L			
			2,6-Dimethyl-4-hydroxybenzaldehyde	24506	070547-87-4	38
			2-Chloro-7-methoxynaphthalene	54369	067061-67-0	30
			5-Formylsalicylaldehyde	24364	003328-70-9	30
11	19.578	2.30	C:\Database\NIST11.L			
			n-Hexadecanoic acid	107548	000057-10-3	99
			n-Hexadecanoic acid	107549	000057-10-3	99
			n-Hexadecanoic acid	107547	000057-10-3	95
12	21.225	0.97	C:\Database\NIST11.L			
			9,12-Octadecadienoic acid (Z,Z)-	127648	000060-33-3	99
			9,12-Octadecadienoic acid (Z,Z)-	127649	000060-33-3	99
			9,12-Octadecadienoic acid (Z,Z)-	127646	000060-33-3	92
13	21.292	3.54	C:\Database\NIST11.L			
			cis-Vaccenic acid	129339	000506-17-2	99
			9-Octadecenoic acid, (E)-	129353	000112-79-8	99
			Oleic Acid	129338	000112-80-1	99
14	22.858	2.54	C:\Database\NIST11.L			
			2(5H)-Furanone, 5-(bromomethyl)-5-phenyl-	103480	053774-22-4	47
			.alpha.-d-Mannofuranoside, methyl-	194112	1000150-79-6	47
			2,3-5,6-bis-O-phenylboranediyl			
			N-Methyl-N-methoxy-5,6,7,8-tetrahy-	76524	185957-97-5	43
			dro-1-naphtamide			
15	23.849	2.80	C:\Database\NIST11.L			
			3-Phenylpropionic acid, 2-formyl-4	162507	1000331-06-4	38
			,6-dichlorophenyl ester			
			3-Oxo-5-phenylpentanoic acid	54210	054680-53-4	37
			Benzenesulfonamide, 4-fluoro-N,N-d	187249	1000295-55-0	35
			ibenzyl-			
16	27.120	3.64	C:\Database\NIST11.L			
			3-(3-Hydroxy-4-methoxyphenyl)-1-al	70055	1000103-80-4	50
			anine			
			Benzenemethanol, 4-methoxy-.alpha.	103005	103130-05-8	50
			-(2-nitrocyclopentyl)-, [1.alpha.(
			R*),2.alpha.)-			
			Propan-2-one, 1-(4-isopropoxy-3-me	79048	1000267-40-3	45
			thoxyphenyl)-			
17	27.263	1.69	C:\Database\NIST11.L			
			Benzofran-3-one, 2-[3,4-dihydroxyb	118911	1000128-63-2	87
			enzylidene]-6-hydroxy-			
			4H-1-Benzopyran-4-one, 3,5,7-trihy	118905	000548-83-4	76
			droxy-2-phenyl-			
			9,10-Anthracenedione, 1,3,8-trihyd	118901	000518-82-1	60
			roxy-6-methyl-			
18	31.959	0.83	C:\Database\NIST11.L			
			.gamma.-Sitosterol	217434	000083-47-6	99
			.beta.-Sitosterol	217432	000083-46-5	91
			5-Cholestene-3-ol, 24-methyl-	212397	1000214-17-4	66