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Research Article

GREEN SYNTHESIS OF COPPER NANOPARTICLES FROM THE FLOWERS OF *MIMUSOPS ELENGI*

Sarah Suganthi Lilian R and Priya R Iyer*

Department of Biotechnology, Women's Christian College, Chennai- 600006, India

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ABSTRACT

The flower sample (*MIMUSOPS ELENGI*) was extracted using Cold and Soxhlet extraction and those extracts were used to find out the Qualitative and Quantitative analysis. For qualitative and quantitative analysis of *Mimusops elengi* was done with 11 tests to find out the presence Alkaloids, terpenoids, glycosides, amino acids, flavonoids, tannins, saponins, steroids, proteins, carbohydrates, phenols in both the samples. Then Synthesis of copper nanoparticles of the extracts of *Mimusops elengi* was done. The copper nanoparticles were given for UV spectrophotometer, FTIR, SEM and XRD for characterization studies. By using the nanoparticles various applications were studied.

Key Words:

Mimusops elengi, nanoparticle,

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INTRODUCTION

The Research work in the field of Nanotechnology is increasing because it plays an important role in many fields. Nanoparticles are particles that exist on a nanometer scale (below 100nm in at least one dimension). Nanotechnology was founded by Richard Feynman. They possess physical properties such as uniformity, conductance or special optical properties that make them desirable in science and biology. Various methods of synthesis have been reported they are Biological, Physical, Chemical, Vacuum vapor deposition etc. Biological synthesis of Nanoparticles is the best way and it also reduces the toxic substance which affects human and environment. A Nanometer is one thousand millionth of a meter.

In this study deals with the synthesis of copper nanoparticles by using *Mimusops elengi* belongs to the family of Sapotaceae and commonly known as Spanish cherry. Trees or shrubs bearing latex, young parts often rusty tomentose. Leaves alternate, petioled, entire, coriaceous. Flowers regular. Stamens epipetalous, either in 1 series and as many as and opposite the corolla-lobes, or in 2 to 3 series and twice or thrice as many as the corolla lobes. Carpels connate in a superior 2 to 8 celled ovary. It is otherwise called as Spanish cherry, Maulsari in Hindi and Magizhampoo in Tamil. Bark contain Tannins, Saponin was isolated from ethanol extract. With ethanol and aqueous extract it gave beta d glucoside and beta sitosterol. The flowers contain D- mannitol on extraction with acetone. With

ethanol it yielded quercitol. It contains Calcium and Phosphorous. Alkaloids was present on flowers but not on other parts of the tree. *Medicinal uses of Mimusops elengi are Flowers, Bark, Fruits are used in Ayurvedic Medicine, Flowers used against Middle Cerebral Occlusion, and in Induced Brain Injury, Used as an antipyretic and asastringent for bowels.*

MATERIALS AND METHODS

Collection of *Mimusops Elengi*

The flowers of *Mimusops Elengi* were bought from Pandy Bazaar, T.Nagar. The flowers were washed and was shade dried for 2 to 3 weeks. Then it was powdered and stored in air tight containers. The Solvents (Ethanol, Acetone, N-Hexane, Chloroform, Aqueous) for the Phytochemical extraction were bought in Parris Corner.

Phytochemical Analysis: The Flower were washed 2-3 times in distilled water, it was cut in small strips and shade dried. Then it was ground into a powder and stored in a closed container.

Cold Extraction

5gms of flower powders (*Mimusops elengi*) was dispensed in 30ml of solvents

- Aqueous – 6ml
- Chloroform – 6ml

*Corresponding author: R Iyer

Department of Biotechnology, Women's Christian College, Chennai- 600006, India

- Ethanol – 6ml
- N-hexane – 6ml
- Acetone – 6ml

It was allowed to soak for 72 hrs in a plant tissue culture bottle. Then the extract was collected by filtering with filter paper.

Soxhlet Extraction

5gms of flower powder (*Mimusops elengi*) were taken and 250ml of solvents was added for each.

- Aqueous – 50ml
- Chloroform – 50ml
- Ethanol – 50ml
- N- hexane – 50ml
- Acetone 50ml

The solvents were poured in a soxhlet reservoir and the temperature was set to 50c. The powder was added over the muslin cloth and was made into a bag and placed inside the thimble. The solvents in the reservoir get condensed in the condenser which drips over the thimble, thus gets extracted. After the solvent becomes pale green the cycle was stopped. It was done at 50c for 6 hours.

Qualitative Analysis

1. Detection of Alkaloids: Dragendroff 's Test Mayer's Test
2. Detection of Glycosides: Concentrated Sulphuric acid Test
3. Detection of Proteins: Biuret's Test
4. Detection of Terpenoids: Salkowski's Test
5. Detection of Saponins: Foam Test
6. Detection of Steroids: Concentrated Sulphuric acid Test.
7. Detection of Carbohydrates: Fehling's Test
8. Detection of Flavonoids: Lead acetate Test
9. Detection of Tannins: Ferric chloride Test
10. Detection of Phenol: 1ml of Ferric chloride was added to 1ml of sample. Formation of brownish black color indicates the presence of Phenols.
11. Detection of Amino acid: Ninhydrin Test

Quantitative Analysis

Estimation for Alkaloid: In 1 ml of sample 2 ml of Dragendroff's reagent was added it was centrifuged at 10,000 rpm for 10 minutes. 1ml of supernatant was taken. 3% (0.5ml) of Thiourea solution was added and the reading were taken in spectrophotometer at 435nm.

Estimation for Tannins: Folin & Ciocalteu's Method.

Estimation of Carbohydrates: Phenol sulphuric acid method

Estimation of Terpenoids: 0.1g of sample powder was macerated with 5ml of Ethanol and filtered. To the filtrate 2.5ml of 5% of aqueous Phosphomolybdic acid solution was added and 2.5ml of Concentrated Sulphuric acid was gradually added and mixed. The mixture was left to stand for 30 minutes and then made upto 12.5ml with Ethanol. The absorbance was taken at 700nm.

Estimation of Proteins: Bradford's Method

Estimation of Amino Acid: Ninhydrin Method

Estimation of Cardiac Glycosides: Cardiac glycoside content in the sample was evaluated using Bulje's reagent. Powder sample (1mg) was soaked in 10ml of 70% ethanol for 24 hrs and then filtered. The extract obtained was then purified using lead acetate and di sodium hydrogen phosphate solution before the addition of freshly prepared Bulje's reagent. Samples gives the absorbance and propotional to concentration of the glycosides.

Estimation of Steroids: 0.2ml of sample was taken 2ml of dilute Sulphuric acid was added, then 2ml of Ferric chloride was added. 0.5ml of Potassium hexagnoferate was added it was incubated in water bath for 30 minutes. Then 5.3 ml of distilled water was added. The absorbance was measured at 780nm.

Estimation of Phenol: 0.2 ml of sample was taken and 0.8ml of folin ciocalteau reagent was added. 75% of 2ml Sodium carbonate was added and 4ml of distilled water was added. It was kept in dark for 2 hours and the absorbance was measured at 765nm.

Estimation of Saponins: Test extract were dissolved in 80% methanol, 2ml of vanillin in ethanol was added, mixed well and 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60C for 10 minutes. Absorbance was measured at 544nm against reagent blank. Diosgenin was used as a standard material.

Synthesis of Copper Nanoparticles

The synthesis of copper nanoparticles, 5 grams of flower powder of *Mimusops elengi* was added to 20ml deionized water and extracted using filter paper. 2ml of extract was added to 8ml of copper sulphate solution kept under constant stirring using magnetic stirrer at 50°C for 3 hours. At the end of the centrifugation process the pellet was obtained and dried in hot air oven at 99°C for 3 hours. Finally the dried powder was stored. This same were carried out with cold and soxhlet extraction extracts.

Characterization of Copper Nanoparticles: The liquid samples of *Mimusops elengi* with the copper nanoparticles, the soxhlet extraction with Nanoparticles and cold extraction with Nanoparticles were characterized by U-Vspectrophotometer. The liquid samples with copper nanoparticles were given for FTIR analysis. The powdered sample of *Mimusops elengi* with Copper nanoparticles was characterized by Scanning electron microscopy. The powdered sample of *Mimusops elengi* with Copper nanoparticles was characterized by X- Ray diffraction Spectroscopy.

Antimicrobial Activities of Copper Nanoparticles: The antimicrobial activity of copper nanoparticles were done by agar well diffusion method and was tested against five different bacterial isolates like *E.coli*, *Streptococcus*, *Straphylococcus*, *Pseudomonas*, *Bacillus subtilus*. The agar plates were incubated at 37°C for 48 hours, and the antifungal activity was tested against four fungal isolates like *Aspergillus flavus*, *Candidaalbicans*, *Penicillium* and *Aspergillus fumigates*.

Antioxidant Activity: The DPPH radical scavenging method was used to evaluate the antioxidant property. The antioxidant activity of each samples was expressed in terms of IC50, and

was calculated from graph after plotting inhibition percentage against extract concentration DPPH assay was carried out after making some modifications in the standard protocol.

Anti Coagulation Assay: Determination of Pt (Prothrombin time): 10ml of blood samples was drawn from healthy volunteers by making vein puncture. To the 9 μ l of blood 1 μ l volume of 3.8% trisodium citrate uses added to avoid natural coagulation process. Immediately centrifuge for 15 minutes at 3000rpm to separate blood cells from plasma and to obtain pure platelet plasma (PPP). PPP was used for PT test.

Negative Control: 0.2ml plasma+0.1ml of 0.9%saline water+0.3ml of 25ml CaCl₂, Positive control: 0.2ml plasma+ 0.1ml of 50mg/ml EDTA+ 0.3ML CaCl₂ (0.5g/ml), Plant extract: 0.2ml plasma+ 200ul of plant extract (stem,leaf) = 0.3ml CaCl₂

All tubes were titled at an angle of 45°, for every 30 seconds to measure the clotting time. This is Prothrombin time.

Thrombolytic Activity: Whole blood was drawn from healthy volunteers without a history of contraceptives or anticoagulant therapy. 1ml of blood was transferred to the sterile white tile and was allowed to form clots. Then 1ml of sample was added and allowed to break the clot.

Anti- Larval Activity: Larvae was taken in a crucible and 50 μ l of sample was added. This activity was watched continuously in every 5 minutes interval. To report killed time of larvae.

Anti-Diabetic Activity: The antidiabetic activity was done by alpha amylase activity

Anti- Inflammatory Activity: Prepare various concentration of extract in buffer solution. Take 1ml of extract in buffer + 1ml of RBC suspension and mixed gently. Duplicate into 2 sets. One set was incubated at 54°C for 20 minutes. Another set was incubated at 10°C for 20 minutes. Centrifuge at 3000 rpm/ 3 minutes at hemoglobin content in the supernatant was measured in Colorimeter at 540nm. Percentage inhibition of hemoglobin by the extract was calculated.

$\frac{OD_2 - OD_1}{OD_3 - OD_1} \times 100$ were, OD₁- Absorbance of test sample unheated, OD₂- Absorbance of test sample heated, OD₃- Absorbance of control sample heated.

In- Vitro Assay for Cytotoxicity Activity (MTT ASSAY): The In- vitro assay for cytotoxicity activity was done by MTT assay method. The % cell viability was calculated using the formula:

% Cell viability = A570 of treated cells/ A570 of control cells x 100.

Hardness of Water by EDTA Titration: Pipette 100ml of tap water into a conical flask. Add 2 cm³ buffer solution followed by 3 drops of Eriochrome Black T indicator solution. Titrate with 0.01M EDTA until the solution turns from wine red to sky blue with no hint of red. This is used as control.

Pipette 50ml of tap water into a conical flask. Add 2ml of sample followed by 3 drops of Eriochrome Black T indicator solution. Titrate with 0.01M EDTA until the solution turns from wine red to sky blue with no hint of red.

Heavy Metals Removal by Copper Nanoparticles: The experiment is carried out with 4ml of Sewage sample in 6 Centrifuge tubes. Then 4ml of Samples were added to each

tubes. It was kept for agitation in centrifuge at 3000rpm for 3minutes. The Concentrations were determined using Colorimeter at 540nm Heavy metal removal was calculated by Heavy metal removal (%) = $\frac{Ci - Ct}{Ci} \times 100$, Where Ci and Ct are Hm concentrations before and after the treatment.

RESULT AND DISCUSSION

Extraction of *Mimusops Elengi*: *Mimusops elengi* flowers were collected and washed 2 to 3 times with distilled water. The flowers were shade dried at room temperature. (RNS Yadav et al, 2011)

Cold Extraction: Solvents (acetone, chloroform, ethanol, n-hexane and aqueous) were used for extraction process (Fig1). After 72 hours the extract was removed from fridge was filtered and stored in bottles. Similar work has been done at 25°C for 240 minutes (Milena M Ramirez et al, 2011)



Fig1

Soxhlet Extraction: Solvents (acetone, chloroform, ethanol, n-hexane and aqueous) were used for extraction process. Flower powders was packed in Soxhlet apparatus, solvents were added and the extracts was collected in the reservoir and stored in storing bottles (Fig 2). Similar work has been done at 90°C 16 minutes (Milena M Ramirez et al, 2011)



Fig 2

Qualitative Analysis

The preliminary phytochemical screening of the flower extract of *Mimusops elengi* revealed that the presence of compounds of alkaloids, glycosides, terpenoids, flavonoids, saponins, tannins, proteins, carbohydrates, steroids and phenols. The flowers indicate the presence of bioactive compounds which has medicinal value. In cold extraction alkaloids glycosides, saponins, steroids were present. In soxhlet extraction Carbohydrates and amino acids were absent. (A. Karthikeyan et al. 2008).

Table 1 Qualitative analysis

S.No	Tests	Cold Extraction	Hot Extraction
1	Alkaloids	Positive	Positive
2	Glycosides	Negative	Positive
3	Proteins	Positive	Positive
4	Treprenoids	Positive	Positive
5	Saponins	Positive	Positive
6	Steroids	Positive	Positive
7	Carbohydrates	Positive	Negative
8	Flavanoids	Negative	Positive
9	Tannins	Negative	Positive
10	Phenols	Positive	Positive
11	Amino acids	Negative	Negative



Fig 3



Fig 4



Fig 5



Fig 6



Fig 7

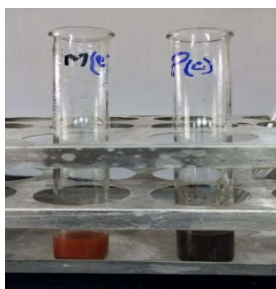


Fig 8



Fig 9

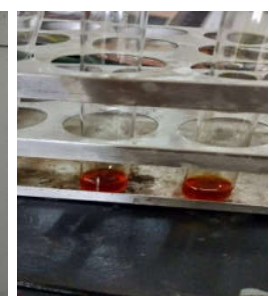
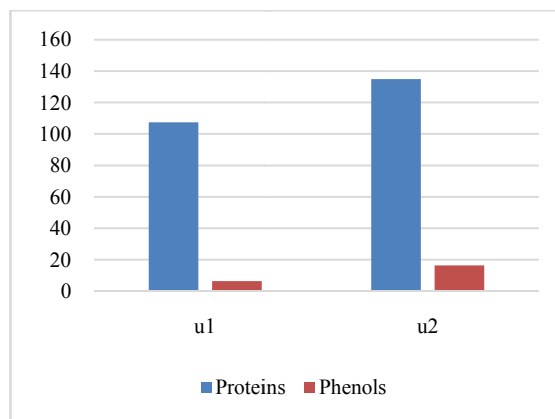
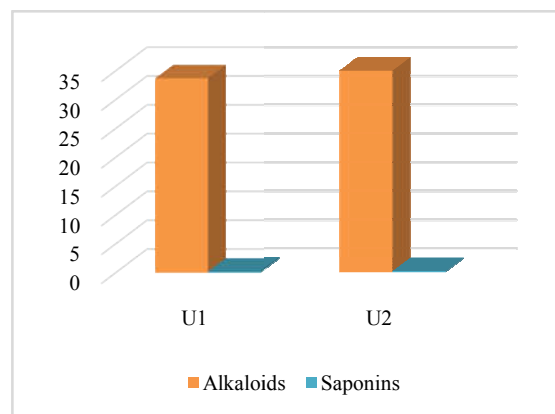


Fig 10

The quantitative test was done to find the quantity of bioactive compounds in the samples. The flower extracts of *Mimusops elengi* revealed that the quantity of alkaloids, terpenoids, saponins, tannins, steroids, flavonoids, phenols and amino acids. For terpenoids and glycosides only the powder was used. The soxhlet extraction sample showed more amount of steroids, tannis and phenols. The cold extraction sample showed more amount of alkaloids and saponins. (Chantana Aromdee *et al*,2009)



Graph 1 Test for Proteins and Phenols



Graph 2 Test for Alkaloids and Saponins

Table 2 Quantitative test for Proteins and Phenols

Phytochemical	U1	U2
Proteins	107.5	135
Phenols	6.25	16.25

Table 3 Quantitative test for Alkaloids and Saponins

Phytochemicals	U1	U2
Alkaloids	33.75	35
Saponins	0.23	0.12

Nanoparticle Synthesis

3gms flower powder of *Mimusops elengi* was taken and 15ml of distilled water was added to the powder. The extract was collected using filter paper. 2.5gms of Copper sulphate was weighed followed by adding 16ml of distilled water, from that 8ml of copper sulphate was added to 2ml of each samples. The samples were kept in Magnettic strier at 50°C for 3 hours and were centrifuged at 10,000rpm for 15 minutes (Fig 12) . The pellet was collected and dried in hot air oven at 99C for 3 hours and the nanoparticles were obtained. Similar work has been done by (S.Rajeshkumar *et al*,2018)

Quantitative Analysis



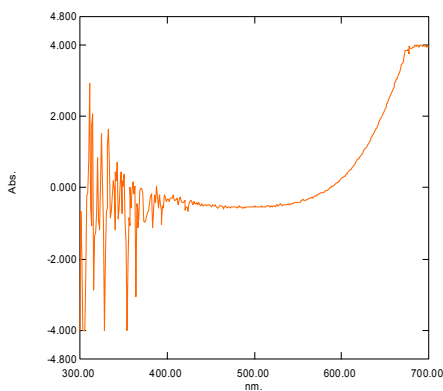
Fig 12 Eme sample with copper sulphate

Note: Eme is *Mimusops elengi*

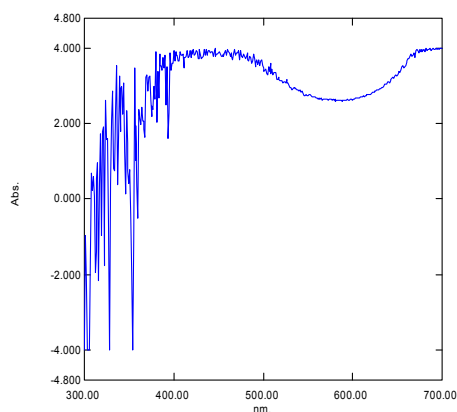
Characterization of Nanoparticles

Uv- Visible Spectrophotometer Analysis

Newly synthesized *Mimusops elengi* with nanoparticles, cold extraction and soxhlet extraction nanoparticles. OD range from 300 to 700nm for copper nanoparticles. Nanoparticles were subjected to UV-visible spectrophotometer and maximum absorption range was from 300 to 600 nm (Jae Young Song et al,2009)



Graph 3 Mec 1



Graph 4 MeH 1

Note: Mec 1 is *Mimusops elengi* cold extract and MeH 1 is *Mimusops elengi* soxhlet extract

Fourier Transform Infrared (Ftir) Spectroscopy: In this study, FTIR was performed to study the active groups compounds of nanoparticles. The FTIR spectrum for the

nanoparticles were analysed and absorption bands were observed at 3089.4cm⁻¹, 1623.7cm⁻¹, 1101.1cm⁻¹ and have been referred to as O-H stretch of carboxylic acid, C-C-C stretch of alkenes and C-O stretch of esters. When similar work was done the C-O stretch was not reported at esters. (Prasad et al)

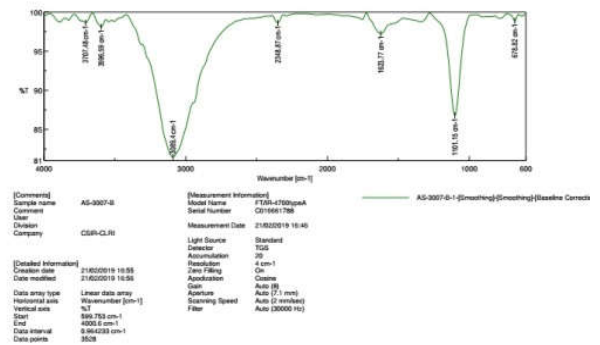


Fig 13 FTIR of Eme

Scanning Electron Microscope Analysis

The SEM micrographs in Fig.14 explain well dispersed, versatile, rod and spherical shape of copper nanoparticles prepared with *Mimusops elengi*. The energy dispersive spectrum of biosynthesized copper nanoparticles recorded. Fig.15 shows the amount of copper nanoparticles present along with other metals. The signal from EDAX spectrum confirms the presence of copper. For Eme the weight composition is 12.32 and the percentage composition is 03.16. The other impurities carbon, oxygen, nitrogen was identified, because of the interaction with the extract during bioprocessing. (S.Rajshkumar et al,2018)

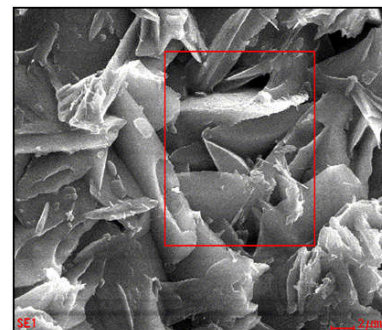


Fig 14 SEM of Eme

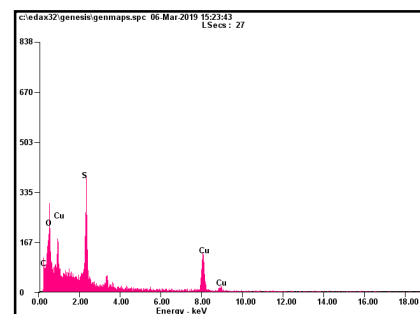


Fig 15 SEM of Eme

X-Ray Diffraction Studies

XRD was performed for copper nanoparticles, using powder X-ray diffractometer instrument. In Eme the peaks were at 25° and

30° correspond to 85. The average diameter of the copper nanoparticles is calculated and found to be in the range 42-90nm. (S.Rajesh kumar *et al* 2018)

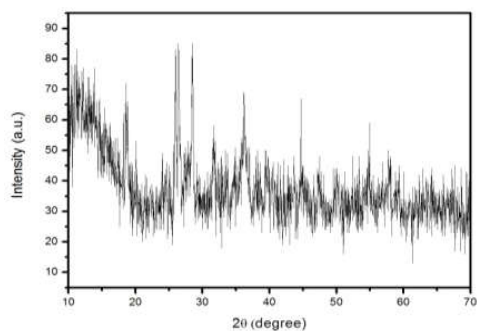


Fig 16 XRD of Eme

Antimicrobial Activity

The agar well diffusion method was used to enumerate the antimicrobial activity of the test organisms but measuring the zone of inhibition. Different types organisms were used in antimicrobial activity. In 50µl concentration 27 mm and 25mm of zone shows the highest activity against *P.aureuginocae*, *S.pneumonia*. The maximum zone was seen in *B.subtilis* and *P.aureuginocae*. The lower zone of inhibition was in *E.coli* (5mm). When compared with cold, soxhlet and extract samples, the cold extraction and soxhlet extracts with nanoparticles had maximum zone of inhibition. Instead of 50µl, 100µl concentration was used. (SA Junaid *et al*,2006)

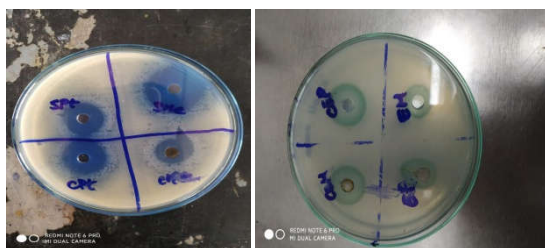


Fig 17

Fig 18



Fig 19

Fig 20



Fig 21

Note: Fig 17 is *B.subtilis*, Fig18 is *E.coli*, Fig 19 is *S.aureus*, Fig 20 *S.pneumonia*, Fig 21 is *P.aeruginocae*.

Table 4 Anti-bacterial activity

S.no	Microorganisms	Eme	Cme	Sme
1	<i>E.coli</i>	9mm	5mm	-
2	<i>S.aureus</i>	8mm	5mm	10mm
3	<i>B.subilis</i>	15mm	25mm	20mm
4	<i>S.pneumonia</i>	24mm	27mm	28mm
5	<i>P.aeruginocae</i>	15mm	9mm	11mm

Antifungal Activity

Different types of organisms were used in antifungal activity. *Mimusops elengi* extraction with nanoparticles showed the maximum (39mm)zone of inhibition in *Candida albicans* and *Aspergillus niger*. These results indicate that copper nanoparticles have excellent potential antifungal activity in treating fungal infections.

Table 5 Anti-fungal activity

S.no	Microorganisms	Eme	Cme	Sme	CuSO ₄
1	<i>A.flavus</i>	12mm	10mm	13mm	11mm
2	<i>Penicillium</i>	14mm	12mm	11mm	14mm
3	<i>A.niger</i>	20mm	-	20mm	18mm
4	<i>C.albicans</i>	32mm	20mm	29mm	30mm



Fig 22

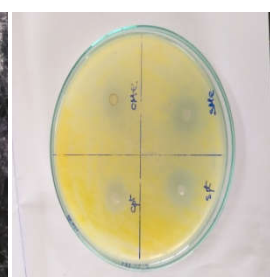


Fig 23



Fig 24



Fig 25

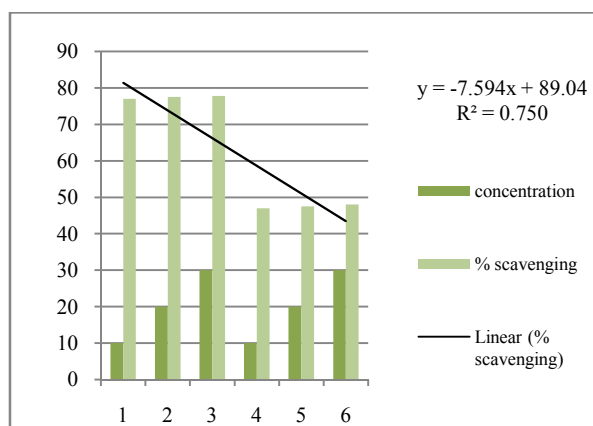
Note: Fig 22 is *Candida albicans*, Fig 23 is *A.flavus*, Fig 24 is *A.niger*, Fig 25 is *Penicillium sps*.

ANTI-OXIDANT Activity

The copper nanoparticles are continuously used for advanced biomedical applications. DPPH has been used extensively as a stable free to evaluate reducing substances and its useful reagent for investigation free scavenging activity of the component. Extracts with *Mimusops elengi*with nanoparticles has the highest anti-oxidant activity (Yosie Andriani *et al* 2015).

Table 6 Anti-oxidant activity

Topic	Concentration(µl)	Absorbance(517nm)	% Inhibition
Control	Negative	0.056	
Control	Positive	2	
Sample Eme	10	0.57	71
	20	0.58	71
	30	0.56	70
Sample Cme	10	1.04	47



Graph 3 Cpt and Cme

Thrombolytic Activity

Thrombolytic activity test was done by nanoparticles. On a tile 1 drop of blood was added and allowed to clot then 1ml of sample was added. It was allowed to break the clot within 45 minutes. On comparing the cold with nanoparticles, soxhlet with nanoparticles and extract with nanoparticles, the extract with nanoparticles showed this activity as in Figure 28. (Mohammad Shahriar et al, 2013)

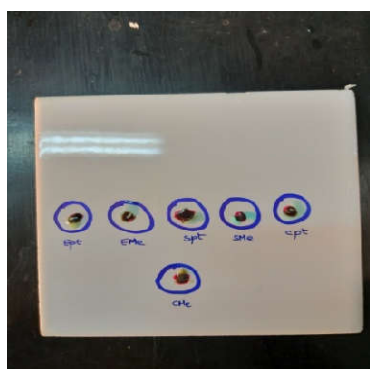


Fig 26

Anti-Coagulant Activity

The results were not observed in the nanoparticles for its anti-coagulant activity. The anti-coagulant activity was not shown in *Mimusops elengi*. The nanoparticles does not contain good anti-coagulant property. Presence of anti-coagulant was reported by (Santhosh Kumar Singh et al, 2014)

Anti-Larval Activity: This activity was done by mosquito larvae. 50µl of sample was added into a few amount of larvae. It was observed continuously in first 30 minutes. Cold extraction with nanoparticles killed larvae within 15 minutes compared to other samples. (Figure 27) A similar work was done with other insects larvae(M J Pascual et al,1998)

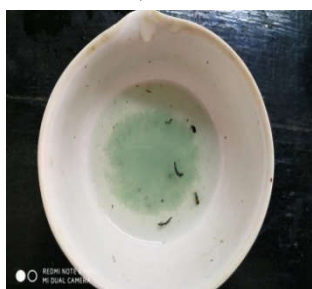


Fig 27 Cold extract with Nanoparticles

In-Vitro Cytotoxicity Activity (Mtt) Assay

Table 7 In- vitro cytotoxicity assay

Topic	Concentration(µl)	% of cell viability in Vero
Control		100
Sample Eme	30	101.80

Table showed the result for cytotoxicity test. When the VERO cells were treated with *Mimusops elengi* extract nanoparticles the cytotoxicity was reported. This tells us that these samples were not toxic to normal cells. (Yosie Andriani et al,2015)



Figure: 26



Figure 27

Note: Fig 26 is VERO cell lines which showed cytotoxic activity, Fig 27 is HEP 2 which did not show anti-cancer activity.

Hep 2 liver cancer cell were treated with Eme. It did not show any anti-cancer activity.

Anti-Diabetic Activity

The results were not observed in the nanoparticles for its anti-diabetic property. The nanoparticles along with samples did not show any alpha amylase inhibitory action. Using medicinal plants an with methanol extracts the presence of anti-oxidant was reported.(MC Sabuet al,2002)

Removal of Heavy Metal

The results were observed in the nanoparticles for its heavy metal removal. The extract samples with nanoparticles had the ability to remove heavy metals from Sewage samples. On comparing the values, the Concentration of heavy metals in treated sewage samples were lesser compared to the concentration of heavy metals in untreated sewage

samples.(Ming Hua *et al*,20112), Heavy metals were removed using effluents. (MA Barakat 2011)

Water Hardness Removal

The activity of Water hardness removal was not shown by the nanoparticles (Figure 28). This nanoparticle does not contain the ability of water hardness removal. (Handout for student activity)



Fig 28

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