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Green synthesis of copper nanoparticles obtained from *Pedalium Murex*. L (Yanai Nerunjil) and their antimicrobial activity

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Abstract: *Pedalium murex* is useful in treatment for urinary diseases. It is distributed in the coastal area of south India. In the present study, we investigated the biosynthesis of copper nanoparticles from *Pedalium murex*. The synthesized nanoparticles were characterized through the UV-Vis Spectrophotometer, SEM, EDAX and FT-IR. The antibacterial activity of copper nanoparticles tested against disease causing five bacterial pathogen like *Escherichia coli, Klebsiella sp., Serratia sp., Pseudomonas sp., and Staphylococcus sp.* and antifungal species like *Aspergillus niger* and *Aspergillus nidulans*. The preliminary phytochemical analysis showed the presence of six compounds namely Tannins, Saponins, Flavonoids, Carbohydrates, Proteins and Steroids. Synthesis of copper nanoparticles from *Pedalium murex* leaf extract was non-toxic and environmental friendly.

Index Terms: *Pedalium murex*, UV-Vis**,** SEM, EDAX, FT-IR and Antibacterial activity

1. INTRODUCTION

Nanotechnology is a multidisciplinary field. It has been used in various fields like biotechnology, physics, chemistry, material sciences, engineering medicine [1]. In the last fifteen years we assisted to the massive advance of nanomaterials in materials science. Every object possessing at least one characteristic dimension between 1 and 100nm can be defined as a "nanoparticles". When dealing with such small structures, the ratio between surface or interface and inner atoms became significant [2].Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Among the various nanoparticles, assume special importance because they are easier and cheaper to synthesize and are the most promising in applications [3]. Copper nanoparticle (CuNPs) synthesis has been targeted now-a-days compared with other NPs. Because their production cost is comparatively lower than that of silver and gold [4]. Research into CuNPs has made significant progress in the areas of nanotechnology and nanomedicine within the last decade due to their excellent catalytic, optical, electrical and antifungal/antibacterial applications [5, 6]. Copper and CuNPs have been synthesized by a variety of plant extracts. Plant extracts from magnolia leaf, *Syzygium aromaticum*, *Euphorbia nivulia*, *Sterculia urens* and latex of *Euphorbiaceae* were reported to have biomolecules, which reduce Cu ions to CuNPs [7, 8, 9].

1.1 Pedalium Murex

Pedalium murex is most useful traditional medicinal plant in India. It grows abundantly on the sea coasts in South India, Srilanka, Ceylon, Mexico and Tropical Africa. In and around Visakhapatnam the plant is very prolific after summer rains. This plant has medicinal attributes [10]. *Pedalium murex* leaves and stems were reported to be used in the treatment of gonorrhoea and dysurea, puerperal diseases, digestive tonics, ulcers, fevers, wounds, etc. *Pedalium murex* have many other names, such as Telugu-Yenugu Palleru, Sanskr-Brihat gokshur, Hindi-Bada goshur, English-Large caltrops [11]. Nowadays Pharmacological studies are the most important studies by using *Pedalium murex* plant. Pharmacological studies are, Anti-feedent effect activity (impact of ethanol extract of *P.murex* root), Anti-hyperlipidemic activity (the anti hyperlipidemic potential of the ethanolic extract from the fruits of *P.murex*), Anti-nephrolithiatic activity (the ethanolic extract from the *P.murex* plant), Nephroprotective activity (the ethanolic extract of dried fruits of *P.murex*), Antiulcer activity (the aqueous extract from leaves of *P.murex*), Anti-inflammatory activity (the ethanolic extract from the *P.murex* root, leaves and seeds), Antioxidant activity (the methanol extract from fruits of *P.murex*), Antibacterial activity (the methanol extract from fruits and leaves of *P.murex*) [12].

2. MATERIALS AND METHODS

2.1 Collection of the plant materials

The medicinal plant *Pedalium murex* free from diseases were collected from the surrounding of Pillaiyarnatham village, Srivilliputhur, Virudhunagar district,

Tamilnadu, India (It is located 10.806624 latitude and 79.071393longitude) (Fig. 1).

Fig. 1. Photography of *Pedalium murex* Leaves

2.2 Preparation of the extract

The *Pedalium murex* leaves were washed several time with water to remove dust particles and then dried for 14 days to remove the residual moisture. The *Pedalium murex* leaf extract was used for the biosynthesis of copper nanoparticles. The *Pedalium murex* leaves were collected and shadow dried. Then it was grounded using mixer grinder. 5g of powder was taken and dissolved in 100ml of distilled water. Then allowed to boil at 50^0C for 30 min after boiling it was subjected to cooled down to room temperature. The extract was filtered by Whatmann No.1 filter paper to get clear solution [13].

2.3 Cleaning of glassware's and sterilization

Glassware's (borosil and schott brands) were washed with detergents, rinsed in tap water and shocked in chromic solution. They were rinsed in tap water and in double distilled water and kept for sterilization in an autoclave at 121°C for 45 minutes. Distilled water of reverse grade, from a reverse osmosis unit (Millipore, USA) was used for preparation of all solutions and media.

2.3.1 Chemicals

All analytical grade chemicals were purchased from Merck (India) and Hi-Media (Mumbai, India) were used for synthesis of copper nanoparticles and tested its antimicrobial activity.

2.4 Taxonomic classification

Kingdom : Plantae
Order : Caryophyllales
Family : Pedaliaceae
Genus : Pedalium
Species : *P. murex* L

2.5 Synthesis of Copper nanoparticles

1mM aqueous solution of copper sulphate was prepared and used for the synthesis of copper nanoparticles. 20ml of *Pedalium murex* leaf extract was added into 80ml of aqueous solution of 1mM copper sulphate (It was purchased from HiMedia) for reduction to copper ions and kept at room temperature for 24 hours. As a result the brown colour of the leaf extract was changed in to green colour which will indicate the formation of copper nanoparticles.

2.6 Characterization of Copper nanoparticles

Initial characterization of copper nanoparticles was carried out by using UV-Visible spectrophotometer. Changes in colour was visually observed in the copper sulphate solution incubated with leaf extracts of *pedalium murex.* The bio reduction of precursor copper ions were monitored by sampling of aliquots (Copper nanoparticles diluted with distilled water) at different intervals. Absorption measurements were carried out on UV-Visible spectrophotometer using UV-1700 Pharma spec, at a resolution of 1nm between 200-800 nm. Distilled water was used as blank.

2.7 Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR measurements, the copper nanoparticles solution was centrifuged at 10,000 rpm for 30 minutes. The pellet was washed three times with 20ml of de-ionized water to get rid of the free proteins or enzymes that are not capping the copper nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on a SHIMADZU model in the diffuse reflectance mode operating at a resolution of $4cm^{-1}$. Background correction was made using a reference blank KBr pellet.

2.8 Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) analysis was done by using Hitachi S-4500 SEM machine. Thin films of synthesis and stabilized copper nanoparticles were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed by using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes and sample was analyzed for morphology and size of the copper nanoparticles.

2.9 Energy Dispersive Absorption X-ray spectroscopy (EDAX)

Pedalium murex leaf extract reduced copper solution was dried and drop coated on to carbon film and tested using Hitachi S-340 N SEM instrument equipped with a Thermo EDAX attachments. Energy Dispersive X-ray Spectroscopy (EDAX) analysis for the confirmation of elemental copper was carried out for the detection of elemental Copper. EDAX was analysed for sample composition of the synthesized nanoparticles.

2.10 Collection of Test Organisms

Five organisms namely *E.coli, Klebsiella sp., Serratia sp., Pseudomonas sp. and Staphylococcus sp.,* were used in this study. The cultures were collected from MTCC (Microbial Type Culture Collection), Pune. The pathogenic culture was subcultured in nutrient broth at 37°C maintained in nutrient agar slants and stored at

4°C for determining antimicrobial activity of these medical plants.

2.11 Antibacterial Activity Assay

The antimicrobial activity of selected medical plant against clinical pathogen was determined by using agar well diffusion method [14]. The Muller Hinton Agar medium was prepared and poured on to the petriplates and allowed to solidify. After solidification, 24 hours nutrient broth grown pathogenic cultures were swabbed on the molten medium using sterile cotton swabs. Well of 6mm diameter were punched over the agar plate using a sterile puncher. 50 µl of each leaf extract was poured in to the wells and the plates were incubated at 37°C for 24 hours. After incubation, the antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

2.12 Anti-Fungal Activity

The synthesized nanoparticles were screened for antifungal activity by agar well diffusion method [14].The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strains such as *Aspergillus niger and Aspergillus nidulans*. Aliquots of inoculum was introduced to molten PDA and spread into a petriplates by spread plate technique. After solidification, the appropriate wells were made on agar plate by sterile well cutter. Keep plate at room temperature for 2 hours to allow diffusion of the synthesized copper nanoparticles and controls in to the nutrient agar medium. Incubation period of 24-48hours at 28°C was maintained for observation of antifungal activity of the synthesized copper nanoparticles. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the synthesized copper nanoparticles extracts. The complete antifungal analysis was carried out under aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm.

2.13 Phytochemical Screening

The preliminary phytochemical screening of *Pedalium murex* leaf sample was investigated and the presence of bioactive compounds [15]. The samples were screened for alkaloids, flavonoids, steroids, tannins, saponin, carbohydrates and proteins.

2.13.1 Test for protein

Ninhydrin test

Leaf extract was when boiled with 2 ml of 0.2 % solution of ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

2.13.2 Test for carbohydrates

Molish test

Leaf extract was mixed with 2ml of H_2SO_4 a reddish brown indicates the presence of carbohydrate.

2.13.3 Test for tannins

1 ml of leaf extract was added with 2 ml of 5 % ferric chloride. Formation of dark blue or greenish black indicates the presence of tannins.

2.13.4 Test for saponins

5-10 ml of distilled water was added with 1 ml of leaf extract for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponin.

2.13.5 Test for flavonoids

2 ml of leaf extract was added with1 ml of 1N aqueous NaOH solution. The formation of yellow-orange colouration showed the presence of flavonoids.

2 ml of dis H_2O was added with 2 ml of of leaf extract and the formation of orange colour indictes the presence of flavonoids.

2.13.6 Test for steroids and triterpenes

Lieberman's test

1 ml of leaf extract was dissolved in acetic anhydride. In that mixture 1ml of conc.H_2SO_4 .The formation of black ring indicated the presence of steroids and triterpenes.

3. RESULTS AND DISCUSSION

The copper nanoparticles were successfully synthesized using leaf extract of *Pedalium murex* plant. The visible observation confirms the synthesis of copper nanoparticles. Initially the leaf extract was brown in colour and later it was changed to green colour after the addition of copper sulphate. It indicates the formation of copper sulphate in the reaction mixture. The colour changes were observed after 24hrs of incubation Fig.2 (A, B). The copper nanoparticles were synthesized by using the *Gloriosa superb* L extract at 24 hrs incubation after the colour change was light green to dark green [16].

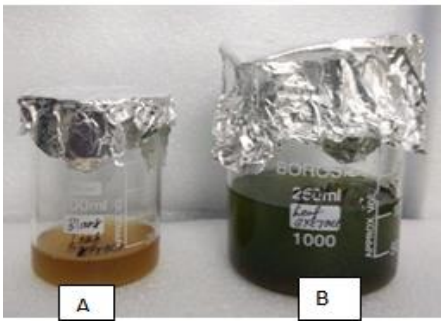

Fig. 2. Leaf extract of *Pedalium murex*

The synthesis of copper nanoparticles was further confirmed by UV-Vis spectrophotometer. The UV-Vis spectra showed the peaks approximately at 205nm and 218nm after 24hrs Fig. 3. This wavelength was characteristic feature of availability of copper nanoparticles in the reaction mixture and the broadening of peak indicates the synthesized copper nanoparticles were polydispersed. Green synthesis of copper nanoparticles using aqueous extract of the leaves of

D*elonix elata* and their catalytic activity for ligand-free Ullmanncoupling reaction and reduction of 4-nitrophenol, had subjected the synthesized copper nanoparticles to UV-visble spectrometry at the range of 200-800nm and recorded the absorption peak at 328nm after 24 hours [17].

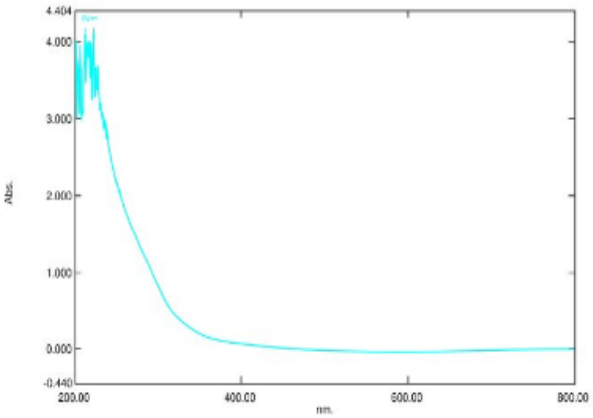

Fig. 3. UV-Vis spectrum of *Pedalium murex* leaf extract

FTIR analysis has become an important tool in understanding the involvement of functional groups in interactions between metal particles and biomolecules. The synthesized copper nanoparticles were confirmed by changes that occurred in FTIR spectra after analysis. This spectrum showed lot of absorption bands indicates the presence of active functional groups in the synthesized copper nanoparticles. Figure. 4 and Table. 1 shows the absorption at 457.1cm^{-1}, 517.85cm^{-1}, 602.71cm^{-1}, 653.82cm^{-1}, 685.65cm^{-1}, 804.26cm^{-1}, 979.77cm^{-1}, 1100.31cm^{-1}, 1124.42 cm^{-1}, 1199.64cm^{-1}, 1382.87cm^{-1}, 1455.19cm^{-1}, 1511.12cm^{-1}, 1642.27cm^{-1}, 1744.49cm^{-1}, 2307.67cm^{-1}, 2854cm^{-1}, 2925cm^{-1}, 3358.8cm^{-1}. Through this peak absorption the functional groups are determined using the FTIR interpretation table 1, the aryl disulfides (S-S stretch), iodo compound C-I, bending =C-H alkene, (C-O stretch) alcohol, phenols, -C-H bending alkane, carboxylic group and N-H bending, C=O ketones (stretches), C-N, C-O stretching, C-H (stretches) alkane, O-H stretch. This indicates that the copper nanoparticles synthesized using *Pedalium murex* leaf extract was proved to have all these functional groups bound in it. Synthesis of copper nanoparticles from *Gloriosa superba* L leaf extract. The FTIR characterization is used to find the molecules and their functional group present in the synthesized copper nanoparticles 1740, 1410, 1250, 1051, 885 and 765 cm-1. The FTIR spectra revealed the presence of different functional groups like alcohol, alkane, amine, nitro compounds, acid and ester [16].

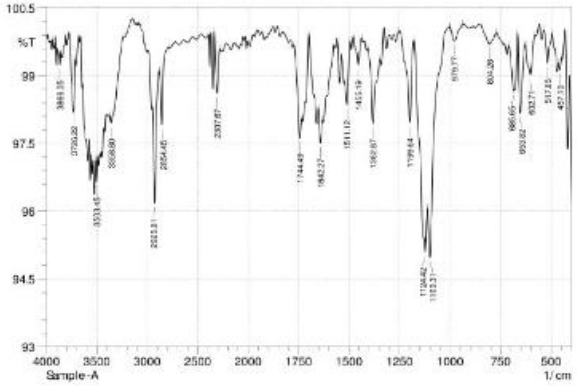

Fig. 4. FTIR spectra of *Pedalium murex* leaf extract

Table 1. FTIR result of *Pedalium murex*

S.No	Peak values	Compound	Functional groups
1	457.1	Aryl disulfides	(S-S STRETCH)
2	517.85	Iodo compound	(C-I)
3	602.71	Iodo compound	(C-I)
4	653.82	Alkenes	BENDING (=C-H)
5	685.65	Alkenes	BENDING (=C-H)
6	804.26	Alkenes	BENDING (=C-H)
7	979.77	Alkenes	BENDING (=C-H)
8	1100.31	Alcohols, phenols	(C-O STRETCH)
9	1124.42	Alcohols, phenols	(C-O STRETCH)
10	1199.64	Alcohols, phenols	(C-O STRETCH)
11	1382.87	R-GCH=CH_2	-C-H (STRETCHING)
12	1455.19	Acids, Ester	C-O (STRETCHING)
13	1511.12	Amide II band	N-H (BENDING)
14	1642.27	Ketones	C=O (STRETCHES)

15	1744.49	Ketones	C=O (STRETCHES)
16	2307.67	Amide III band	C-N (STRETCHES)
17	2854.45	Alkanes	C-H (STRETCHES)
18	2925.81	Alkanes	C-H (STRETCHES)
19	3358.8	Alcohols	O-H (STRETCH)

The synthesized copper nanoparticle by leaf extract was lyophilized and pelletized. It was examined by SEM. The magnification was done in 5.60x. From the SEM image more or less crystal in shape and the range was 5 µm. The surface morphology and interior cross sectional structure of leaf extract was visible and the results were shown in Fig 5. The synthesis and characterization of copper nanoparticle using *Saraca indica* spherical in shape and confirmed at the size ranging from 40-70nm [18].

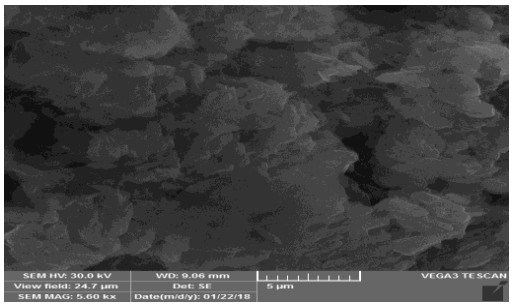

Fig. 5. SEM analysis of *Pedalium murex* leaf extract

Energy Dispersive Absorption Spectroscopy (EDAX) of synthesized copper nanoparticles of *Pedalium murex* was showed in the Fig.6. The presence of expected element in the final products Cu was confirmed. The present analysis revealed that the nano-structures were formed as peak (Table 2) at 2.3 keV solely of copper nanoparticles for *pedalium murex*. EDAX analysis Energy-dispersive X-ray report confirms the elemental composition for the synthesized CuNPs from the leaf extract of *Passiflora foetida*. This analysis also gave information about the weight percentage of CuNPs, depicting 49.95 weight % of Cu and 50.05 weight % of oxygen [19].

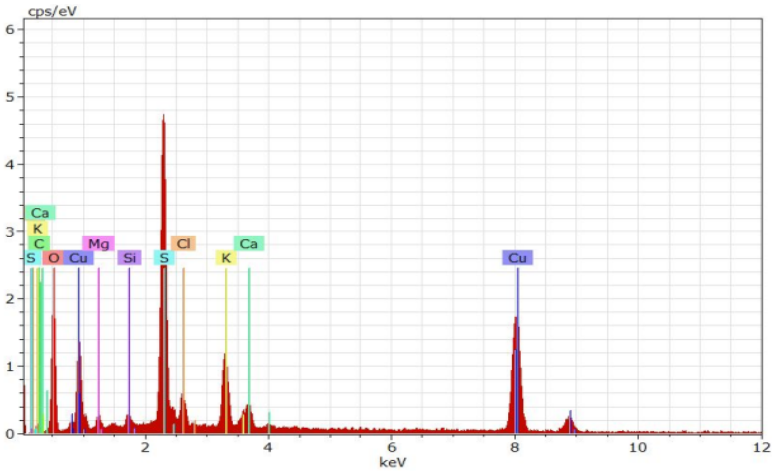

Fig. 6. EDAX analysis of *Pedalium murex* leaf extract

Table 2. EDAX result of *Pedalium murex*

ELEMENTS	SERIES	UNN. [wt %]	Cnorm. [wt %]	CAtom. [wt %]	C Error (1Sigma) [wt%]
Carbon 6	K series	8.56	9.65	17.39	3.04
Oxygen 8	K series	39.14	44.09	59.69	6.79
Potassium 19	K series	3.58	4.03	2.23	0.16
Sulphur 16	K series	11.99	13.51	9.13	0.49
Copper 29	K series	20.94	23.60	8.04	0.61
	Total	84.21	94.88	96.48	11.09

The antibacterial study was performed by Agar-well diffusion method. The effect of *Pedalium murex* leaf extract on standard strain of five human pathogenic *Escherica coli, Klebsiellae sp., Seratia sp., Pseudomonas sp.,* and *Staphylococcus sp*. were investigated for the antibacterial assays. Tetracycline was used as standard reference antibacterial compounds respectively. In the disc diffusion method, the diameter of the inhibition zone was measured (Table 3). The copper nanoparticles at different concentration such as 5µl, 10µl and 15µl showed antimicrobial activity against human pathogens. *Pedalium murex* was most sensitive against *Seratia sp., and Staphylococcus sp.* with a maximum zone of inhibition in diameter (19mm) compared with other strains (Figure.7). The antibacterial activity of synthesized copper nanoparticles using *Vitis vinifera* leaf extract was evaluated against *E. coli, S. aureus* and *K. pneumonia* with zone of inhibition of 14 mm, 16mm and 12 mm respectively [20].

Table 3. Antibacterial activity of *Pedalium murex* (Zone of inhibition)

S.No	Particulars	Concentration(µg)	Zone of inhibition (mm)				
1	Control	-----	-----				
2	Antibiotic	5	*E.coli*	*Kleb*	*S.a*	*Pseu*	*Stapy*
			14	14	20	20	14
3	Plant (leaf) extract	5	13	12	18	15	15
4	Copper Nanoparticle extract (T_1)	5	12	12	15	15	15
5	Copper Nanoparticle extract (T_2)	10	14	13	17	16	16
6	Copper Nanoparticle extract (T_3)	15	15	16	19	16	19

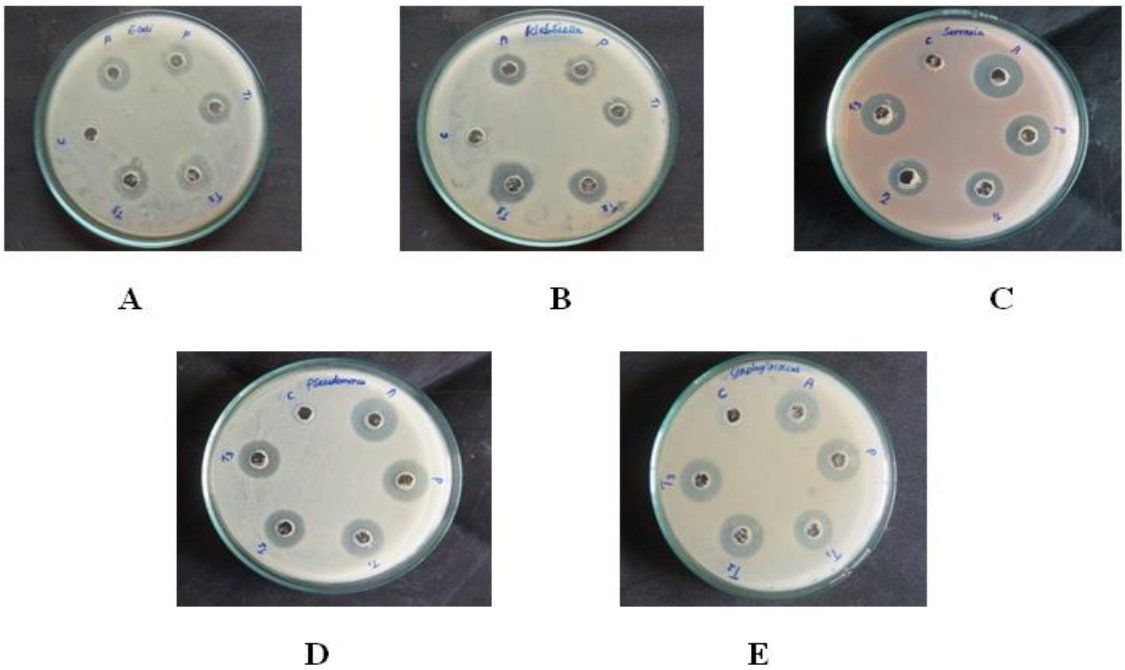

Fig.7. Antibacterial activity of leaf extract of *Pedalium murex* against (A). *E.coli*, (B). *Klebsiella*, (C). *Serratia*, (D). *Pseudomonas putida* and (E).*Staphylococcus*, C – Control; A – Antibiotic; P – Plant (leaf) extract; Test sample (CuNPs) (T1, T2, T3)

Antifungal activity of *Pedalium murex* derived copper nanoparticles were performed by Agar-well diffusion method. In this study, two fungal cultures were used namely *Aspergillus niger* and *Aspergillus nidulans.* The copper nanoparticles were taken in different concentration such as 5µl and 10µl showed antifungal activity with zone of inhibition of 18mm and 17mm Figure. 8 and Table 4. Similarly, the antifungal activity was determined by using the agar well diffusion method for different concentration of copper nanoparticles against *Aspergillus niger* with zone of inhibition in 25µl concentration at 8mm in *Persea Americana* seeds [21].

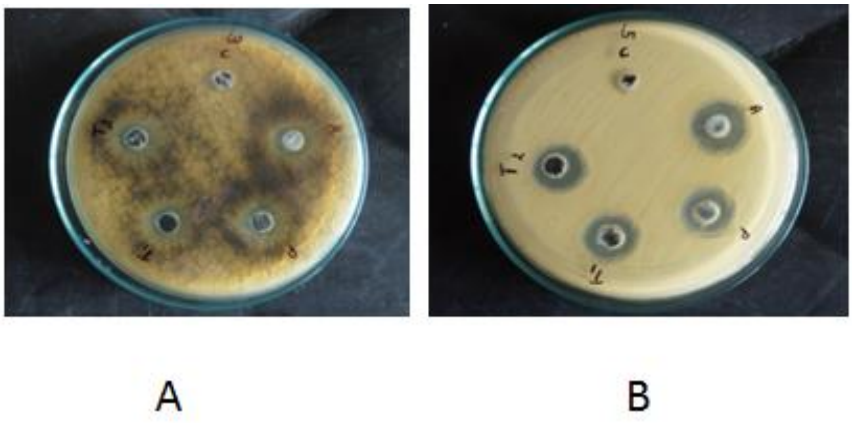

Fig. 8. Antifungal activity of leaf extract of *Pedalium murex* against (A). *Aspergillus niger*, (B). *Aspergillus nidulans*, C – Control; A – Antibiotic; P – Plant (leaf) extract; Test sample (CuNPs) (T1 and T2)

Table 4. Antifungal activity of *Pedalium murex* (Zone of inhibition)

S.No	Particulars	Concentration(µg)	Zone of inhibition (mm)	
1	Control	-----	-----	
2	Antibiotic	5	*A.niger*	*A.nidulans*
			15	20
3	Plant (leaf) extract	5	14	18
4	Copper Nanoparticle extract (T_1)	5	16	16
5	Copper Nanoparticle extract (T_2)	10	18	17

The phytochemical screenings of *Pedalium murex* were investigated and the results were plotted in the Table 5. In the preliminary phytochemical analysis of the *Pedalium murex* leaf chloroform extracts were found to show the presence of tannins, saponins, flavonoids, carbohydrates, proteins and steroid Fig.9. Similarly, *Ziziphus mauritiana* L. leaves revealed and highlighted the presence of tannins, saponins, steroid, protein and carbohydrates which may be responsible for the efficient stabilizing agent of nanoparticles [22].

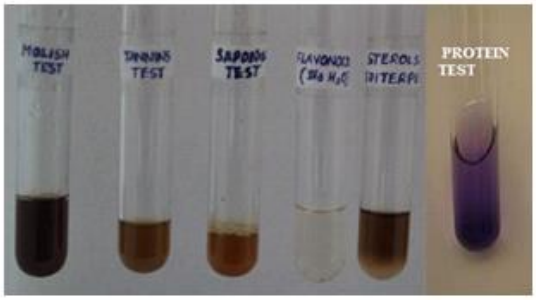

Fig. 9. Phytochemical analysis of *Pedalium murex* Leaf extracts

Table 5. Phytochemical investigation of *Pedalium murex*

S.No	Phyto chemical screenings	Reagents	Inference	Result
1	Tannin	Ferric chloride test	Green colour	positive
2	Saponins	Distilled water	Foam formation	Positive
3	Flavinoids	NaOH	Yellow colour	positive
4	Protein	Ninhydrin test	Blue colour	positive
5	Carbohydrate	Molish test	Red colour	positive

6	Steroids	Acetic unhydrin and sulphuric acid	Brown ring formation or black colour	positive

4. CONCLUSION

In conclusion, here we reported that the biosynthesis of copper nanoparticles using *Pedalium murex* aqueous extract provides a simple, efficient and environment friendly method for the synthesis of nanoparticles. UV–Vis showed that maximum peak was obtained at 218nm. SEM image showed the synthesized copper nanoparticles were relatively crystal in shape and it enhances antibacterial and antifungal activity which is compared with standard antibiotics. Qualitative analysis of phytochemicals revealed the presence of six compounds namely tannins, saponins, flavinoids, carbohydrates, proteins and steroid. Presence of these compounds suggests the medicinal properties of this plant and their biological effects which could be beneficial in the treatment of various diseases.

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