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ANTIMICROBIAL ACTIVITY OF *PILA GLOBOSA* MUCOUS STABILIZED IRON OXIDE NANOPARTICLES AGAINST FRESHWATER PATHOGENS

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ABSTRACT:

P.globosa is also commonly known as the Indian apple snail, it occurs all types of temporary and permanent fresh water bodies. Snails can produce 'mucin' in their mucus secretion which have antimicrobial properties. The present study investigates the eco-friendly synthesis of Iron oxide nanoparticles from the mucus of *Pila gloposa.* The detailed characterization of the resultant samples by UV -Visible Spectroscopy, FTIR Spectroscopy techniques confirms the formation for FeNPs stabilized snail mucus. The prepared nano particals were tested against fresh water pathogens like negative bacteria, *Bacillus subtilis, Escherichia coli and positive bacteria Klebsiella pneumonia Staphylococcus aureus*, and fungus *Aspergillus terreus*, *Aspergillus flavus* and compared with standard of Cefoperazone and pure mucus of snail by disc diffusion method. The resultant FeONPs showed antibacterial and anti fungal activity inhibition zone was maximum observed in standard, when compared to different concentration of FeONPs like 50 µL, 75 µL ,and 100µL, the higher concentration of 100 µL shows highest antibacterial inhibition zone in *Bacillus subtilis*(18 mm) and lowest zone was in *Klebsiella pneumonia* (10mm*)* in 50 *and 75*µL, it indicate the differences in zone formation is FeONPs concentration dependant. The pure mucus also showed antibacterial activity it indicate mollusk also naturally have antimicrobial properties these are necessary for production of new drugs for antibiotic production in future.

Keywords: Mollusks, Mucous, Iron oxide Nanoparticles, Antibacterial, UV–Vis, FTIR

INTRODUCTION

The phylum mollusca is one of the largest invertebrates phyla within the animal kingdom which has evolved successfully and presenting widespread distribution being able to survive in both aquatic and terrestrial environments. Gastropods are largest class of the phylum mollusk (Dharanikota Malleswar *et al.,* 2014). *P.globosa* is also commonly known as the Indian apple snail because of its wide distribution in India (Tapan Sarkar, *et al.,* 2021). The apple snail, *pila globosa* occur in all types of temporary and permanent water bodies such as ponds, canals, and ditches (Jahan *et al*., 2001) . Freshwater mollusks are used as supplementary protein sources in many countries like India (Rao Subba and Dey 1989). Moreover, snails can produce 'mucin' in their mucus secretion, which includes antimicrobial proteins, providing a degree of resistance against infection by microorganisms Adikwu and Alozine (2007). However, there are only a few reports on the chemical components and properties of its mucus (Takeichi *et al.,* 2007; Wasiq-Hidayat and Parman, 2015). While the price of the synthetic antimicrobial drugs is relatively high and increasing, snails that can produce mucin containing mucus secretions are widespread in Thailand. (Nattawadee Nantarat, *et al.,* 2019).

Nanotechnology is concerned with the synthesis of nanoparticles of various sizes and shapes and their potential applications (De,D,Mandal *et al.,* 2010: Dixon *et al*., 2011). Though synthesis of nanoparticles by physical and chemical methods offers well distinct and pure nanoparticles (Saranya *et al.,* 2017). However, due to the increasing interest of these nanomaterials to be used as potential devices for biomedical applications, water soluble iron NPs is an active area of research(Karthikeyeni *et al.*,2013). Fe nanoparticles have been reported to possess potential application in the cancer diagnosis as magnetic resonance imaging agents. Although nanomaterials are currently being widely used in modern technology, there is a serious lack of information concerning the human health and environmental implications of manufactured nanomaterials (Karthikeyeni,*et al.,* 2013).

Metal nanoparticles are reported to have magnetic, catalytic, and optical, anti inflammatory and antimicrobial properties. Among these properties antimicrobial property is considered to be one of the potential in animal and human medicine (Saranya *et al.,*2017). The synthesis iron nanoparticles had antibacterial activity against fresh water pathogenic bacteria like, *Staphylococcus agalactiae, Escherichia coli, Salmonella enteric, pneumonia,*and *Staphylococcus aureus* by well diffusion method. This biosynthesis approach has been found to be cost effective, eco-friendly and promising for application in various fields (Saranya *et al.,*2017). In the present study deal, iron oxide nanoparticles (FeONPs) were synthesized using a *Pila gloposa* mucus as both the reducing and capping agent. Further, the synthesised nanoparticles were tested for antimicrobial activity against fresh water pathogenic organisms like positive, Negative bacteria and fungi.

MATERIAL METHODS

BIOLOGY OF *PILA GLOBOSA*

Pila globosa or the apple snail (Fig-1) is one of the largest freshwater mollusks. *Pila globosa* inhabits freshwater ponds and lakes. They are quite abundant in water having succulent aquatic vegetation such as *vallisnaria and pistia* on which they feed. They live most of the time in water but they can also thrive well on land hence amphibious mode of life. The body of pila globosa is enclosed by a thick spirally-coiled globular shell which is exoskeleton. It is devoid of internal skeleton. A single revolution of the shell is called whorl. The whole body is located within the whorls of the shell and attached to the columella of the shell by columellar muscle. The columellar muscle arises from the foot and is attached with columella. The columellar muscle plays a vital role. It creeps with its ventral muscular foot at 5cm/min. At dry surface, the snail secrets slime while moving leaving a silvery trail. The exhibit two - fold respiratory adaptations. They respire in water by ctenidium and by pulmonary sac on land. During prolonged drought they may remain torpid for long time and during rains they return to normally. At this time shell aperture is closed. It is called as summer sleep or aestivation.

STUDY AREA:

Pila globosa (fresh water snails) were collected from Arasalar River (Fig-2) in kumbakonam near our college campus. The study area is located 39 km away from Thanjavur. Our college campus is located the bank of Arasalar river, hence the water source is available throught the year except April and May shows very little water source. The study animal are available throught the year. Snail abundance is higher in raining season when compare to summer.

MAINTANCE OF PILA GLOBOSA AND COLLECTION OF MUCOUS:

The collected snails were kept in glass boxes, with 5 snails in each box. The glass boxes were sprinkled with water daily in order to maintain humidity. Subsequently, the snails were housed individually in glass boxes and kept without food for 3 days to avoid contamination. The snails were then manually stimulated at their pedal glands, and approximately 2ml of mucous secretion was collected per individual and pooled for each species.


Fig- 1


Fig-2

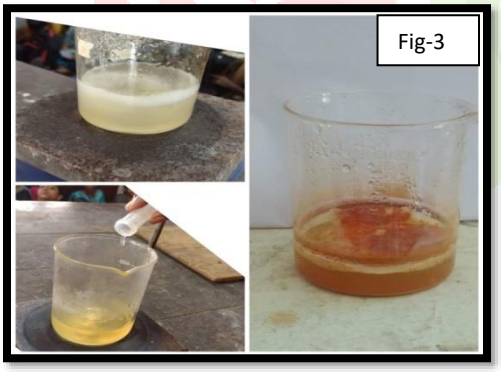


Fig-3

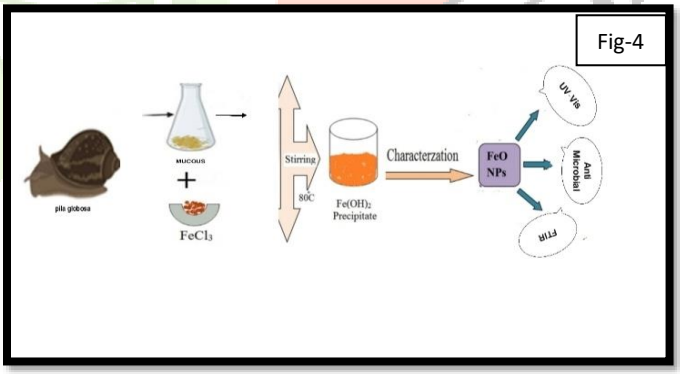


Fig-4

SYNTHESIS OF MUCOUS STABILIZED IRON NANOPARTICLES (FeNPs):

Synthesis of pila mucous stabilized iron oxide nanoparticles was carried out according to the literature. Different volumes of mucous were used to prepare Iron oxide using 0.1M Ferrous sulphate solution. For the synthesis, 2ml of mucous was added to ferrous sulphate solution at stirred at 60º C followed by the addition of 35 wt% Ammonium hydroxide solution. Formation of iron hydroxide was confirmed the appearance of red dish brown color of the reaction mixture (Fig-3&4). The precipitate was centrifuged and thoroughly washed with distilled water. The sample synthesized using 2ml of mucous of *Pila globosa*. A pure sample FeONPs was prepared without mucous for comparative study (Chinnadurai *et al.,* 2020).

EXPERIMENTAL DESIGN:

Various concentration of mucous stabilized iron oxide nanoparticles were (50ug, 75ug and 100ug) treated against gram negative, gram positive fresh water pathogenic bacteria and fungus, distilled water used as negative control, Cefoperazone used as positive control and Pila pure mucous also used for antibacterial activity.

DISC PREPARATION FOR ANTI MICROBIAL ACTIVITY:

The 6mm (diameter) disc was prepared from whatman **No.1** filter paper. 6 discs were made on each plate the disc was sterilized by autoclave at 121ºC. After the sterilization the moisture disc were dried on hot air oven at 50ºC. Then various FeONPs concentrations were loaded in each disc and +ve and –ve control discs were arranged, in addition pure mucus disc were loaded in each plate. The plates were then incubated at 37° for 24 h and the zone of inhibition was measured.

COLLECTION OF TEST MICROORGANISMS:

The Fresh water pathogenic negative bacterial strain of *Klebsiella pneumonia, Escherichia coli* and positive strain of *Staphylococcus aureus*, *Bacillus subtilis* were obtained from microbial type culture collection centre (MTCC), Chandigarh. The fungal strain of *Aspergillus flavus* and *Aspergillus niger* were obtained from microbial type culture collection centre (MTCC), Chandigarh.

ASSAY OF ANTIBACTERIAL ACTIVITY:

Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.*, (1966). Muller Hinton Agar (MHA) was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45ºC. The cooled media was poured on the sterile petriplates and allowed for solidification. The plates with media were added with the respective microbial suspension using sterile swab. The various concentration of FeONPs prepared discs, positive and negative control discs and pure mucus loaded disc individually were placed on the each petriplates. The plates were incubated at 37ºC for 24 hours. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

ASSAY OF ANTIFUNGAL ACTIVITY:

Antifungal activity test was carried out following the modification of the method originally described by Bauer *et al.*, (1966). Potato Dextrose Agar (PDA) was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45ºC. The cooled media was added 10ml/L tartaric acid (10%) act as antibacterial agents and poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various concentration of prepared discs individually were placed done each petriplates and also placed control and standard (Cefoperazon (10µg)) discs. The plates were incubated at 28ºC for 72 hours. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

UV/Vis SPECTROPOTOMETRIC ANALYSIS OF FeONPs:

Synthesis of FeONPs was preliminarily confirmed by recording the absorbance in VU/Vis spectra at a range of 200 – 800 nm. Change in surface Plasmon resonance (SPR) of nanoparticles in the dispersion was recorded using UV/Vis spectrophotometer.

FOURIER TRANSFORM INFRA – RED SPECTROPHOTOMETRIC (FTIR) ANALYSIS OF FeONPs:

The lyophilized samples of Pila globosa (10mg) were mixed with 100mg of dried potassium bromide (KBr) pellet technique in the range of 400-4000cm-1. Then read spectrophotometrically.

STATISTICAL ANALYSIS:

All analyzes were carried out in triplicate, and results are reported as the mean+ standard deviation (SD). Significance differences were analyzed by one-way ANOVA. Differences at $P< 0.05$ were considered significant, at $p<0.005$ were considered highly significant .

RESULT

Antimicrobial effect of the mucous stabilized Iron oxide nanoparticles were tested against selected fresh water pathogens. The effect of FeONPs against Gram negative, Gram positive bacterial strains and fungal strains showed inhibition of zone measurements given below (Table -1)

Table 1: Antimicrobial Activity using FeONPs at selected fresh water pathogens.

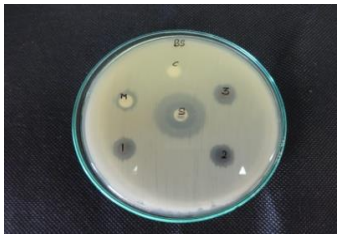
S. No	S. No.	Microbes		Zone of Inhibition (mm in diameter)					
			Control	Standard* Cefoperazone	Mucus	50 µL	75 µL	100 µL	Mean ± SD
1	Bacteria	*Bacillus subtilis*	-	24	10	14	16	18	16.4 ± 5.2
2		*Escherichia coli*	-	22	10	12	14	16	14.8 ± 4.6
3		*Klebsiella pneumonia*	-	23	12	10	10	16	14.2 ± 5.5
4		*Staphylococcus aureus*	-	26	12	13	16	14	16.2 ± 5.8
1	Fungi	*Aspergillus flavus*	-	28	-	-	-	-	
2		*Aspergillus terreus*	-	24	-	-	-	-	

***CFS** - Cefoperazone

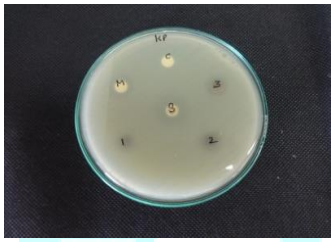
The zone of inhibition values were compared with standard (Cefoperazone) and control. The activity was measured in terms of zone of inhibition in mm. Then the observed values are tabulated. From the present study each strain shows result of zone inhibition was given below.

Gram negative Bacteria

5.BACILLUS SUBTILIS

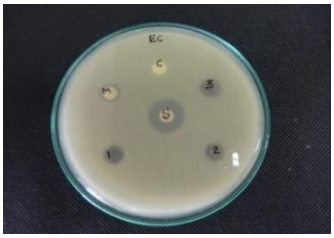


6.ESCHERICHIA COLI

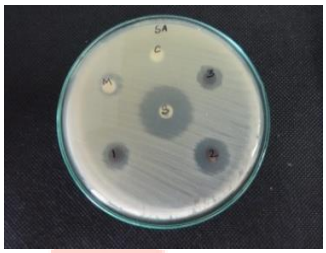


Gram positive Bacteria

7.KLEBSIELLA PNEUMONIAE

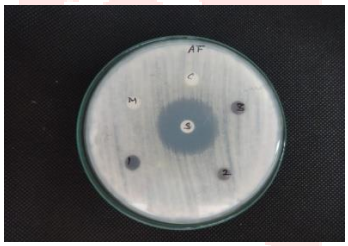


8. STAPHYLOCOCCUS AUREUS

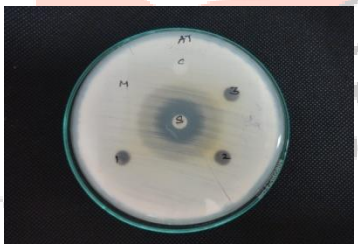


Fungi

9.*ASPERGILLUS FLAVUS*:



10.ASPERGILLUS TERREUS



PATHOGENIC BACTERIA

Bacillus subtilis	-	Standard(24mm) > 100µL(18mm) > 75µL(16mm) > 50µL(14mm) > Mucus (10mm).
Escherichia coli	-	Standard(22mm)>100µL(16mm)>75µL(14mm)> 50µL(12mm)> Mucus (10mm)
Klebsiellapneumonia	-	Standard(23mm)>100µL(16mm)> 75µL(10mm)>50µL(10mm)> Mucus(12mm)
Staphylococcus aureus	-	Standard(26mm)75µL(16mm)> 100µL(14mm)> 50µL(13mm)> Mucus(12mm).

PATHOGENIC FUNGI

Aspergillus flavus - standard (28mm)

Aspergillus terreus - standard(24 mm).

The pathogenic fungus doesn't shows antimicrobial inhibition zone in case of FeONPs, and pure mucus treated groups.

Table 2: BACILLUS SUBTILIS

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	321.6	4	80.4	7.178571	*0.005414	3.47805
Within Groups	112	10	11.2			
Total	433.6	14				

Table 3: ESCHERICHIA COLI

ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	*254.4*	*4*	*63.6*	*4.96875*	**0.01818*	*3.47805*
Within Groups	*128*	*10*	*12.8*			
Total	*382.4*	*14*				

Table 4: KLEBSIELLA PNEUMONIAE

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	362.4	4	90.6	9.244898	**0.002157	3.47805
Within Groups	98	10	9.8			
Total	460.4	14				

Table 5: STAPHYLOCOCCUS AUREUS

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	386.4	4	96.6	15.09375	**0.000306	3.47805
Within Groups	64	10	6.4			
Total	450.4	14				

*- Significant at p<0,05, **-Highly significant at p<0.005

Fig:11: UV- Vis SPECTROPHOTOMETRIC ANAYSIS OF FeONPs:

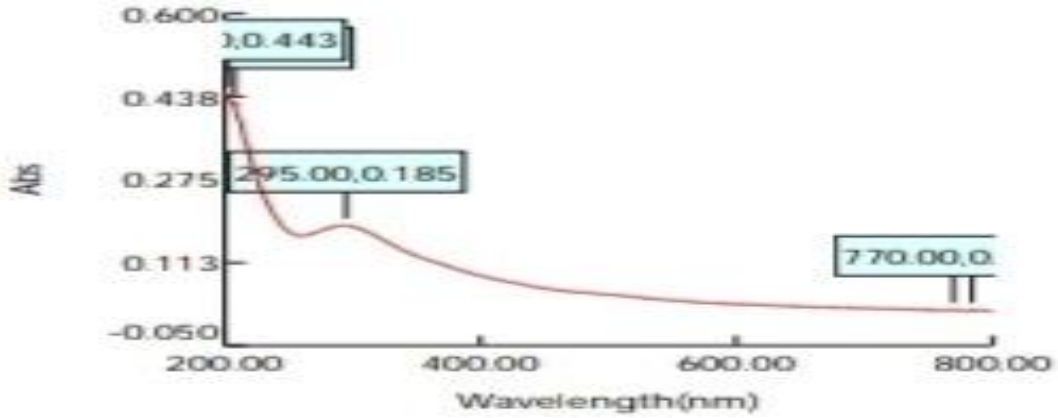
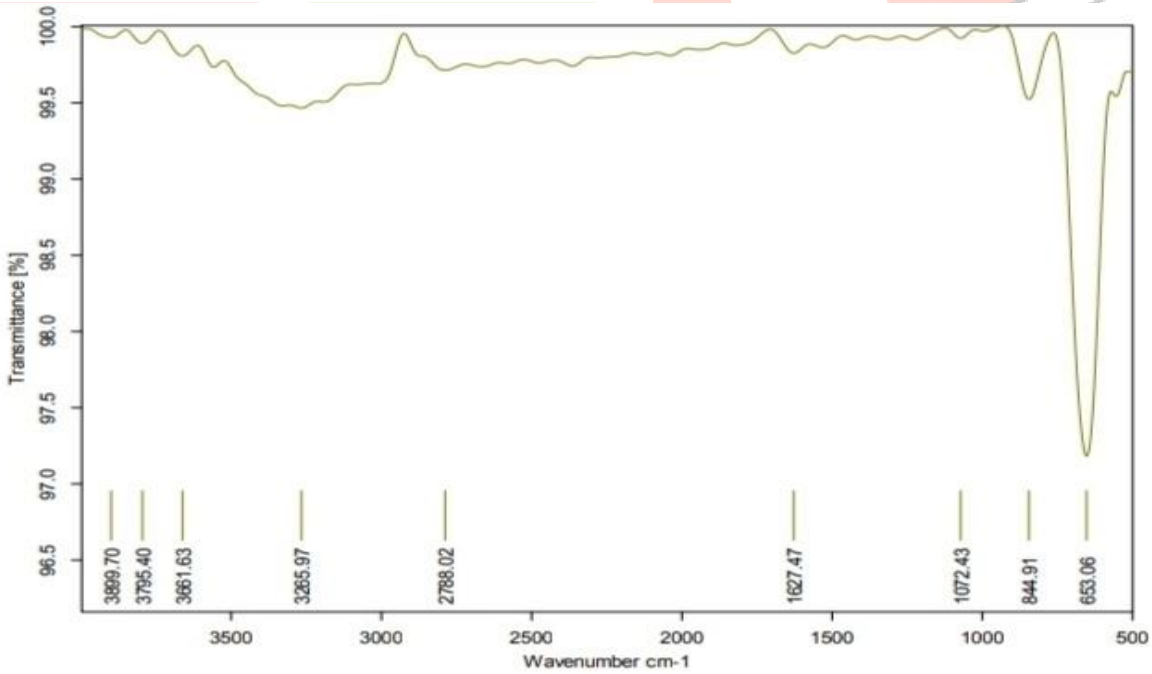


Fig: 12: FOURIER TRANSFORM INFRA RED SPECTROPHOTOMETRIC (FTIR) ANANLYSIS OF FeONPs:



DISCUSSION

Mollusks are widely used in world research institution for various studies, but only recently they have been recognized as potential sources of antibacterial substances. The antimicrobial components in these egg masses could realistically play an ecological role in the prevention of microbial infection (Suresh *et al.,* 2012). Since antimicrobial resistance is a global public animal health concern, there is a growing interest in marine ecosystem to find new antimicrobial agents which will be essential drugs for human and health welfare. The secondary metabolites derived from number of mollusks possess antibiotic, anti – parasitic, antiviral and anti – cancer activities (Harikrishna Jana *et al.,* 2017). Antibacterial and antiviral activities have been previously described in the hemolymph of several molluscan species such as hares, sea slung, oysters and mussels (Amutha and Selvakumari 2016).

Many mollusks mantle cavity produces mucus e.g. Muricid gastropods which defend the developing larvae against microbial infection (Benkendorff, *et al.,* 2011). Protein is a major biochemical constituent in all invertebrates and received highly attention due to their potential bioactive and functional properties (Kiran, *et al.,* 2014). Nattawadee Nantarat *et al.,* 2019 reported that proteins extracted from the terrestrial snail *C.bistrialis* showed the highest antimicrobial activity against pathogenic bacteria and fungi, compared to the other snails proteins. Snails have specific proteins that help their survival in their environment, including preventing bacterial contamination. Their mucus consists of mucin, which includes antimicrobial proteins (Cilia and Fratini 2018). The antibacterial activity of mucin found in the mucous secretions of *L.fulica* was found be related to anti bacterial factors in the protein components, instead of its activity on the cell surface of bacteria (Etim *et al.,* 2016). The present investigation showed antibacterial, antifungal activity of different concentration of FeONPs stabilized with mucus of Apple snail, standard- Cefoperazone and pure mucus of *Pila globosa.*

Nanoparticulate technology has been found to have a wide range of application since most of the biological processes occur at nanoscale level. Few metallic nanoparticles like MgO, CaO, and ZnO have been reported to have antibacterial activities, which has prompted studies to assess the utility of metallic nanoparticles for their antibacterial activities (Saranya *et al.,* 2017). Numerous studies showed the ability of the iron oxide in targeted site drug delivery with improved therapeutic competence than drugs. Iron oxide is less toxic to the human body at a lower dosage for prolonged time Biofabrication of metal (Patil, *et.al.,* 2018). Nanoparticles are attracted by the researchers around the globe due to its essay and eco-friendly procedure. Iron oxide nanoparticles and their composites are well known for their antimicrobial and toxicity (Chinnadurai, *et.al.,* 2020) . Numerous applications of metal/ metal oxide nanoparticles have been reported recently. Role of various nanoparticles have been explored in the field of dye degradation, photocatalytic activity, heavy metal removal, humic acid removal and targeted contrast agent for cancer diagnosis in magnetic resonance imaging (Chinnadurai, *et.al.,* 2020)

The bio reduction of Fe in aqueous solutions was monitored by measuring UV/Vis spectra. FTIR is used to study the surface interaction between synthesized nanoparticles with other molecules involved in the synthesis and stabilization of the nanoparticles. Various characterization techniques were carried out such as UV-Vis, FTIR, XRD, and SEM-EDAX proved formation of iron oxide nanoparticles (Chinnadurai *et.al.,* 2020). The present investigation formation and functional groups responsible for reduction and stabilization of nanoparticles were confirmed by UV-Vis spectra and FTIR analysis. In this study the bio reduction of FeO in aqueous solution was monitored by measuring UV/Vis spectra. UV/ Vis spectra analysis was done at a wavelength range of 200-800 mm to study the absorption spectra of synthesized FeONPs and the absorbtion peaks were observed at 250-350 nm ranges due to the excitation of surface Plasmon vibrations in FeONPs as has been reported. Effect of precursor salt solution on nanoparticles synthesis revealed that 0.1N concentration of $FeSO_4$ resulted in maximum nanoparticles synthesis with absorption peak around 310mM (Fig-11).

FTIR is used to study the surface interaction between synthesized nanoparticles with other molecules involved in the synthesis and stabilization of the nanoparticles (Sarkar *et al*., 2015). The major absorption peaks in FTIR spectra *of M. ornata* leaf extract were mainly located at 3254.05, 1635.17, 525.09, 474.54 and 419.00 cm−1. The presence of peak at 3254.05 cm−1 indicate the possible O-H stretching vibration of phenol groups, which might be responsible for the formation and stabilization of nanoparticles (Latha and Gowri 2014) . The present investigation showed the FTIR spectrum of synthesized FeONPs displayed three strong bands around 3265.97, 1627.47 and 653.06 cm^{-1}. Based on the study the vibration bands could be assigned as 653.06 cm^{-1} (Fe), 1627.47cm^{-1} (H_2O bending vibration) and broad peak at 3265.97 cm^{-1}. Presence of organic molecule on the surface of FeONPs has been reported to have an influence on the FTIR peaks and the broad peak observed around 653.06 cm^{-1} instead of two sharp peaks, might be due to the organic molecule from the extract on the surface of FeONPs. The weak band at 3265.97cm^{-1} could well be attributed to the unsaturated nitrogen (C-N) compound from the extract (Fig-12).

Pattanayak and Nayak (2013) reported that The bio reduction of Fe ions in aqueous solutions was monitored by measuring UV/Vis spectra. UV/Vis spectral analysis was done at a wavelength range of 200-800 nm to study the absorption spectra of green synthesized FeONPs and the absorption peaks were observed at 250-350 nm ranges due to the excitation of surface plasmon vibrations in FeONPs as has been reported earlier. Effect of precursor salt solution on nanoparticles synthesis revealed that 5 mM concentration of FeSO4 resulted in maximum nanoparticles synthesis with the absorption peak around 310 nm. The FTIR spectrum of synthesized FeNPs displayed three strong bands around 3383.42, 1634.15 and 480.69 cm−1(Kumar and Singhal) have reported the presence of similar bands at 472, 1634. FTIR is a useful tool to assess the functional groups of mucous extract involved in the reduction of Fe ions into FeO during the synthesis.

In this study FeONPs showed maximum inhibition zone against freshwater pathogens like positive bacteria at 100µl *B.subtilis* (18mm) followed by *E.coli* (16mm), negative bacteria like *K.pneumoniae* (16mm), *S.aureus* (14mm) and fungi showed no inhibition zone. The zone of inhibition value was compared with standard. Standard of Cefoperazone showed maximum inhibitory zone in positive and negative bacteria including 26 mm in *S.aureus*, 24 mm in *B.subtilis 23 mm in K.pneumoniae and 22mm in E.coli* likewise in fungi 28 mm in *A.flavus and* 24 mm in *A.terreus* which showed highest inhibition zone *w*hen compare to different concentration of mucus stabilized FeONPs and pure mucus groups (Fig-5 to 10). From this study fresh water fungi not showed any inhibition zone in case of different concentration of FeONps. Minimum inhibitory zone was observed in pure mucus treated groups in all selected pathogens, it shows that synthesis of FeONPs stabilized with apple snails mucus had antibacterial activity, which is used for new drug synthesis and delivery, it also indicate the zone of inhibition was concentration dependent. Because the higher concentration 100µl shows high inhibition zone when compare to 75 µl and 50 µl. The difference in the antimicrobial activity between snail species or extracts may be related to the amount of mucin contained in the mucus secretion Etim *et al.,* (2016) or differences in its composition.

Hence diffusion rates versus effective concentration between different compounds may be critical factors (Nattawadee Nantara, *et al.,* 2019). Similar result was observed by Chinnadurai *et.al., (*2020), he stated that the synthesis FeONPs was examined for their size dependent antibacterial activity. The FeO stabilized with mucous exhibited zone of inhibition against *Bacteriodes fragilis, Escherichia coli, Proteus vulgaris* and *Staphylococcus faecalis.* Similarly (Saranya *et al.,*2017) reported that the magnetic nanoparticles were found to be oriented in one direction due to the magnetic properties. The FeONPs did not have appreciable antibacterial activity at 5mg/ml concentration compared to 10, 15, 20 mg concentration. Dhanaraj *et al.,* (2009) reported that the skin and intestinal mucous of *Channa* sp showed a strong inhibition against the selected pathogens. The skin mucous of *C. punnctatus* and *C. marulius* exhibited a maximum zone of inhibition against *V. fischeri* (29 ±3mm) and *E. coli* (24± 2.5mm) respectively followed by *C. striatus* against *A. hydrophila* (19.5 ± 2mm*), P. aeruginosa* (24.5 ± 2.4mm).

The present study Analysis of Variance showed statistically significant value ($p<0.05$) at *B.subtillis and E.coli of negative bacteria (Table-2 &3)and highly significant value (p<0.0005) at K.pneumoniae and S.aureus of positive bacteria (Table 4 &5).* similar result were observed by (Gray Antonio Cenidoza *et al.,* 2018) the result of agar overlay-well diffusion assay showed that there are statistically significant differences

between the effectively of all extracted mucous with antibiotic control, Cefoperazone, against *Aeromonas hydrophila, Escherichia coli, Klebsiella pneumonia, Micrococcus luteus, Pseudomonas aeruginosa, Serratia marcesscens* and *Staphylococcus aureus* as determined by one- way ANOVA at $\alpha = 0.05$.

While the price of synthetic antimicrobial drugs is relatively high and increasing, snails that can produce mucin containing mucus secretions are widespread in Thailand. Hence, it is of medical and commercial interest to discover their potential use as an alternative source of antibacterial agents, as well as understanding how to control these agricultural pests (Nattawadee Nantarat et al., (2019). Molluscs are considered as one of the important sources to derive bioactive compounds that exhibit antitumor, antimicrobial, anti- inflammatory, and antioxidant activities. Most of the pathogens are increasingly resistant to the major classes of the routinely used antibiotic (Marimuthu Gayathri, *et al.*, 2020). Hence, the present study focused more attention in FeONps stabilized apple snail mucus act as antibacterial activities which may reduce fresh water pathogens by using different concentration, mucus of Apple snail also had antibacterial properties, it indicate presence of snail in aquatic medium naturally benefit for aqua culturist it may reduce pathogenic problems.

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