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Green synthesis of copper nanoparticals from the flowers of *Pandanus tectorius*

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Abstract
The flower sample (*Pandanus Tectorius*) 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 *Pandanus tectorius* 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 *Pandanus tectorius* 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.

Keywords: *Pandanus Tectorius*, nanoparticles, spectrophotometer, SEM, XRD

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 *Pandanus tectorius*. It belongs to the family *Pandanaceae* and commonly known as Screw pine. This consists of palm- like often branched trees or shrubs with tristichous long leaves spinous at the margin, apex and often at the keel or back of the mid- rib. The flowers are minute and lack perianths. Male flowers contain numerous stamens with free or fused filaments. Female flowers have a superior ovary, usually of many carpels in a ring, but may be reduced to a row of carpels or a single carpel. It is otherwise called as Kewra in hindi and Thalampoo in tamil. Flower buds and inner parts of leaves are eaten as vegetable, Anthers are use to treat headache, earache.Oil is used for application to scalp to treat alopecia and improve the luster of hair. Oil of flower- Used as nasal drops to treat convulsions due to epilepsy, flowers used for making perfumes and used to make garland or wreath.

Materials and Methods
Collection of *Pandanus tectorius*
The flowers of *PANDANUS TECTORIUS* were bought from Koyambedu market. The flowers were washed, cut into strips and was shade dried for 2 to 3 weeks. Then it was powdered and stored in air tight containers.

Phytochemical Analysis
Cold Extraction: 5gms of flower powder (*Pandanus tectorius)* was dispensed in 30ml of solvents. Aqueous – 6ml, Chloroform – 6ml, 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 (*Pandanus tectoriusi)* was 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 50◦c. The powder was added over the muslin cloth and was made into a bag and placed inside the thimble.

Qualitative Analysis
1. Detection of Alkaloids: Dragendroff 's Test
2. Mayer's Test
3. Detection of Glycosides: Concentrated Sulphuric acid Test
4. Detection of Proteins: Biuret's Test
5. Detection of Terpenoids: Salkowski's Test
6. Detection of Saponins: Foam Test
7. Detection of Steroids: Concentrated Sulphuric acid Test:.
8. Detection of Carbohydrates: Fehling's Test
9. Detection of Flavonoids: Lead acetate Test
10. Detection of Tannins: Ferric chloride Test
11. Detection of Phenol: 1ml of Ferric chloride was added to 1ml of sample. Formation of brownish black colorindicates the presence of Phenols.
12. 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 Buljce'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 Buljet'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 60Oc for 10 minutes. Absorbance was measured at 544nm against reagent blank. Diosgenin was used as a standard material.

Synthesis of copper nanoparticles: 5 grams of flower powder of *Pandanus tectorius* 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℃ for 3 hours. At the end of the centrifugation process the pellet was obtained and dried in hot air oven at 99℃ 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 samples of Pandanus tectorius with nanoparticles were given for U-V spectrophotometer and Fourier transform infrared spectroscopy. The powdered samples with copper nanoparticles was characterized by Scanning electron microscopy and 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, Staphylococcus, Pseudomonas, Bacillus subtilus.* The agar plates were incubated at 37℃ for 48 hours, and the antifungal activity was tested against four fungal isolates like *Aspergillus flavus, Candida albicans, Penicillium and Aspergillus fumigates*.

Anti-coagulation assay: Determination of Pt (Prothrombin time): 10ml of blood samples was drawn from healthy volunteers by making vein puncture. To the 9µl of blood 1µl volume of 3.8% tri sodium citrate was 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 $Cacl_2$, Positive control: 0.2ml plasma+ 0.1ml of 50mg/ml EDTA+ 0.3ML $Cacl_2$ (0.5g/ml), Plant extract: 0.2ml plasma+ 200ul of plant extract (stem, leaf) = 0.3ml $Cacl_2$
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 50µl 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℃ for 20 minutes. Another set was incubated at 10℃ for 20 minutes. Centrifuge at 3000 rpm/ 3 minutes at haemoglobin content in the supernatant was measured in Colorimeter at 540nm. Percentage inhibition of haemoglobin by the extract was calculated.

- $\frac{OD2-OD1}{OD3-OD1} \times 100$ were, OD_1- Absorbance of test sample unheated, OD_2- Absorbance of test sample heated, OD_3- 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 cm^3 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 *Pandanus tectorius*: *Pandanus tectorius* 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).

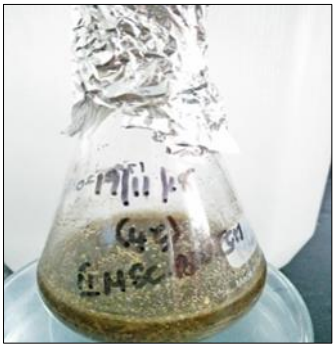


Fig 1

Soxhlet Extraction: Solvents (acetone, chloroform, ethanol, n-hexane and aqueous) were used for extraction process. Flower powder 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 *Pandanus tectorius* revealed that the presence of compounds of alkaloids, glycosides, flavonoids, saponins, tannins, proteins, steroids and phenols. The flowers indicate the presence of bioactive compounds which has medicinal value. In cold extraction alkaloids glycosides, saponins, flavonoids were present. In soxhlet extraction Glycosides and amino acids were absent**.** (A. Karthikeyan et al. 2008).

Table 1: Qualitative analysis

S.no	Tests	Cold extraction	Soxhlet extraction
1	Alkaloids	Positive	Positive
2	Glycosides	Positive	Negative
3	Proteins	Positive	Positive
4	Trepenoids	Negative	Positive
5	Saponins	Positive	Negative
6	Steroids	Negative	Positive
7	Carbohydrates	Negative	Positive
8	Flavanoids	Positive	Positive
9	Tannins	Positive	Positive
10	Phenols	Positive	Positive
11	Amino acids	Negative	Negative

Fig 3: Glycosides

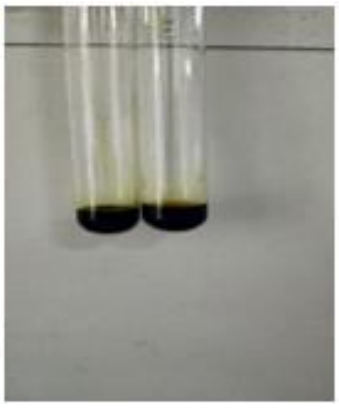


Fig 4: Tannins

Fig 5: Saponins

Fig 6: Terpenoids

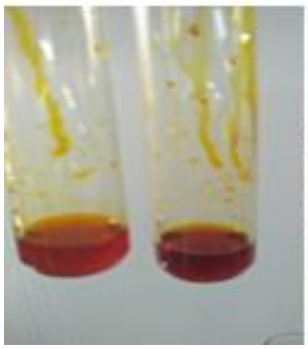


Fig 7: Steroids

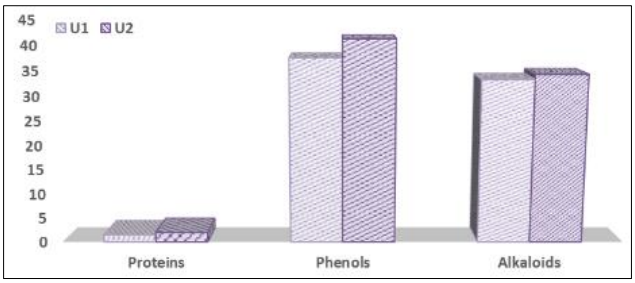
Fig 8: Amino acids

Fig 9: Biurit's test

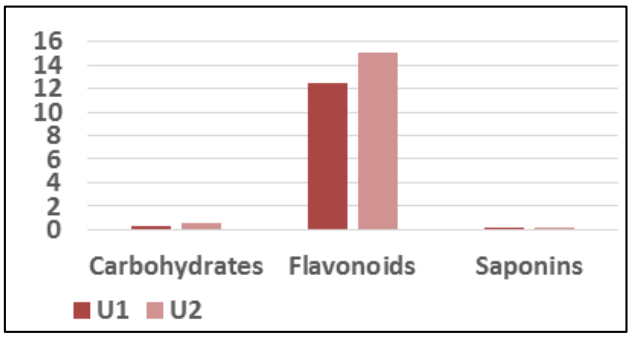
Fig 10: Phenol

Quantitative Analysis

The quantitative test was done to find the quantity of bioactive compounds in the samples. The flower extracts of *Pandanus tectorius* 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 saponins, flavonoids and phenols. The cold extraction sample showed more amount of alkaloids and carbohydrates. (Chantana Aromdee et al, 2009).



Graph 1: Test for proteins, phenols and alkaloids



Graph 2: Test for flavonoids, saponins

Table 2: Quantitative test for Proteins, Phenols, alkaloids

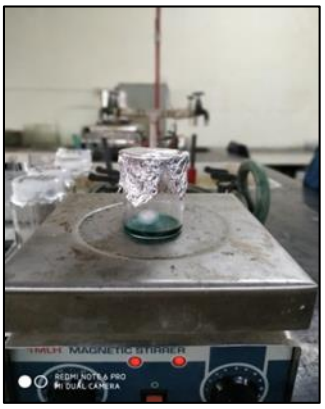
Phytochemicals	U1	U2
Proteins	1.47	1.97
Phenols	37.5	41.25
Alkaloids	33.25	34.25

Table 3: Quantitative test for Carbohydrates, Flavonoids, Saponins

Phytochemicals	U1	U2
Carbohydrates	0.31	0.62
Flavonoids	12.5	15
Saponins	0.23	0.12

Nanoparticle Synthesis

3gms flower powder of *Pandanus tectorius* was taken and 15ml of distilled water was added to the powder. The extract was collected using filter paper. 1.25gms of Copper sulphate was weighed followed by adding 8ml of distilled water, it was added to 2ml of samples. The samples were kept in Magnetic stirrer 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 99·C for 3 hours and the nanoparticles were obtained. Similar work has been done by (S. Rajeshkumar et al, 2018) [4].

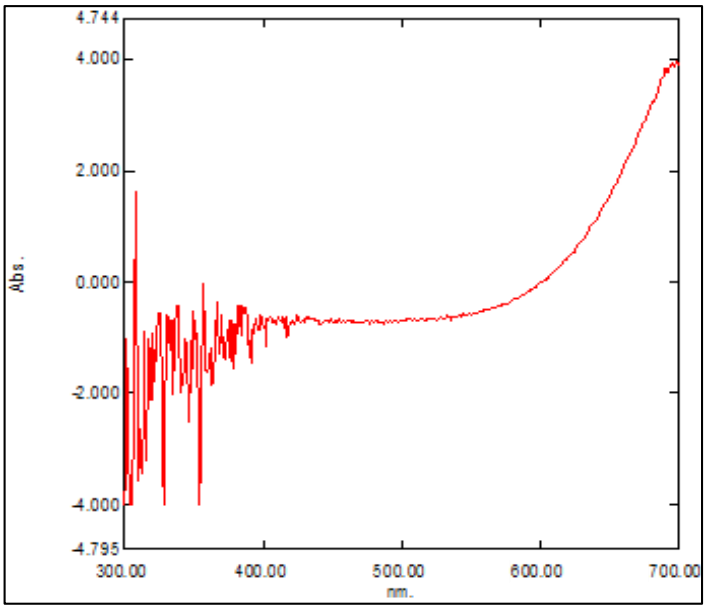


Note: Ept is *Pandanus tectorius*

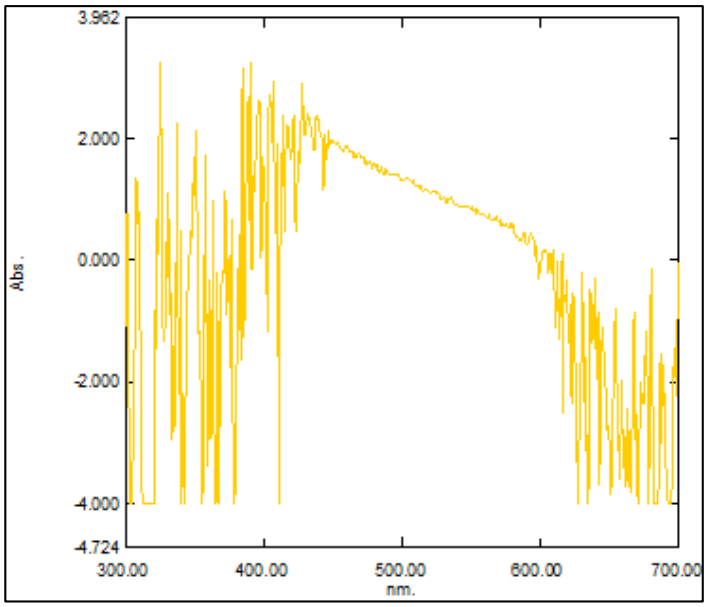
Fig 11: Ept sample with copper sulphate

Characterization of nanoparticles

UV- Visible spectrophotometer analysis: Newly synthesized *Pandanus tectorius* with naoparticles, cold extraction and soxhlet extraction nanoparticles. OD range from 300 to 700nm for copper nanoparticles. Nanoprticles were subjected to UV-visible spectrophotometer and maximum absorption range was from 300 to 600 nm (JaeYoung Song et al, 2009)



Graph 3: Ptc 1



Graph 4: PtE 1

Note: Ptc 1 is Pandanus tectorius cold extract and PtE 1 is extract samples.

Fourier transform infrared (FTIR) spectroscopy: In this study, FTIR was performed to study the active group's compounds of nanoparticles. The FTIR spectrum for the nanoparticles were analyzed and absorption bands were observed at (Ept) 3093.26cm^{-1}, 1617.02cm^{-1}, 1100.19cm^{-1}correspond to O-H stretch of carboxylic acid, H-C-H stretch of alkanes, C-C-C stretch of alkenes. When similar work was done the C-O stretch was not reported at esters. (Prasad et al)+

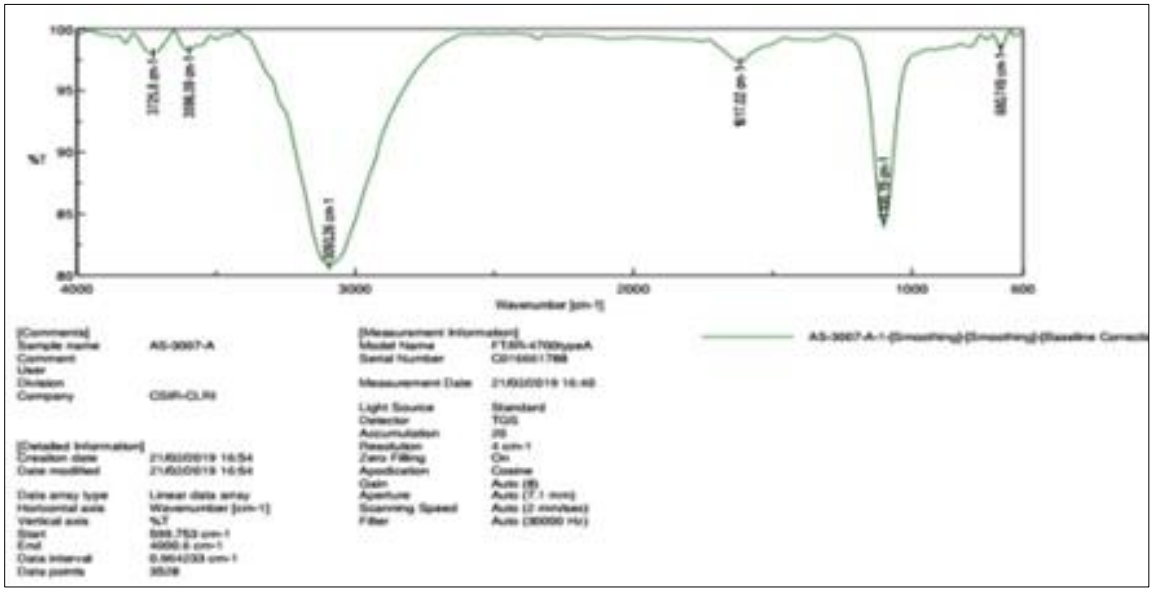


Fig 12: FTIR for Ept

Scanning electron microscope analysis: The SEM micrographs in Fig.13 explain well dispersed, versatile, rod and spherical shape of copper nanoparticles prepared with *Pandanus tectorius.* The energy dispersive spectrum of biosynthesized copper nanoparticles recorded. Fig14 shows the amount of copper nanoparticles present along with other metals. The signal from EDAX spectrum confirms the presence of copper. For Ept the weight composition of copper is 13.79 and the atomic percentage is 03.53. The other impurities carbon, oxygen, nitrogen was identified, because of the interaction with the extract during bioprocessing. (S. Rajshkumar et al, 2018)

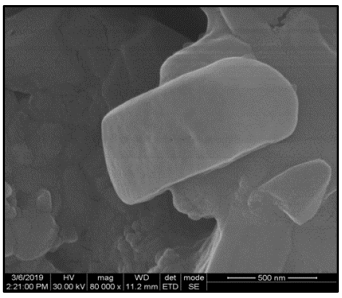


Fig 13: SEM of Ept

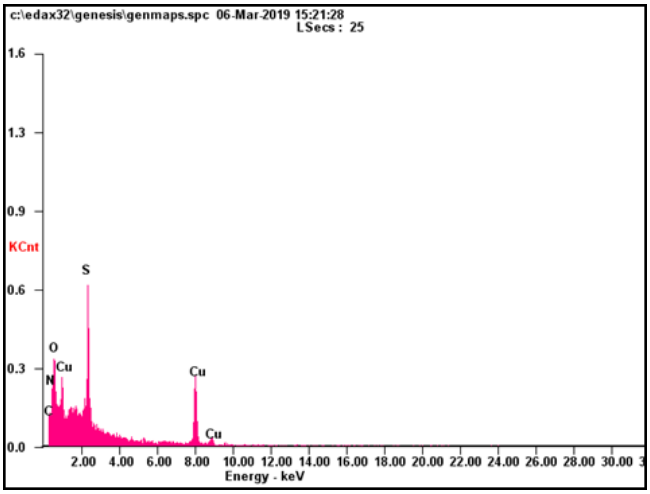


Fig 14: SEM of Ept

X-Ray Diffraction Studies: XRD was performed for copper nanoparticles, using powder X-ray diffractometer instrument. In Ept one peak was observed at 25° correspond to 75, the sample was noisier. The average diameter of the copper nanoparticles is calculated and found to be in the range of 42-90nm. (S.Rajesh kumar et al 2018) [4]

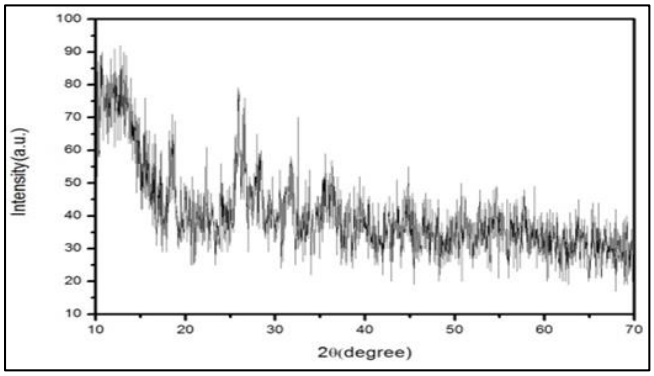


Fig 15: XRD for Ept

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 type's organisms were used in anti-microbial activity. In 50µl concentration 27 mm and 25mm of zone shows the highest activity against *Bacillius subtilius*, *S. pneumonia*. The maximum zone was seen in *B. subtilis* and *S. pneumoia*. 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 16

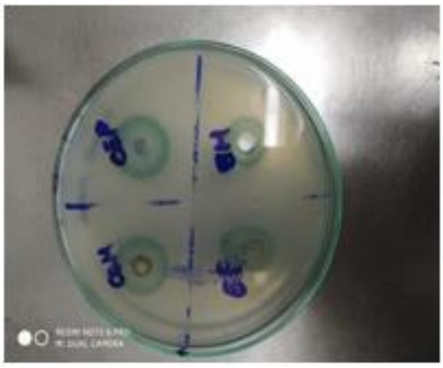


Fig 17

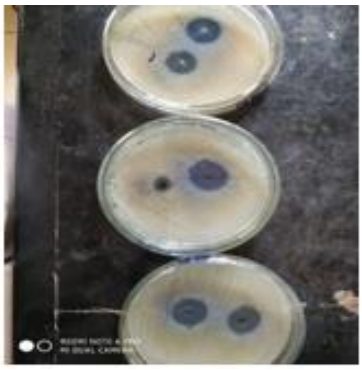


Fig 18

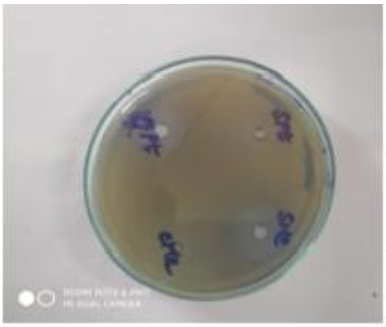


Fig 19

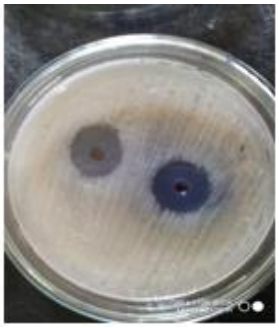


Fig 20

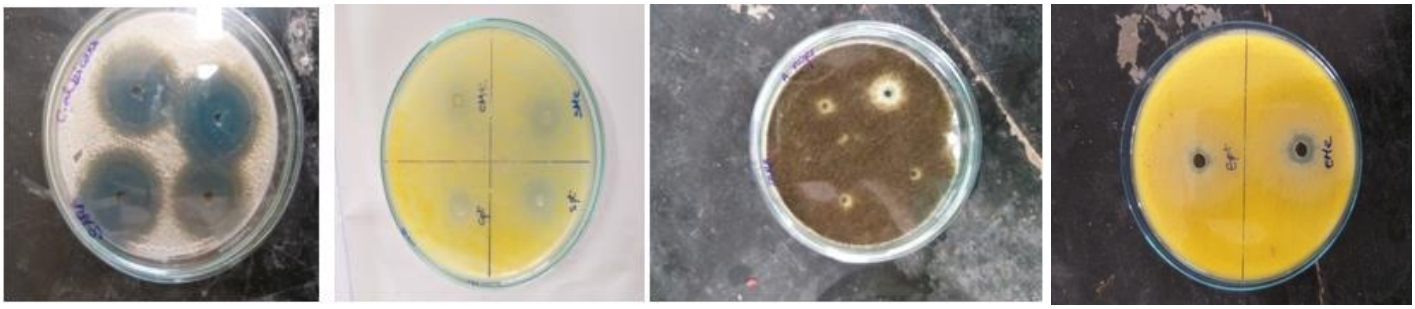
Table 4: Antibacterial activity

S.no	Microorganisms	Ept	Cpt	Spt
1	*E. coli*	4mm	6mm	-
2	*S. aureus*	8mm	8mm	-
3	*B. subilis*	18mm	25mm	20mm
4	*S. pneumonia*	20mm	29mm	28mm
5	*P. aeruginocea*	13mm	18mm	23mm

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.



Note: Fig 21 is *Candida albicans*, Fig 22 is *A.flavus*, Fig 23 is *A.niger*, Fig 24 is *Penicillium sps.*

Fig 21 **Fig 22** **Fig 23** **Fig 24**

Table 5: Antifungal activity

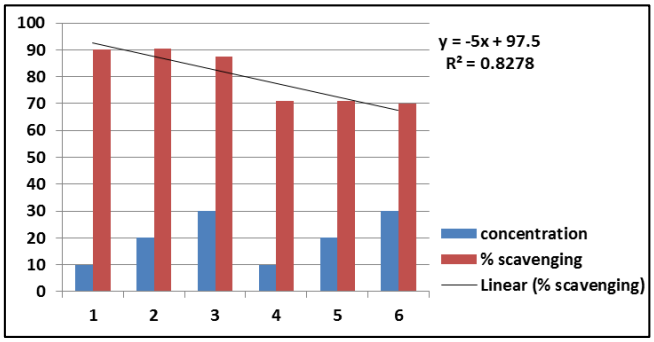
S.no	Microorganisms	Ept	Cpt	Spt	$CuSO_4$
1	*A. flavus*	11mm	9mm	13mm	11mm
2	*Penicillium*	15mm	10mm	13mm	14mm
3	*A.niger*	20mm	-	14mm	18mm
4	*C.albicans*	39mm	20mm	24mm	32mm

Anti-Oxiant 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 *Pandanus tectorius* with nanoparticles has the highest anti-oxidant activity (Yosie Andriani et al 2015).

Table 6: Antioxidant activity

Topic	Concentration(µl)	Absorbance(517nm)	% Inhibition
Control	Negative	0.056	
Control	Positive	2	
Sample Ept	10	0.18	90
	20	0.19	90.5
	30	0.17	87.5
Sample Cpt	10	0.44	77
	20	0.45	77.5
	30	0.46	77.8



Graph 5: Ept and Cpt

Thrombolytic Activity

Thrombolytic activity test was done by nanoparticles. On a tile 1drop 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 26. (Mohammad Shahriar et al, 2013).

Fig 25

Anti-Coagulant Activity

The results were not observed in the nanoparticles for its anti-coagulant activity. The anti-coagulant activity was not shown in *Pandanus tectorius.* The nanoparticles do 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 insect's larvae (M J Pascual et al, 1998)

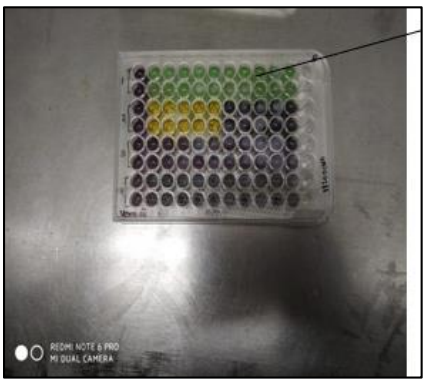
Fig 26: Cold etxract with nanoparticles

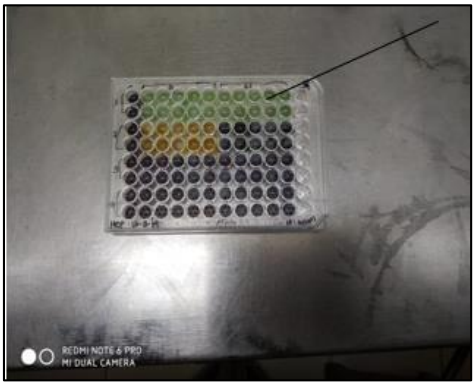
***In vitro* cytoxicity activity (MTT) assay**

Table 7: *In-vitro* cytotoxicity assay

Topic	Concentration(µl)	% of cell viability in Vero
Control		100
Sample Ept	10	177.51
	20	118.08

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





Note: Fig 27 is VERO cell lines which showed cytotoxic activity, Fig 28 is HEP 2 which did not show anti-cancer activity.
HEP 2 liver cancer cell were treated with Ept. It did not show any anti-cancer activity.

Fig 27 **Fig 28** **Fig 29**

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 Sabu et 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 29). This nanoparticle does not contain the ability of water hardness removal. (Handout for student activity)

Conclusion
- The flowers of Pandanus *tectorius* were washed and cut into strips then it was shade dried and ground into powder.
- 5 different solvents (acetone, chloroform, n-hexane, ssethanol and aqueous) were used for both cold and soxhlet extraction.
- The phytochemical screening was done for *Pandanus tectorius* and *Mimusops elengi*. In the qualitative analysis it was confirmed the presence of glycosides, steroids and saponins in cold extraction. In soxhlet all the 10 were there.
- In quantitative analysis of samples revealed the cold extraction samples has more of alkaloids, flavonoids and

saponins. The soxhlet extraction showed more amount of tannins, steroids and phenols.

- Synthesis of nanoparticles were done for all the 6 samples.
- UV was done for all the six samples in the range from 300 to 700nm
- FTIR was done with extract with nanoparticles
- SEM was done with extract powder with nanoparticles.
- XRD was done with extract powder with nanoparticles.
- Antibacterial activity was studied against 5 bacterial organisms such as *B. subtilis, S. aureus, P. aeruginoceae, E. coli, S. pneumonia* .The cold extracts with nanoparticles and the extracts with nanoparticles has maximum zone of inhibition.
- Antifungal activity was studied against 4 fungal organisms such as *Candida albicans, A. niger, A. flavus* and *Penicillium SPS*
- Antioxidant activity was found highest in *Pandanus tectorius* extracts with nanoparticles.
- Anti-inflammatory activity was not present in all the samples
- Anti-diabetic activity was absent in all the samples.
- Thrombolytic activity was present in the extracts containing the nanoparticles.
- Anti larvicidal activity was done in which the larvaes died in10 minutes with the addition of sample
- In vitro cytotoxicity was absent so the normal cells viability increased after the incubation period with the samples.
- The anticancer activity was absent so the cancer cells viability was increased after the incubation period with the samples.
- The removal of water hardness property was not present.

References

1. Bharat Gami, Smita Pathak Minoo Parabia Author. Ethnobotanical, phytochemical and pharmacological review of Mimusops elengi Linn. 2012; 2(9):743-748.
2. Ammar Altemimi, Naoufal Lakhssassi, Azam Baharlouei, Dennis G. Watson, and David A. Lightfoot Ulrike Mathesius, Academic Editor. *Phytochemicals:* Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts, 2017. Revised: 17 September 2017
3. Xiaopo Zhang, Chongming Wu, Haifeng Wu, Linghui Sheng, Yan Su, Xue Zhang, Hong Luan, *et al*. *Anti-Hyperlipidemic* Effects and Potential Mechanisms of Action of the Caffeoylquinic Acid-Rich Pandanus tectorius Fruit Extract in Hamsters Fed a High Fat-Diet.
4. Mangesh Khond, J.D. Bhosale, Tasleem Arif,T.K. Mandal, M.M. Padhi and Rajesh Dabur. Screening of Some Selected Medicinal Plants Extracts for *In-vitro* Antimicrobial Activity. Middle-East Journal of Scientific Research, 2009; 4(4):271-278.
5. Singh Gurmeet, Parle Amrita. *Unique pandanus* - Flavour, food and medicine. 2015; 5(3):08-14
6. Saha MR, Hasan SMR, Akter R, Hossain MM. *In vitro* free radical scavenging activity of methanol extract of the leaves of Mimusops elengi Linn, 2008, 6(2).
7. BiswakanthKar RB, Suresh Kumar, Indrajit Karmakar, Narayan Dola, Asis Bala, *et al*. Antioxidant and in vitro anti-inflammatory activities of Mimusops elengi leaves. 2012; 2(2):S976-S980.
8. Chaiyan Boonyuen, Sunanta Wangkarn, Oranart Suntornwat, Rasamee Chaisuksant. Antioxidant Capacity and Phenolic Content ofMimusops elengi Fruit Extract. Kasetsart J (Nat. Sci.). 2009; 43:21-27.
9. Usman H, Abdulrahman FI, Ladan AA. Phytochemical and Antimicrobial Evaluation of *Tribulus terrestris L. (Zygophyllaceae)* Growing in Nigeria. Year. 2007; 2(3):244-247
10. Sulekh Chandra, Avdhesh Kumar, Praveen Kumar Tomar. Synthesis and characterization of copper nanoparticles by reducing agent. 2014; 18(2):149-153.
11. Amit Kumar Mittal, Yusuf Chisti, Uttam Chand Banerjee, Synthesis of metallic nanoparticles using plant extracts, Biotechnology Advances. 2013, 31(2):346356.
12. Kalpana VN, Poulami Chakraborthy, Palanichamy V, Devi Rajeswari V. Synthesis and characterization of copper nanoparticles using Tridaxprocumbensand its application in degradation of bismarck brown. 2016; 9(09):498-507.
13. Shashikanta Behera, Biswaranjan Behera, Durga Prasad Barik. Phytochemical Analysis Of The Leaf, Flower, And Bark Of A Medicinal Plant, Crataeva Magna (Lour.), 2016, 9(1).
14. Guogang Ren, Eileen Cheng, Dawei Hu. Characterisation of copper oxide nanoparticles for antimicrobial applications. 2009; 33(6):587-90.
15. Thi My Dung Dang, Thi Tuyet Thu Le, Eric Fribourg-Blanc, Mau Chien Dang. Synthesis and optical properties of copper nanoparticles prepared by a chemical reduction method. Published, 2011.