Biosynthesis of silver nanoparticles from medicinal plant, ~~*Pedalium murex*L.~~ *Pedalium murex* L., and evaluation of its antibacterial activity against selected pathogens

ABSTRACT

Silver nanoparticles play an important role in controlling mosquito population as well as multidrug resistant pathogens. ~~An aqueous~~ Aqueous extract of ~~*Pedalium murex*L.~~ *Pedalium murex* was used for the synthesis of silver(Ag) nanoparticles and its antibacterial activity. The green synthesis of nanoparticles in plants is cost-effective and eco-friendly approach. To identify the compounds responsible for the reduction of silver ions, the functional groups present in plant extract were investigated by Fourier Transformation Infra Red Spectroscopy (~~FT-IR~~ FTIR) and characterization of nanoparticles was done using standard analytical methods. UV-visible spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 350-600 nm. The compounds observed from ~~FT-IR~~ FTIR spectra are correlated with the reduction and capping material of silver nanoparticles. The observed results suggested that the bioactive compounds present in ~~*Pedalium murex*L.~~ This information is not necessary. This was introduced earlier. Replace by *P. murex*., was found to exhibit a significant inhibitory activity against selected ~~pathogens~~. Replace by sensitive microorganisms. Add species nomes.

Keywords: *Pedalium murex*, silver nanoparticles, FTIR, SEM, Antibacterial activity

INTRODUCTION

Not just "In ancient times". Currently also. Adjust text.

In ancient times, plants have provided a source of idea for the production of new drug and medicines derived from different plants have made to improve the human health and well-being (Igbinosa *et al*., 2009). India, a biodiversity nation embraces the indigenous knowledge of traditional herbs. Since decades, the empirical knowledge of utilizing the medicinal plants for the treatment of various ailments was in wide use (Mohana *et al*., 2008). The World Health Organization estimated that 80% of people employ the plant extracts as folk medicine in conventional therapies (World Health Organization, 1993). Furthermore, 900 types of valuable medicinal plants are said to be establish in Nepal among 7,000 species of medicinal plants exist in the world (Joshi *et al*., 2011 and Manandhar, 2000). ~~From the different~~ Different parts of the world, the antimicrobial properties of medicinal plants have been reported (Adiguzer *et al*., 2005; Ahmad *et al*., ~~2001and~~ 2001 and Saxena *et al*., 1999). The most common infection produced by microorganisms are fever, mouth infections, jaundice, guinea worm sores, joint pain, food borne disease, kidney infection and etc. In recent years, several drugs resistance was developed to human and plant caused by pathogenic microbes due to randomly usage of viable antimicrobial drugs used for the treatment of diseases (Davis 1994 and Service 1995). These antibiotics had some side effects in the host which includes hypersensitivity reaction, immuno-suppression and some allergic reactions (Adiguzer *et al*., 2005 and Ahmad *et al*., 1998). Due to this reason, there is a need to develop different antimicrobial drugs for the treatment of several diseases from a variety of sources, including medicinal plants (Clark, 1996 and Cordell, 2000).

Confused phrase.

Which antibiotics?

Whict host? Does not require readers to guess what are they thinking....

Wouldn't it be or get/to explore?

Confused phrase.

An extensive research has been carried out recently with secondary plant metabolites (Krishnaraju *et al*., 2005) for the treatment of many diseases (Balandrin *et al*., 1985). Phytochemicals are mainly divided into two groups, i.e. Primary and Secondary constituents; based on their functions of plant metabolism. Phytochemicals are natural bioactive compounds present in all medicinal plants. These compound work with nutrients and fibers act as resistance against various diseases and in stress conditions. Primary metabolites contain common sugars, amino acid, proteins and chlorophyll while secondary metabolites consists of Alkaloids, Terpenoids, Saponins, Phenolic compounds, Flavonoids, Tannins etc (Koche *et al*., 2010).

The medicinal importance of a plant is due to the presence of bioactive compound such as primary and secondary metabolites and these active compounds usually present in the storage

Not always... Many seeds have anti-nutritional factors, including proteins and other molecules... Many plant are toxic... Furthermore, not all molecules are potents remedy.

organs of the plants like fruits, roots, seeds, bark, leaves etc. Plant products are always hailed to be a potent remedy against dreadful diseases with lesser or no side effects (Bibitha *et al*., 2002 and Maghrani *et al*., 2005). Hence, more studies are pertaining to the use of the plant as therapeutic agents ~~should be emphasized~~; especially those related to the control of antibiotic resistant microbes. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. Add a reference: e.g. (https://dx.doi.org/10.31533/pubsaude5.a119)

Nano-biotechnology has emerged as an important divisionof nanotechnology. One of the important aspects in thefield of nanotechnology is the development of a moreconsistent process for the synthesis of nanomaterials morethan a range of size (with good monodispersed) andchemical composition (~~Rai *et al*. 2011~~ Rai *et al*., 2011). Noble metalnanoparticles have been gaining a lot of significance inthe past few years due to their applicability in the field ofphysics, chemistry, medicine, biology and material science (Yokohama and Welchons 2007). Metal nanoparticles have a high specific surface area and surface atoms,because of their outstanding physicochemical characteristics, including optical, catalytic, electronic, magneticand antibacterial properties. Synthesis of metal nanoparticles is enormous due to their potential applicability indifferent areas such as electronics, chemistry, energy, andmedicine development (Saxena *et* ~~*al.*~~ *al.,* 2012). Metal nanoparticles, especiialy with inert metals, have been extensively studied, due to their strong optical absorption in the visible region, caused by the group excitation of the free electron gas (Mohamed *et* ~~*al.*~~ *al*., 2000).

The silver nanoparticles have nonlinear optics, spectrally selective coating for solar energy absorption, biolabeling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions etc. (Zargar *et* ~~*al* .2014~~ *al*., 2014). Silver is well known for possessing an inhibitory activity towards many bacterial strains and microorganisms (Jiang *et* ~~*al*.2004~~ *al*., 2004). The process of synthesizing silver nanoparticles by chemical reduction is as colloidal dispersions in water or organic solvents (Sharma *et al*. 2009). Silver and its nanoparticles have broad applications, especially in skin ointments. Various green synthetic approach using plant leaf extracts from *Alternanthera sessilis* (Niraimathi *et al*. 2013), ~~*Morindacitr ifolia*~~ *Morinda citrifolia* (Sathishkumar *et al*. 2012), *Mukia scabrella* (Prabakar *et al*. 2013), *Iresine herbstii* (Dipankar andMurugan 2012), *Tribulus terrestris* (Gopinath *et al*. 2012), *Azadirachta indica* (Khan *et al*. 2012), *Cycas*

Mukia scabrella is a heterotypic synonym to *Cucumis maderaspatanus*... Therefore, I strongly suggest replacing as this is the current nomenclature.

Lippiano diflora

circinalis,*Ficus* *amplissima*, *Commelina benghalensis*, ~~*Lippianodiflora*~~ (Johnson and Prabu 2015), *Ocimum sanctum*and *Aloe vera* (Medda *et al*.2014)were widely reported. *Pedalium murex* is a luscious herb originated from different parts of the globe such as India, tropical Africa, Sri Lanka, Mexico and Pakistan. In India, it occurs primarily in the Western and Corommandal coastal area. *Pedalium murex* Linn is mainly used in the treatment of disorders of urinary systems such as gonorrhea, incontinence of urine, dysuria, etc. (Subramanian *et al*., 1972; Sermakkani *et al*., 2010; Satyavathi *et al*., 1987; Haravey 1996; Chopra 1999 and Shukla *et al*., 2004). It act as antibilious agentand is widely used to promote lochial discharge from juice of fruits. It is also used to control white discharge due to excessive body heat in the decoction of leaf. Pedalin dinatin-7-glucuronide. Glycosides present in the seeds of plant showed the mild diuretic activity.

Ocimum sanctum is a heterotypic synonym to *Ocimum tenuiflorum*... Therefore, I strongly suggest replacing as this is the current nomenclature.

What is it? Be understandable...

Meaningless...

The secondary metabolite, phytosterol is present only in this plant and large amounts of gums and mucilage is also present. ~~It purifies the blood and removes stone in the bladder~~. According to Unani system of medicine, Phytosterols find significant therapeutic application as diuretic and it enriches blood and useful against various ailmets (Hemalatha *et al*., 2011). ~~In thepresently investigation, the synthesis of silver nanoparticlesfrom *Pedalium murex* L. extract and ascertain theircharacterizationand also to evaluate the efficacy of antimicrobial activity against several Gram Positive and Gram Negative bacteria~~.

Phytosterol is capable of purifies the blood and stimulate the elimination of solid masses (small crystals, formed during urolithiasis events).

In the present investigation, we synthesized silver nanoparticles from *Pedalium murex* extract, characterized and predicted antimicrobial activity against Gram-positive and negative bacterial strains.

2. MATERIALS AND METHODS

2.1. Collection and identification of plant

The plant *Pedalium murex* ~~(L.)~~ was collected from Thanjavur district, Tamil Nadu in the month of September. It was taxonomically identified and authenticated by Rev Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular systematic, St. Joseph College (Autonomous), Tiruchirappalli, Tamilnadu, India (Fig 1).

Add voucher number/identifitation.

I suggest using as figure 1: A representation containing a morphological characteristics analyzed in exsiccatae.

Fig 1: Collection of *Pedalium murex* (L.)

2.2. Preparation of extract

Describe how the leafs were sprayed...

For aqueous extraction, add 50 g of dried leaf powder immersed in 250 ~~ml~~ mL of distilled water for 24 hrs at room temperature and then boiled for 15 min on slow heat. Then the residue was removed by filtering through four layer of muslin cloth to obtain filtrate. Again, the filtrate was centrifuged at 5,000 rpm for 15 min and supernatant was collected. Finally, the extract was preserved at 4ºC for further use (Gopalakrishnan *et al*., 2013).

Room temperature is relative. Add actual temperature to this experimental stages.

replace by *g*-force

2.3. Synthesis of Zinc oxide nanoparticles

Preparation of ~~1mM~~ 1 mM AgNO3 solutions

One ~~milimolar~~ millimolar solution of AgNO3 (0.017 gms) was prepared by dissolving in ~~100ml~~ 100 mL deionized water (DIW) and stored in amber colored bottle in cool and dry place.

Green synthesis of AgNPs

10 ~~ml~~ mL of aqueous leaf extract was added into ~~20ml~~ 20 mL aqueous solution of 1 mM AgNO3 aqueous solution into 170 ~~ml~~ of DIW for reduction into Ag+ ions at room temperature.

Add actual temperature to this experimental stage.

Reduction of Ag+ to AgO was confirmed by the colour change of solution from colourless to brown. Its formation was also confirmed by using UV–Visible spectroscopy.

Preliminary phytochemical screening for plant extract

The presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins in the plant *Acalypha indica* ~~(L.)~~ were analysed by the standard methods of Harborne, 1973.

But that would not be the *P. murex*?

Alkaloids:

Hager's test

About 2 ~~ml~~ mL of Hager's reagent (Saturated aqueous solution of picric acid) was added to the extract, and the presence of alkaloids was indicated by the formation of yellow precipitate.

Wagner's test

To the plant extract, few drops of Wagner's reagent was added by the side of the test tube. A reddish brown precipitate confirmed positive test.

What is the volume?

Volume?

Test for Steroids:

Salkowski's test

About ~~100mg~~ 100 mg of ~~Pedalium murex~~ *P.murex* dried extract was dissolved in ~~2ml~~ 2 mL of chloroform. Sulphuric acid was carefully added along the sides to form a lower layer. A reddish brown colour ring formed at the interface was an indicative of the presence of steroid.

Test for Cardiac glycosides:

Keller killiani's test

About ~~1mL~~ 1 mL of glacial acetic acid was added to 100 mg of the plant extract containing one drop of ferric chloride solution. To which, ~~1mL~~ 1 mL of concentrated sulphuric acid was added slowly. A brown ring at the interface indicated the presence of a deoxy sugar, which is characteristic of cardenolides.

Volume?

Concentrated how much? add concentration.

Test for Saponins:

Foam Test:

20 mL How was it shaken or stirred? Vortex, sheker (vertical or horizontal?), or manually? Add...

The extract diluted with ~~20ml~~ of distilled water was agitated for 15 minutes. Foam formation indicates the presence of saponins.

Test for Resins:

mL 10 mL Concentration? Add...

To 20 ~~ml~~ of plant extract, 5 to ~~10ml~~ of acetic anhydrite was added and dissolved by gentle heating. After cooling, ~~0.5ml~~ 0.5 mL Concentration? Add... of H_2SO_4 was added. A bright purple colour confirms the presence of resins.

Test for Phenols:

Lead Acetate Test:

3 mL lead

The extract (50 mg) dissolved in distilled water with ~~3ml~~ of 10% ~~Lead~~ acetate solution was added. A bulky white precipitate showed the presence of phenol compound.

Test for flavonoids:

Alkaline Reagent Test

mL add the volume... Concentration? Add...

To the 2 ~~ml~~ of aqueous solution of plant extract add few drops of Ammonium hydroxide. Formation of a yellow fluorescence indicated the presence of flavonoids.

Test for Tannins:

Lead acetate test

5 mL add the volume...

To ~~5ml~~ of an aqueous extract, a few drops of 1% solution of lead acetate were added. Formation of a yellow or white precipitate revealed the presence of tannins.

Test for Terpenoid:

mL 1 mL Concentration of H_2SO_4? Add... 1 mg

2 ~~ml~~ of chloroform and ~~1ml~~ of conc. H_2SO_4 was added to ~~1mg~~ of extract and observed for reddish brown colour that indicated the presence of terpenoids.

Redo the text... What is concentrated?

Fehling's test

Which are this volumes? Does not require readers to guess such volumes...

Equal volume of Fehling solution A (copper sulphate in distilled water) and Fehling solution B (potassium tartarate and sodium hydroxide in distilled water) reagents were mixed with the plant extract and boiled. The appearance of the brick red precipitate confirms the presence of reducing sugar.

Test for Gum and Mucilage:

The extract (~~100mg~~ 100 mg) was dissolved in ~~10ml~~ 10 mL of distilled water, then add ~~25ml~~ 25 mL of absolute alcohol with constant stirring. The presence of gum and mucilage was confirmed by the formation of white/cloudy precipitate How was it shaken or stirred? Sheker (vertical or horizontal?), or manually? For how long? Add...

Test for Quinone:

A few drops of conc?? H_2SO_4 or NaOH Concentrations? solution was added to the test solution. The change of colour was taken as an evidence for the presence of quinone. ??? Quinone?

Test for phlobatannins:

The test solution was mixed with 1 ~~ml~~ mL of 1% magnesium acetate solution. A purple colouration was formed to show positive result for the presence of anthraquinone.

Test for Coumarins:

One percent of alkaline KOH solution Concentrations? was added to the test solution. The formation of golden yellow colour indicates the presence of coumarins.

Characterization of phytochemicals: Leave no doubt in the minds of readers...

UV-VIS Spectral analysis

The ZnO-NPs were characterized using various spectroscopic and microscopic techniques. UV–visible spectrum was evaluated using UV–Visible spectrophotometer (Shimadzu UV-2450) and the spectrum was recorded between 300 and 800 nm (Huzaifa *et al*., 2019).

FTIR Analysis

Fourier transform infrared (FTIR) analysis of the NPs was carried out with Fourier transform spectrometer (Shimadzu ~~FT-IR~~ FTIR Prestige-21 Model) at a frequency range of 400–500 cm^{-1} (Huzaifa *et al*., 2019).

SEM Analysis

Morphological analysis of the synthesized ZnO NPs coated with platinum was carried out using scanning electron microscope (SEM) (JOEL JSM 6335-F) equipped with 150 kV acceleration voltage, and energy-dispersive (Huzaifa *et al*., 2019).

Antibacterial Activity

Test Organisms

For this study, both Gram-Positive [*Staphylococcus haemolyticus* (MTCC 3383) and ~~*Staphylococcus hominis*(MTCC 96s)~~] S. hominis (MTCC 96s)] and Gram-Negative [*Escherichia coli* (MTCC 433), ~~Vibrio cholerae~~ (MTCC 3906), ~~*Salmonella typhi*~~ (MTCC 733)] bacteria were used to determine the antibacterial activity of different extracts of plant ~~*Pedalium murex*~~. Vibrio cholerae Salmonella enterica subsp. enterica serovar Typhi P. murex. Extracts are not the same? Leafs extracts? At least that's what it says in the Materials...

Preparing Inoculum

About 1.3 g of nutrient broth (NB) was dissolved in 100 ml of distilled water to prepare the bacterial broth. The bacteria was inoculated in the broth medium and incubated for 18 - 24 h at 37°C. mL

Determination of Antibacterial Assay

Agar-well diffusion method

Antibacterial activity of plant ~~*Pedalium murex*~~ P. murex. extracts was carried out by a modified agar method (Sinclair and Dhingra, 1995; Ahmad *et al*., 1998). Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with Twenty four hour old broth culture of respective bacteria. C~~c~~onsequently, using sterile borer, well of 0.5 cm diameter was made into the each agar plate and then 90 ~~µl~~ µL containing 500 ~~µg/ml~~ µg/mL concentration of each extract (Aqueous) in aseptic condition filled into the well. The plates were placed at room temperature Exact temperature... for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 hrs at ~~37 °C~~ 37ºC. The results were recorded by measuring the diameter of inhibitory zone ~~using a transparent meter rule at the end of the 24 – 48 hrs~~ (using a digital pachymeter) after 24 and 48 hrs. Tetracycline was used as standard drugs for antibacterial activities, respectively.

RESULTS AND DISCUSSION

Phytochemical evaluation of the silver nanoparticles of aqueous leaf extract of ~~Pedalium murex *L.*~~ *P. murex* were done for the presence of steroids, alkaloids, phenols, tannins, flavonoids, ~~T~~t erpenes, ~~C~~c ardiac glycosides, saponins, ~~G~~g um and mucilage and absence in the ~~C~~c arbohydrates, ~~P~~p hlobatannins and results were indicated in Fig 2 ~~&~~and Table 1.

Maybe it's fig 1...

The active component alkaloids contain ~~∝ tocopherol~~ α-tocopherol and ~~I~~i satin which act as antioxidant and antifungal properties. These activities have not been evaluated. The bioactive compound, Saponin possess antifungal, anticancer, anti-inflammatory and antifungal properties. Tannin also possesses secondary metabolites which contains antimicrobial and antioxidant properties (Riviere *et al*., 2009). Flavonoids contain Dinatin–7-glucuronide, Diosmetin–7– glucuronide, Pedalitin (Subramanian and Nair, 1972) which act as antimicrobial, anti allergic and anti-cancer properties. The primary antioxidants or free radical scavenger's properties are present mainly in the metabolites such as tannins and flavonoids which are major groups of compounds (Polterait, 1997).

Steroid which contain diosgenin component play a vital role in antimicrobial activities. Anthraquinones also has antimicrobial properties (Cowan, 1999), antidepressant, antiparasitic (Pieters and Vlietinck, 2005) and bacteriostatic. The medicinal plants with gum and mucilage are widely used as pharmaceutical adjuvant, due to its properties to act as a suspending agent (Yeole *et al*., 2010). These secondary metabolites play a vital role in the antimicrobial activity against human pathogenic bacteria which is used in these studies.

The reduction of AgNO3 was visually evident from the color change (Brownish-Yellow) of the reaction mixture after 24 h (Fig 3). The intensity of the brown color increased in direct proportion to the incubation period. This may be due to the excitation of the Surface Plasmon Resonance (SPR) effect and the reduction of AgNO3 (Vijayaraj *et al*., 2016). The control AgNO3 solution (without *Pedalium murex* extract) showed no color change. The silver nanoparticles obtained were characterized by UV–visible spectroscopy and the characteristic absorption peaks at 350-600 nm in the Spectrum confirmed the formation of silver nanoparticles. In the present study After 24 h incubation in dark room condition??, the light colored reaction mixtures turned into dark brown for indicating AgNPs formation. The surface plasmon resonance (SPR) of AgNPs produced a peak at 440 nm, which suggests the dispersal of AgNPs,

which corroborates with the characteristic peaks of silver nanoparticles (Kong and Jang, 2006; Petit *et al*., 1993).

The phytochemical analysis of ~~Pedalium~~ *P.* murex indicates the presence of flavonoids, alkaloids, steroids, rosins, saponins and proteins (Rajashekar *et al*., 2012). Figure -2 reveals that strong absorbance were observed at peak 3379.29cm^{-1}, 3163.26 cm^{-1}, 3005.10 cm^{-1}, 2627.05 cm^{-1}, 2252.86 cm^{-1}, 2148.70 cm^{-1}, 1442.75 cm^{-1}, 1375.25 cm^{-1}, 1037.70 cm^{-1}, 918.12 cm^{-1}, 748.38 cm^{-1} which are characteristic of N–H stretch, –C ≡ C–H: C–H stretch, =C–H stretch, H–C=O: C–H stretch, C≡ N stretch, –C≡C– stretch, C–H bend, C–H rock, C–N stretch, O–H bend, C–Cl stretch and Presence of functional groups such as 1°& 2° amines, amides, alkynes (terminal), Alkenes, Aldehydes, Nitriles, Alkanes, aliphatic amines, carboxylic acids and alkyl halides. In overall spectral profile is similar except for the variation in intensities of the bands. The most widely used modes in protein structural studies are 1°& 2° amines and amides. The broad band at 3297 cm^{-1} has been assigned in the present study to O–H stretching, the bands of proteins have made a small contribution to it. The bands observed at 2923 cm^{-1} and at 3250 cm^{-1} confirms the presence of asymmetric and symmetric starching modes of the methylene chain in the membrane lipids (Patrick *et al.,* 1993). The sharp bands observed at 1653 cm^{-1} are assigned to the in plane C=O stretching vibration (amide) and to the aliphatic amines and carboxylic acid (Parveez *et al.*, 1999). The phenolicgroups participating in ion replacement response are placedin the 1315–1037 and 1456–1600 cm-1 regions for theplant extract (Jeeva *et al*. 2014b).

The very strong band at1592 cm-1 is due to C=C stretching in the aromatic ring,confirming the presence of the aromatic group (Reddy *et al*.2014). The silver nanoparticles of O–H stretching in carboxylic acids vibration is shifted from 3785 to 3881 cm-1. The immediate reduction and capping of silver ion into silver nanoparticles in the present analysis might be due toflavonoids and proteins. The flavonoids present in the leaf extract act as a strong reducing agents, which may be suggestive of the formation of AgNPs by reduction of silver nitrate. The flavonoid compounds in the water extract of *M.pendans*might be actively involved and responsible for thereduction of Agto Ag0 (Zuas *et al*., 2014). The involvement of water-soluble flavonoid in the reduction of metalions using plant extracts is also evidenced from anotherstudy (Prabhu *et al*., 2010).

In SEM analysis AgNPs have been observed with quasi-spheres shape with uniform distribution (Fig 5). Nevertheless, agglomeration of the particles was observed probably due to the presence of a weak capping agent, which stabilizes the nanoparticles (Mani *et al.*, 2016). This may be due to availability of different quantity and nature of capping agents present in the AgNPs synthesized *pedalium murex.* The above findings were corroborative by the observations from the peaks obtained in the FTIR analysis.

The antibacterial property of silver nanoparticles and aqueous extract of whole plant *Pedalium murex* was analysed against bacterial pathogens using tetracycline as control. Out of these five bacterial pathogens four were found to be Gram-Negative (*Escherichia coli, Vibrio cholera, Salmonella)* and one were Gram-Positive (*Staphylococcus haemolyticus, Staphylococcus hominis*). Agar well method was used to evaluate the antibacterial activity of taken samples. After twenty-four hours the minimum inhibitory zone of aqueous extract and silver nanoparticles of P. *murex and* control were measured (Table: 3& Fig 6).

Escherichia coli were tested in *P. murex* were found to be AgNPs of aqueous extract with a maximum inhibitory zone (15mm each), followed by aqueous extract did not show any inhibition against of *E. coli* and control shows of maximum inhibitory zone (33.3 mm each). *Vibrio choleraewas* found to be more susceptible towards the AgNPs of aqueous extract of *P. murex* maximum inhibitory zone (15mm), followed by control (42.3mm), and aqueous extract did not show any inhibitory against in *Vibrio cholerae*. *Staphylococcus haemolyticus* were tested were found to be that AgNPs has highest significatory effects (18.3mm) and aqueous extract of *P. murex* did not show any inhibitory zone and control shows maximum zone (38.3 mm each).

Salmonella typhi was found to be more susceptible towards the AgNPs of aqueous extract of *P. murex* has some inhibitory zone (15 mm), followed by control (40mm), and aqueous extract did not show any inhibitory against in *Salmonella typhi* (Fig 4). *Staphylococcus hominis*were found to be AgNPs of aqueous extract with a maximum inhibitory zone (15mm each), followed by aqueous extract did not show any inhibition activity and control shows of maximum inhibitory zone (43.3mm each).

The well diffusion method maximum susceptibility with 15-20 mm zone of inhibition was observed at the level of 500 µg/ml concentrations against tested bacteria. It was clearly evident

that our experimental plant has the antimicrobial/antibacterial property against bacterial species. Likewise, *in-vitro* antimicrobial activity of methanolic extract of showed 17 mm, 12 mm and 17 mm inhibitory zone of diameter against *S. aureus*, *E. coli* and *K. pneumonia* respectively and there was no zone of inhibition observed in aqueous extract. Likewise, the methanolic extract of *Solanum palinacanthum* revealed the zone of inhibition against *A. hydrophila*, *B. subtilis* and *S. aureus*. In terms of specific inhibition by petroleum ether extract of *Capparis zeylanica* produced the inhibitory zone ranging from 10 to 16 mm at a concentration of 16.5 µg/ml against *S. aureus, B. subtilis, K. pneumoniae* and *P. vulgaris*, whereas chloroform, ethanol and water extracts showed inhibitory activity against all six bacterial strains at concentrations of 13.5, 14.0 and 14.0 µg/ml respectively. MIC value also revealed that almost all tested bacterial strains were sensitive to the ethyl acetate and petroleum ether extracts of our study plant *P. murex*.

The results showed that the aqueous plant extract have not possessed any antibacterial activity. These results supported that the organic solvent extract is better than aqueous extracts. Similarly, the ethanolic extract of *Punica granatum* was most active against *E. coli* and the methanolic extract of *Euphorbia fusiformis* root possessed significant antibacterial activity against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa,K. pneumonia, Proteus vulgaris, S. typhii* A *and S. typhii* B; *Solanum torvum* showed antimicrobial activity against *B. subtilis, B. cereus, P. aeruginosa* and *S. aureus*, while *S. nigrum* was active against *Salmonella typhi*. Now, the present study also supported that the ethyl acetate and petroleum ether effectively controls the bacterial growth than the other extracts. This probably indicated that the bioactive ingredients are able to inhibit the growth of the common pathogens.

According to this study, plant based antimicrobial drug with silver nanoparticles have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobial agents. The results revealed that the extract of AgNPs *P. murex* effectively produced inhibitory activities against both gram positive and gram negative bacteria. Presence of chemical components of *P. murex* associated with silver nanoparticles may inhibit the bacterial growth. These bacterial group incubations around the wall are due to the release of diffusible inhibitory compounds from silver nanoparticles. These biosynthesized nanoparticles are widely used in cancer therapy, wound healing, antimicrobial activity, water paints, cotton fabrics and textiles, etc. The green synthesis of AgNPs has also

paved a better methodological approach in the medical field. The present study provides the scientific information about the plant extract of *P. murex* and supports the usage of this plant for folkloric treatment of traditional healers. Now, the trend is switched over towards the animal disease management. The results of the present study explore the antibacterial potential of *P. murex* which leads to further study in this direction.

CONCLUSION

The study proves to be an eco-friendly, rapid green approach for the synthesis providing a cost effective and an efficient way for the synthesis of silver nanoparticles. Therefore, this reaction pathway satisfies all the conditions of a 100% green chemical process. The biosynthesized AgNPs were found to have a pronounced antibacterial activity against selected pathogens. In this present study, proteins and flavonoids in the*Pedalium murex* extract play an important role in the formation of silver nanoparticles. Such eco-friendly method could be a better alternative to the conventional physical/chemical methods used for the synthesis of silver nanoparticles and thus has a potential to use in biomedical applications. Hopefully, such green therapeutic approaches will play an important role in opto-electronics and medical devices in the near future.

NOTE:

The study highlights the efficacy of “traditional herbs" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable

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Table 1: Preliminary phytochemical analysis for AgNPs of *Pedalium murex*

Compound	AgNPs sPM
Alkaloids	++
Flavonoids	+
Steroid	+
Saponins	+
Quinones	+
Resins	+
Phenols	+
Cardiac glycosides	+
Tannin	-
Terpenes	+
Phlobatannins	-
Carbohydrates	-
Gum and Mucilage	+
Coumarin	+

figure without explanation or description

(+) Present; (-) Absent

Table 2: FTIR analysis of AgNPs synthesized *Pedalium murex*

Frequency, cm^{-1}	Bond	Functional group
3390.86 cm^{-1}	O–H stretch, H–bonded	Alcohols, phenols
2920.23 cm^{-1}	C≡N stretch	Nitriles
2850.79 cm^{-1}	C–H stretch	Alkanes
1456.26 cm^{-1}	C–H bend	Alkanes
1381.03 cm^{-1}	C–H rock	Alkanes
1230.58 cm^{-1}	C–H wag (–CH2 X)	Alkyl halides
1037.70 cm^{-1}	C–N stretch	Aliphatic amines
545.85 cm^{-1}	C–Br stretch	Alkyl halides

figure without explanation or description

Table 3: Antibacterial assessment of AgNPs of *Pedalium murex* against bacterial pathogens

S. No	Antibacterial activity of AgNPs of *Pedalium murex* L. in mg/ml (Mean ± S.D)*				
	Pathogens	**AgNPs APM**	**APM**	**Tetracycline**	**DMSO**
1.	*E. coli*	15.0±0.00	-	33.3±5.77	-
2.	*Vibrio cholerae*	15.0±0.00	-	42.3±6.42	-
3.	*Staphylococcus haemolyticus*	18.3±2.88	-	38.3±2.88	-
4.	*Salmonella typhi*	15.0±0.00	-	40.0±0.00	-
5.	*Staphylococcus hominis*	15.0±0.00	-	43.3±11.5	-

figure without explanation or description

FIGURES:

Fig 2: Preliminary phytochemical analysis of synthesized AgNPs *Pedalium murex*.

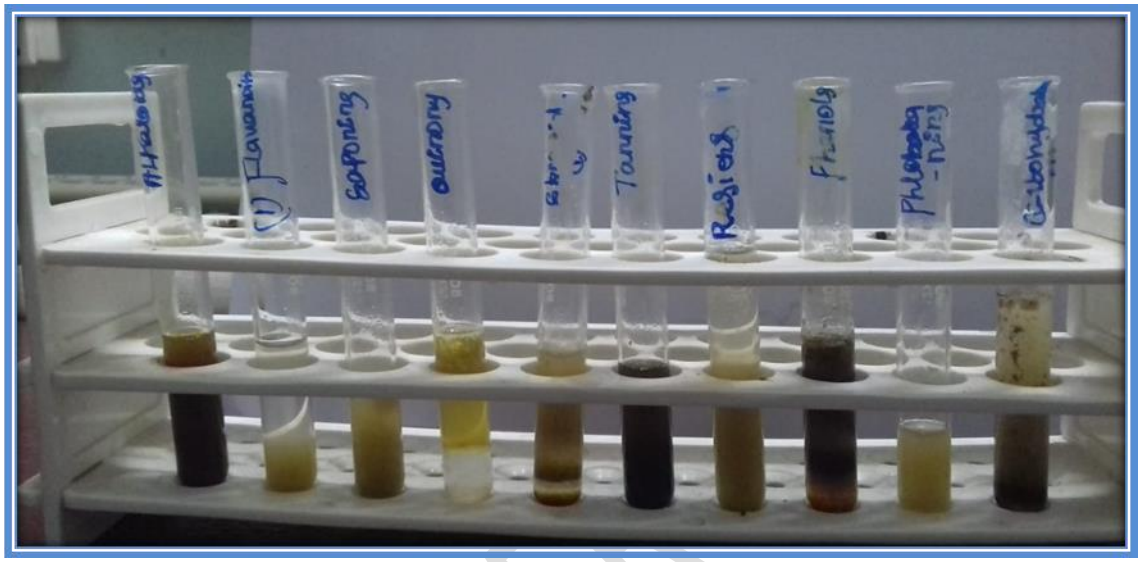

figure without explanation or description

Fig 3: UV spectra of AgNPs of *Pedalium murex* L.

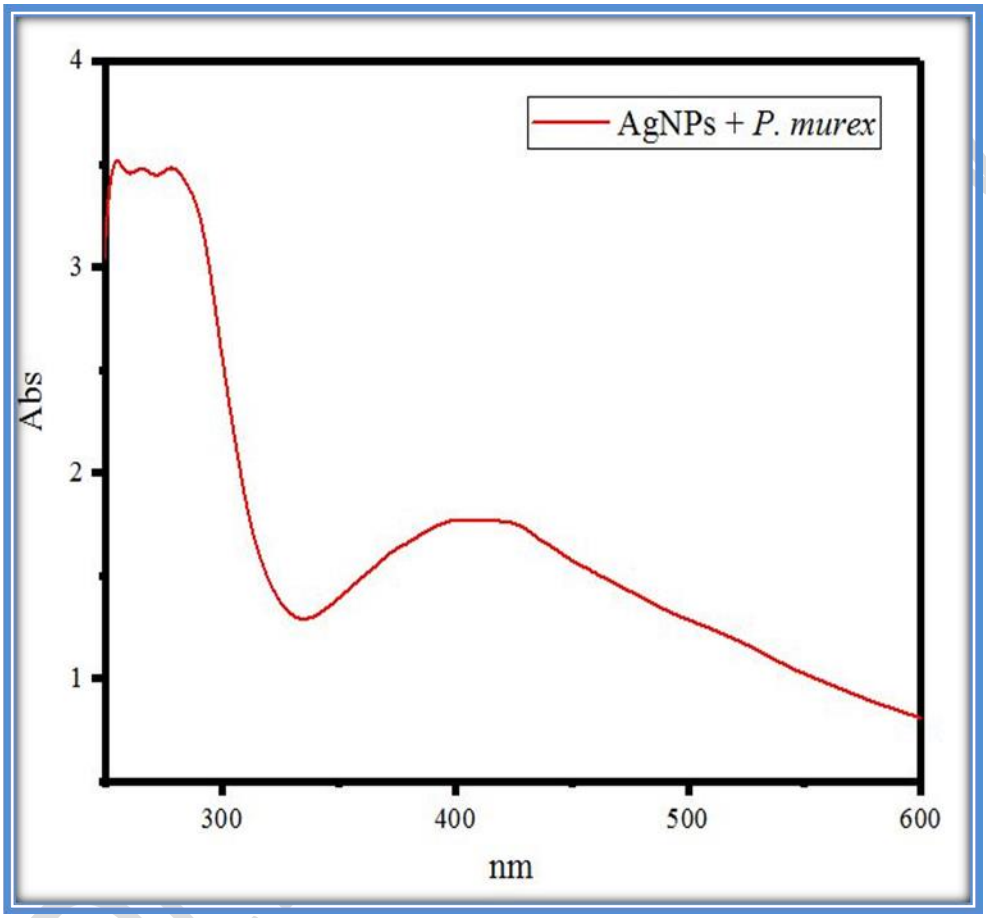

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Fig 4: FTIR analysis of AgNPs synthesized *Pedalium murex*

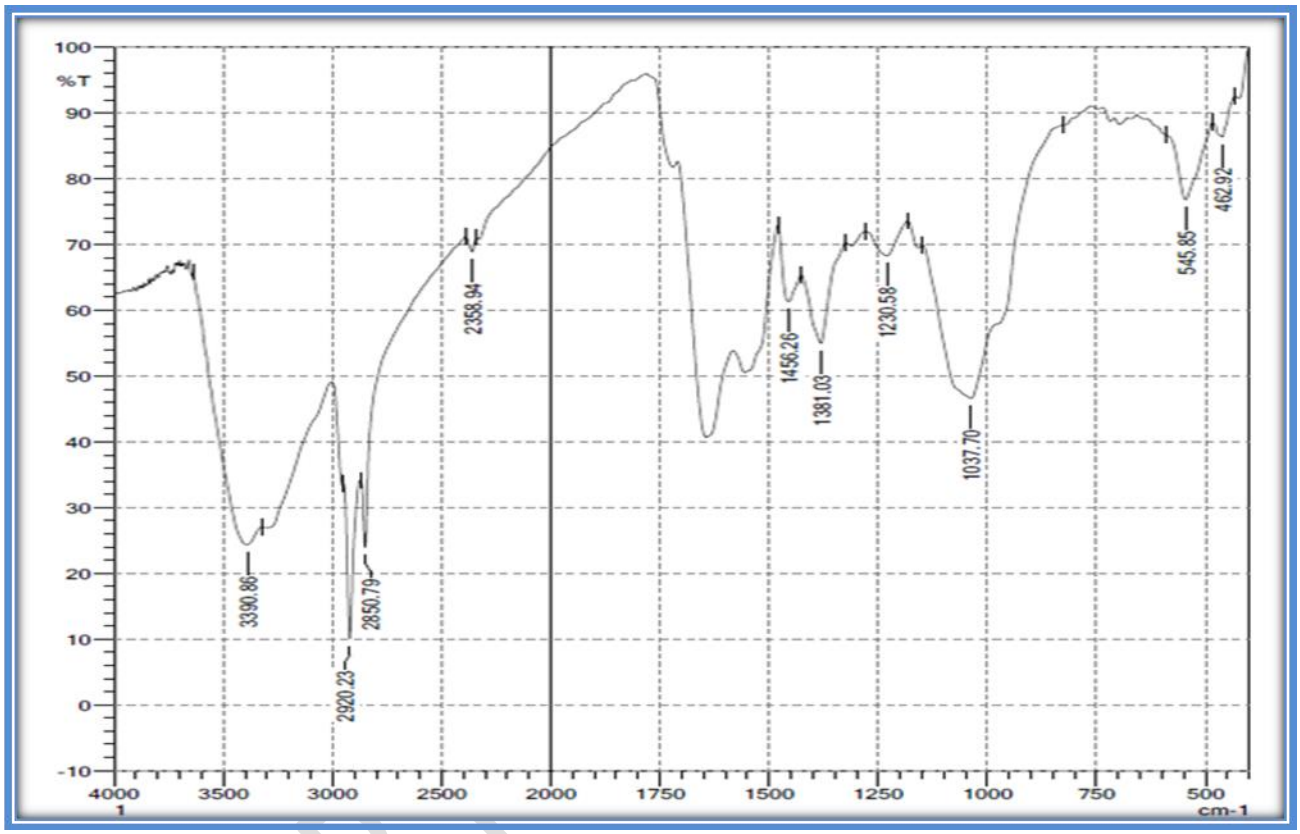

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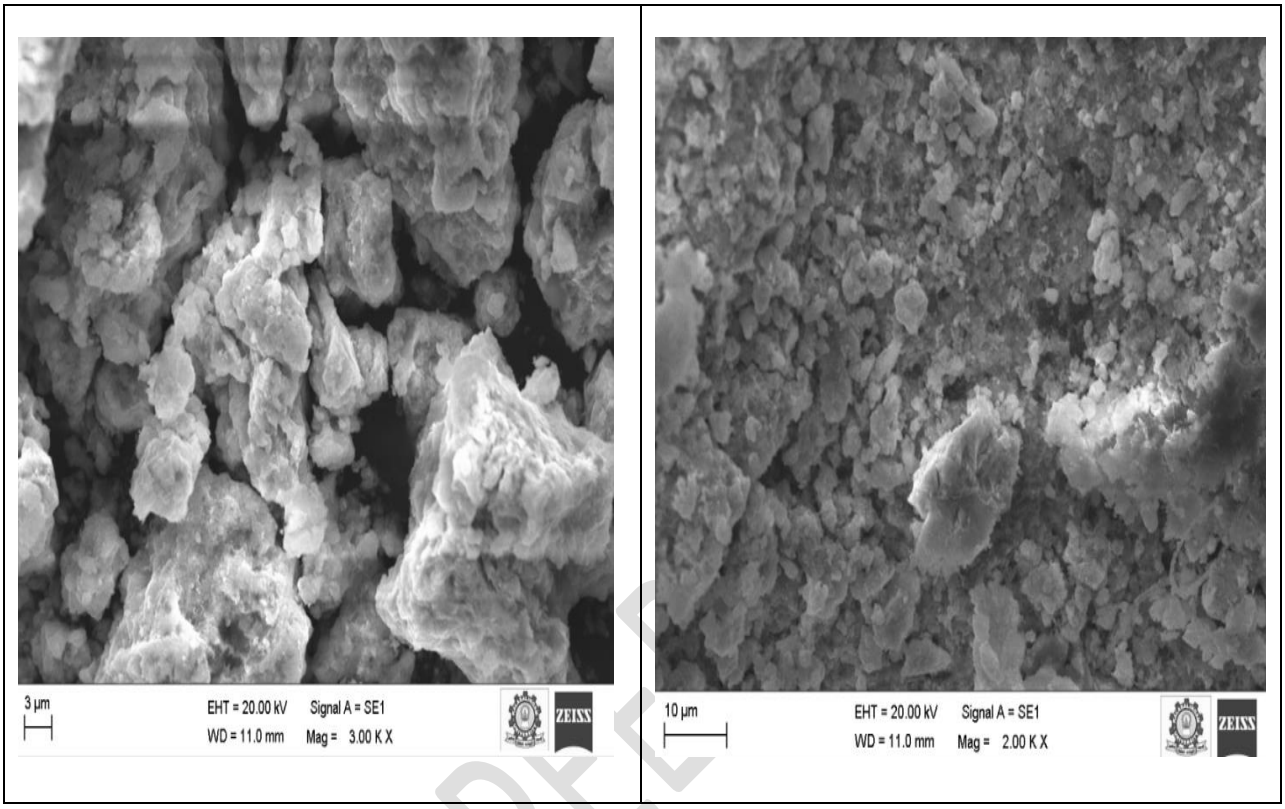
Fig 5: SEM Analysis of AgNPs of *Pedalium murex*



AgNPs PM– Silver nanoparticles of *Pedalium murex* L extract; **APM –** Aqueous extract of*Pedalium murex* L. ; **Tetracycline –** Positive control**; DMSO –**Dimethyl sulphoxide (Negative control); *Three replicates of mean

Fig 6: Antibacterial activity of AgNPs of aqueous extract of *Pedalium murex*

a. *E.coli*
b. *V.cholera*
c. *S.parahaemolyticus*
d. *S.typhi*
e. *S. hominis*

figure without explanation or description