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Synthesis of Silver Nano-Particle with Poly-Herbal Plant Extracts for Anti-Fungal and Anti-Microbial Activity on Candida Albicans and E. coli

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PREFACE

Rapid compounding of silver nanoparticles through economically feasible green chemistry proposition is highly desirable. In this study we have developed a method to synthesize silver nanoparticles by mixing silver solution with leaf extract of Pisum sativum without using any surfactant or external energy. In this method, physiologically steady, bio-compatible Ag nanoparticles were set upped. These functionalized POLY HERBAL Ag-NPs could be used for targeted drug delivery with enhanced therapeutic efficacy and minimal side effects. Extracellular synthesis of metal nanoparticles using extracts of plants like Pisum sativum (Pea), and Zingiber officinale (Ginger) and Curcuma longa (turmeric) has been successfully carried out. In this article Ag-NPs formation using Pisum sativum (Pea), and Zingiber officinale (Ginger) and Curcuma longa (turmeric) has been thoroughly discussed. It is well recognised that on treating the metallic salt solution with some plant extracts, a rapid reduction take place leading to the origination of highly stable metal nanoparticles. With this method quick synthesis of nanoparticles was noticed to occur; i.e., reaction time was 1–2 h ascontrasted to 2–4 days required by microorganisms. These nanoparticles were examined by various characterization techniques to disclose their morphology, chemical composition, and antimicrobial activity. TEM image of these NPs shows the evolution of spherical, non- uniform, poly dispersed nanoparticles. A comprehensive study of anti-microbial activity of nanoparticles was performed.

Key words- Silver nanoparticles, Poly Herbal, Anti-microbial

INTRODUCTION

Nanoparticles are globular, polymeric particles composed of natural or artificial polymers. They scale in **size between 1-1000 nm**.

As a consequence of their globular shape and high surface area to volume ratio, theseparticles have a wide range of potential applications

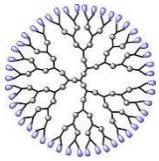
Nanoparticle technology is rapidly facilitating, providing novel and effective treatments for various diseases, including neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. [1]

Nevertheless, effectively and territorially targeting drugs to the brain remain a challenge due to the

restrictive properties of the blood–brain barrier (BBB). This barrier, mostly formed by endothelial cells that are physically joined by tight junctions in their external membranes, limits the molecular interchange to transcellular transport, thus narrowing the passage of molecules across the barrier. The healthy BBB also mostly protects the brain from blood- borne nanoparticle disclosure; however, a number of pathologies, including hypertension and allergic encephalomyelitis, have been shown to enlarge BBB permeability to nanoparticles. The probable widespread future applications and nearing commercialization of nanoparticles of different constitution also pose risks both to humans and to environmental systems. [2]

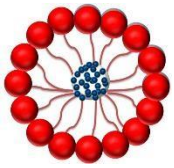
TYPES OF NAOPARTICLE-ORGANIC

- Polymeric-
- Dendrimers- Biological molecules such as genes, drugs, vaccines, and mono-polymers or co-polymers such as chitin, polyethyleneimine, polyamide amine, and poly (propylene amine) are currently used to form dendrimers. [3]

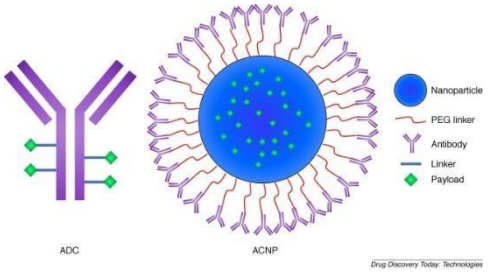


- Micelle- Micellar solutions are most frequently used to carry low- solubility therapeutic agents. Micelles are roughly 100 μm in diameter and

form aggregates in the solvent. The elemental molecules of polymeric micelles are constructed in a spherical configuration, where a head of hydrophilic groups surrounds the hydrophobic centres. [4]

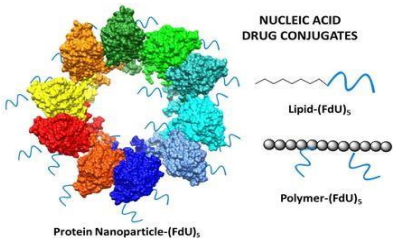


- Drug Conjugates- For continual drug release and upgraded drug capacity, covalently conjugated polymer drugs are particularly stable. For low- molecular-weight vehicle, conjugation of polymers with drug molecules is familiar, especially in cancer treatments. [5]



- Protein Nanoparticle- Protein polymers are self-gatheredinto usable drug delivery porters by genetic modification, with polymer-based nanoparticle interest. Viruses are

natural porter systems for transporting genetic material encapsulated by capsid proteins. Virus-like particles (VLPs), a type of protein nanoparticle, are arranged as nanocarrier systems with a morphologically alike, virus-isolated structure but do not hold viral genetic material. [6]



ADVANTAGES

1. 1.. The nanoparticle surface can be changed to alter biodistribution of drugs with subsequent clearance of the drug so as to reach maximum therapeutic efficacy with minimal side effects of the drug.
2. Controlled release and particle degradation properties can be willingly modulated by the alternating of matrix constituents.
3. Drug loading is higher and drugs can be incorporated into the systems without any chemical reaction; this is a key factor for preserving the drug activity.
4. Site-specific targeting can be got by attaching targeting ligands to surface of particles oruse of magnetic guidance.
5. Liposomes and polymer based nano particulates are normally biodegradable, do notassemble in the body and so are possibly risk free. [7]

LIMITATIONS

1. Adjusted physical properties which guide to particle-particle aggregation, making physicalhandling of nanoparticles difficult in liquid and dry forms due to small-scale size and large- scale surface area.
2. Small-scale the particles size greater the surface area and this property makes nanoparticles very reactive in the cellular environment.
3. Less particles size results in limited drug loading and blast release. [8]

APPLICATIONS

- Magnetic nanoparticles have been utilised to replace radioactive technetium for tracking the dispersion of cancer along lymph nodes.
- superparamagnetic iron oxide nanoparticles is used in magnetic resonanceimaging (MRI).
- Enhance fluorescent imaging or to increase images from positron emission tomography (PET) or ultrasound.
- The evolution of nanoparticles to aid in the delivery of a drug to the brain via inhalation holds considerable promise for the treatment of neurological disorders such as Parkinson disease, Alzheimer disease, and multiple sclerosis.
- Design and manufacture of novel scaffold constructions for tissue and bone repair.
- Development of health-related products.
- Drug and gene delivery
- Bio detection of pathogens
- Detection of proteins
- Probing of DNA structure
- Tissue engineering
- Tumour destruction via heating (hyperthermia). [9]

PREPARATION OF NANOPARTICLES

Emulsion/ EvaporationDouble Emulsion
Salting Out
Emulsification – DiffusionSpray Drying

PURIFICATION METHODS

Purification by filtration:

Polymerization method
Super critical fluid technology Coacervation or Ionic Gelation MethodEmulsion-Diffusion-Evaporation
Solvent Displacement/ Nanoprecipitation

- Briefly, 10mL of polymersomes at a polymer concentration of 10mg/mL were diluted to50mL with PBS.
- The dilute polymersome solution was aliquoted into polystyrene sample tubes and attached to the research system with a 50 nm hollow fibre filter module.
- The filtration was started with the flow rate of 2mL/minute.
- After the retained volume was reduced to 2mL it was re-diluted to 50mL and the process was repeated.
- To concentrate polymersome samples, a hollow fibre module with pores of 10 kDa was utilised. [10]

Purification by centrifugation:

- The initial step of polymersome purification by size involved removal of micelles from the solution using the Crossflow filtration system.
- The polymersomes were centrifuged at 500 Rotational Centrifugal Force (RCF) for20minutes.
- The resulting pellet was removed and resuspended in PBS.
- This fraction contained the largest aggregate fraction.
- The supernatant was then re-centrifuged at 2000 RCF for 20minutes and the pellet was removed and re-suspended, constituting fraction 1.
- This was repeated with further 20-minute centrifugations at 5000, 10000, 15000 and 20000 RCF. [11]

Purification by GPC:

- For separation of polymersomes by GPC, micelles and aggregates were removed as described above and the remaining polymersome solution was concentrated to approximately 200 µL using a 500kDa MicroKros filter module.
- The solution was then placed in a glass liquid chromatography column containing Sepharose 4B.
- The fractions were collected in a 96-well plate. Dynamic Light Scattering (DLS) measurements were performed. [12]

GINGER

SYNONYMS Gingerin, Rhizoma zingiberis, Zingibere,

Family Zingiberaceae .

CHEMICAL CONSITITUENTS

The ginger is consisting with 1-2% of volatile oil, 5-8%pungent principle, starch and resinous mass. Aromatic smell is responsible for volatile oil and it contains the Zingiberener 6% sesquiterpenes hydrocarbon zingiberol a sesquiterpene alcohol and besaabolene. The gingirol are the chemical which is a yellow pungent oil liquid and give gingerone a ketone and aliphatic aldehyde. Shogaols and ginger are less pungent as compare the gingirol. The bitterness of ginger and gingerol is destroyed, which is boiled with 5%KOH or other alkaloids. [13]

PHARMACEUTICAL USES

The ginger is basically used for treatment of some types of- "**Stomach problems**," including motion sickness, morning sickness, colic, upset stomach, gas, diarrhoea, irritable bowelsyndrome (IBS), nausea, Nausea caused by cancer treatment, Nausea caused by HIV/AIDS treatment, Nausea and vomiting after surgery, as well as loss of appetites. [14] **Other uses of ginger-**

- Bronchitis
- Diabetes
- Chest pain

ANTIMICROBIAL ACTIVITY OF GINGER

Ginger (Zingiber officinale) has long been used as naturopathy due to their potential antimicrobial activity against different microbial pathogens. The present study showed the potent antimicrobial activity of the ginger extract against the all tested bacterial pathogens. Ginger has direct anti-microbial activity and thus can be used in treatment of bacterialinfections.

Synonyms

TURMERIC

Saffron Indian; haldi (Hindi); Curcuma; Rhizoma cur-cumae.

Biological Source

Turmeric is the dried rhizome of *Curcuma longa* Linn. (syn. *C.domestica* Valeton)., belonging to family Zingiberaceae.

Chemical Constituents

Turmeric contains yellow colouring matter called as curcuminoids (5%) and essential oil (6%). The chief constituent of the colouring matter is curcumin I (60%) in addition with small quantities of curcumin III, curcumin II and dihydrocurcumin. The volatile oil contains mono- and sesquiterpenes like zingiberene (25%), α-phellandrene, sabinene, turmerone, arturmerone, borneol, and cineole. Choleretic action of the essential oil is attributed to β- tolylmethyl carbinol.

The volatile oil also contains α- and β-pinene, camphene, limonene, terpinene, terpinolene, caryophyllene, linalool, isoborneol, camphor, eugenol, curdione, curzerenone, curlone, AR- curcumenes, β-curcumene, γ-curcumene. α- and β-turmerones, and curzerenone. [15]

ANTIMICROBIAL ACTIVITY OF TURMERIC

Curcumin, a principal bioactive substance of turmeric (*Curcuma longa* L.), is reported as a strong antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral agent.

However, its antimicrobial properties require further detailed investigations into clinical and multidrug-resistant (MDR) isolates. In this work, we tested curcumin's efficacy against over 100 strains of pathogens belonging to 19 species. This activity was

determined by the broth microdilution method and by calculating the minimum

inhibitory concentration (MIC). Our findings confirmed a much greater sensitivity of Gram-positive than Gram-negative bacteria. This study exhibited a significantly largervariation in the curcumin activity than previous works and suggested that numerous

clinical strains of widespread pathogens have a poor sensitivity to curcumin. Similarly, the MICs of the MDR types of *Staphylococcus aureus, S. haemolyticus, Escherichia coli,*

PEAS

BOTANICAL CLASSIFICATION

Botanical Name- Pisum sativum

Family Name - Fabaceae

CHEMICAL CONSTITUENT

- Kaempferol
- 0.19% coumaric acid
- 6.83% ellagic acid
- 6-prenylpinocembrin

- Beta-amyrine

REQUIREMENTS

Materials

Heating mental, Centrifugation chamber ,Suction pump, Magnetic stirrer Microscope with glass slides. Spade. BOD Incubator , Sprit Lamp. UV Laminar Air Flow, Autoclave **Chemicals**

Silver nitrate was purchased from Iso chemical laboratories kochi.

METHODS

Isolation and identifications of herb samples:

Site location: Herb samples were collected between 20th FEBREARY 2023and 10th APRIL 2023 from one of the MARKET areas located in CHINPAI, BIRBHUM, West Bengal, India.

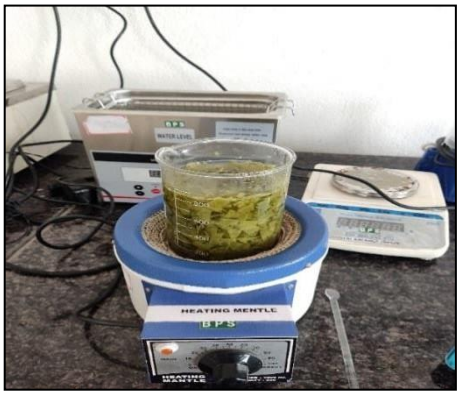
Herb sample collected:

- Peas sample
- Ginger sample
- Turmeric sample

EXPERIMENTAL PROCEDURE

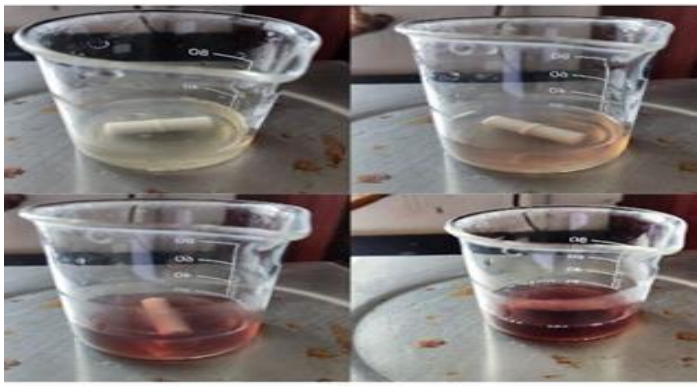
Extraction of herbal constituents:

- Peas peels, Ginger is cute into small fine pieces.
- Herbs are separately boiled in different beaker on heating mental for approximately **4 hr** at a temperature of **50 degree centigrade**.



Filtration of extract:

- Filtration is done of extracts by a suction pump using a funnel and Whatman filter papers.
- Collected in a conical flask.
- This process is done for 3 times for getting accurate measures.



Preparation of AgNo3 solution:

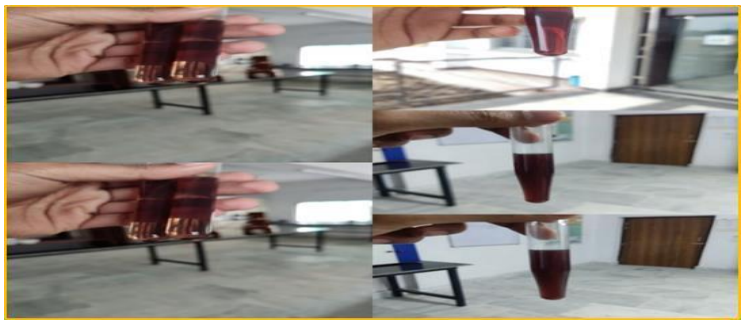
- Add **0.17 gm** of **AgNo3** crystals in **100 ml of demineralizedwater**.
- Stare is until they produce a complete solution.

Preparation of nano-particles:

- Herbal extract and AgNo3 solution are mixed in 3 differentratios-
- ✓ 10:5 (2:1)
- ✓ 15:5 (3:1)
- ✓ 20:5 (4:1)
- Continuous stirring is done of this mixture for approximately **3 hr** on a magnetic stirrer for production of nano-particles.

Purification of nano-particle:

- Remove all impurities and macro molecules by precipitating them by centrifugation withthe help of a centrifugation chamber at a speed of **5000 rpm for 30 min**.
- Collect the supernatant solution and disperse in demineralizedwater



PREPARATION OF NUTRIENT AGAR MEDIA

Preparation nutrient agar

- Suspend 28g of nutrient agar powder in 1L of distilled water.
- Mix and dissolve them completely.
- Sterilize by autoclaving at 121°C for 15 minutes.
- Pour the liquid into the petri dish and wait for themedium to solidify.
- Preparing the agar in the clean environment to prevent any contamination.
- Once the agar solidifies, the agar is ready to use.

Storage condition and shelf life for nutrient agar

Store the dehydrated medium at 10-30°C.

Once the nutrient agar is prepared in the petri dish, store at 2-8°C. [16]

Composition of Nutrient Agar

Typical Formula	**Nutrient Agar (gm/litre)**
Beef Extract	5 gm
Distilled Water	100 ml
Peptone	5 gm
Sodium chloride	3 gm
Agar	25 gm

INOCULATON OF BACTERIAL SUSPENSIONS:

1. 3 sterile petri plates are taken and numbered as (i), (ii) & (iii).
2. he nutrient agar media which is cooled to 45° C after autoclaving, is poured on the sterilepetri plates to form a thick layer (6 mm) and allowed to rest undisturbed for 2 hours.
3. After 2 hours when the media solidifies, from test tube 0.2 ml of bacterial suspension istaken and inoculated in petri plate (I), in an aseptic condition (laminar air-flow chamber).
4. In the same way inoculation in done in petri plate (ii) and (iii) from suspension of testtube 2 &3.
5. In the petri plates hole are bored with cork borers and the plated are placed in BODincubator to incubate for 24 hours. [17]

Bacterial species used

Petri plate no.	**Bacterial species used**
Petri plate I	S. aureus (GIFTED FROM CDL)
Petri plate II	E. coli (GIFTED FROM CDL))
Petri plate III	C. albicans (GIFTED FROM CDL)

Table 2: different bacterial culture used in this experiment

METHOD OF ANTIBIOTIC ASSAY

After 24 hr of incubation, herbal nanoparticles are added to the culture media indifferent media

$AgNo_3$ Treated	**Micro organisms**	**Amount of sample added**
PHF treated with $AgNo_3$	S. aureus	10µl, 30µl, 50µl
PHF treated without $AgNo_3$	E. coli	10µl, 30µl, 50µl
Only $AgNo_3$ treated	C. Albicans	10µl, 30µl, 50µl

Table 4: different combination of samples with different amount are used for detectionof

antimicrobial and antifungal study of poly-herbal silver nanoparticle.


RESULT AND DISCUSSION

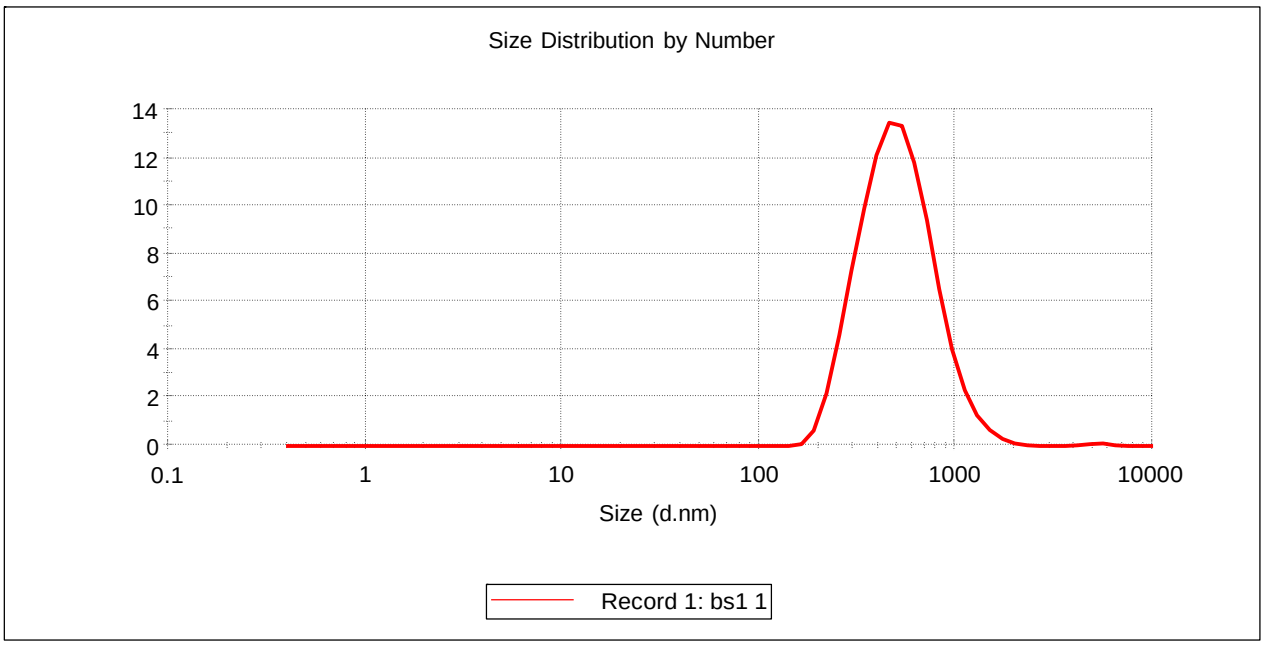
Size Distribution Report by Number

v2.2

Sample Details				
	Sample Name:	bs1 1		
	SOP Name:	mansettings.nano		
	General Notes:			
	File Name:	03.04.23	Dispersant Name:	Water
	Record Number:	1	Dispersant RI:	1.330
	Material RI:	0.20	Viscosity (cP):	0.8872
	Material Absorbtion:	3.320	Measurement Date and Time:	Monday, April 03, 2023 12:38:20 AM
System	Temperature (°C):	25.0	Duration Used (s):	50
	Count Rate (kcps):	229.7	Measurement Position (mm):	1.05
	Cell Description:	Disposable sizing cuvette	Attenuator:	5

Results						
				Size (d.nm):	% Number:	St Dev (d.nm):
	Z-Average (d.nm):	873.9	Peak 1:	544.9	99.7	247.7
	PdI:	0.539	Peak 2:	5158	0.3	740.1
	Intercept:	0.860	Peak 3:	0.000	0.0	0.000

	Result quality :	Good				



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Zetasizer Ver. 7.11
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Serial Number : MAL1022382
Record Number: 1
03 Apr 2023 12:54:17 AM
Size Distribution Report by Intensity
v2.2

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	SOP Name:	mansettings.nano		
	General Notes:			
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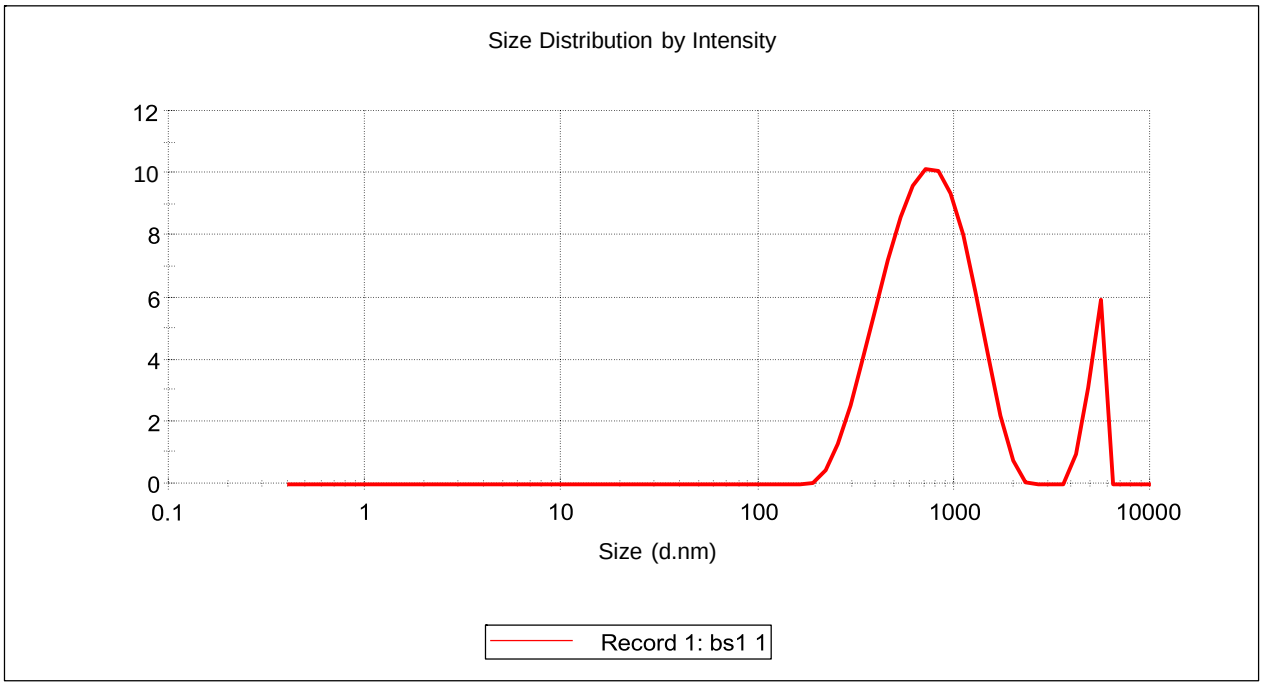
	Record Number:	1	Dispersant RI:	1.330
	Material RI:	0.20	Viscosity (cP):	0.8872
	Material Absorbtion:	3.320	Measurement Date and Time:	Monday, April 03, 2023 12:38:20 AM
System	Temperature	25.0	Duration Used (s):	50

	(°C):			
	Count Rate (kcps):	229.7	Measurement Position (mm):	1.05
	Cell Description:	Disposable sizing cuvette	Attenuator:	5
Results				

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 873.9	Peak 1:	790.4	90.0	368.3
PdI: 0.539	Peak 2:	5187	10.0	483.0
Intercept: 0.860	Peak 3:	0.000	0.0	0.000
Result quality : Good				



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Record Number: 1
03 Apr 2023 12:53:52 AM
Size Statistics Report by Number
v2.0
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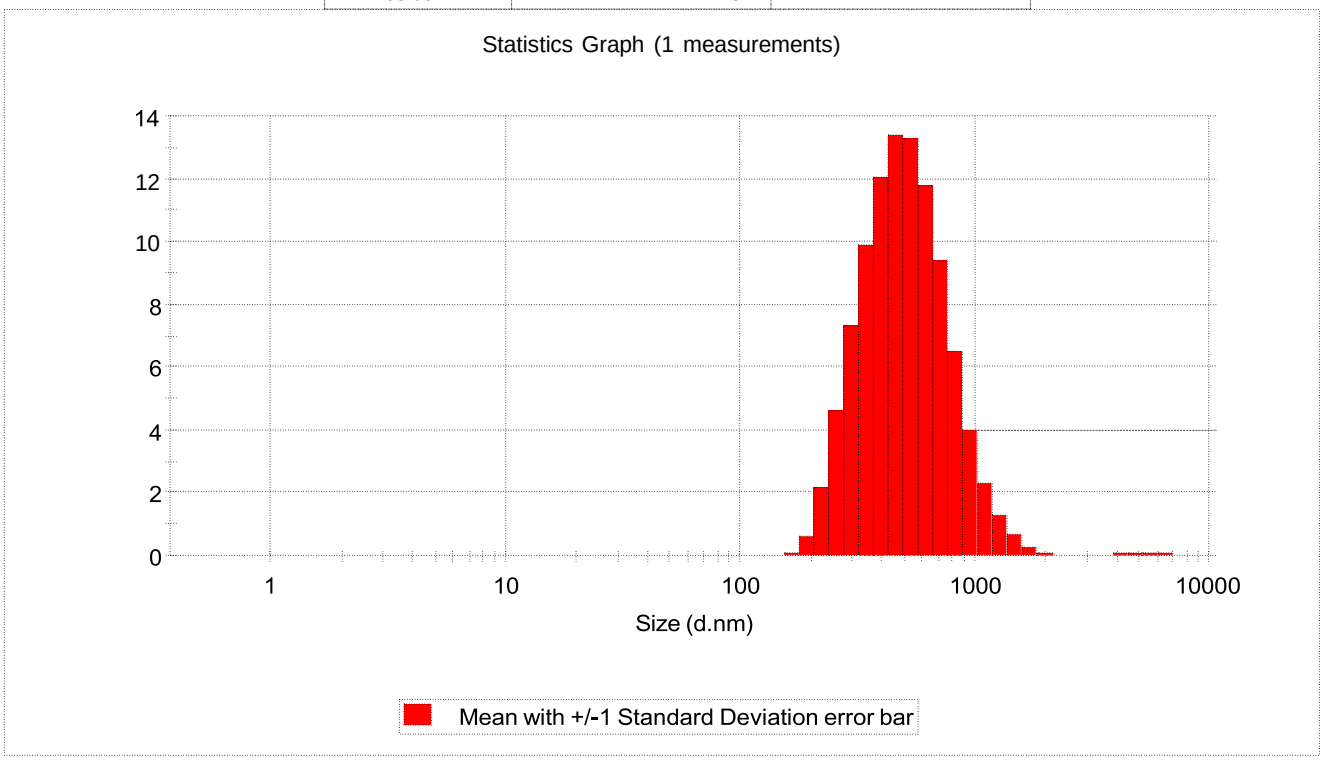
Sample Details

nple Name: File Name: SOP Name: Measurement Date and Time:	bs1 1 03.04.23 mansettings.nano Monday, April 03, 2023 12:38:20 AM		
Z-Average (nm):	873.8616	Derived Count Rate (kcps):	162122.144723352
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Number Percent	Std Dev Number Percent
0.4000	0.0	
0.4632	0.0	
0.5365	0.0	
0.6213	0.0	
0.7195	0.0	
0.8332	0.0	
0.9649	0.0	
1.117	0.0	
1.294	0.0	
1.499	0.0	
1.736	0.0	
2.010	0.0	
2.328	0.0	
2.696	0.0	
3.122	0.0	
3.615	0.0	
4.187	0.0	
4.849	0.0	
Size d.nm	Mean Number Percent	
5.615	0.0	
6.503	0.0	
7.531	0.0	
8.721	0.0	
10.10	0.0	
11.70	0.0	
13.54	0.0	
15.69	0.0	
18.17	0.0	
21.04	0.0	
24.36	0.0	
28.21	0.0	
32.67	0.0	
37.84	0.0	
43.82	0.0	
50.75	0.0	
58.77	0.0	
68.06	.0	



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Size Distribution Report by Number
v2.2

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	SOP Name:	mansettings.nano		
	General Notes:			
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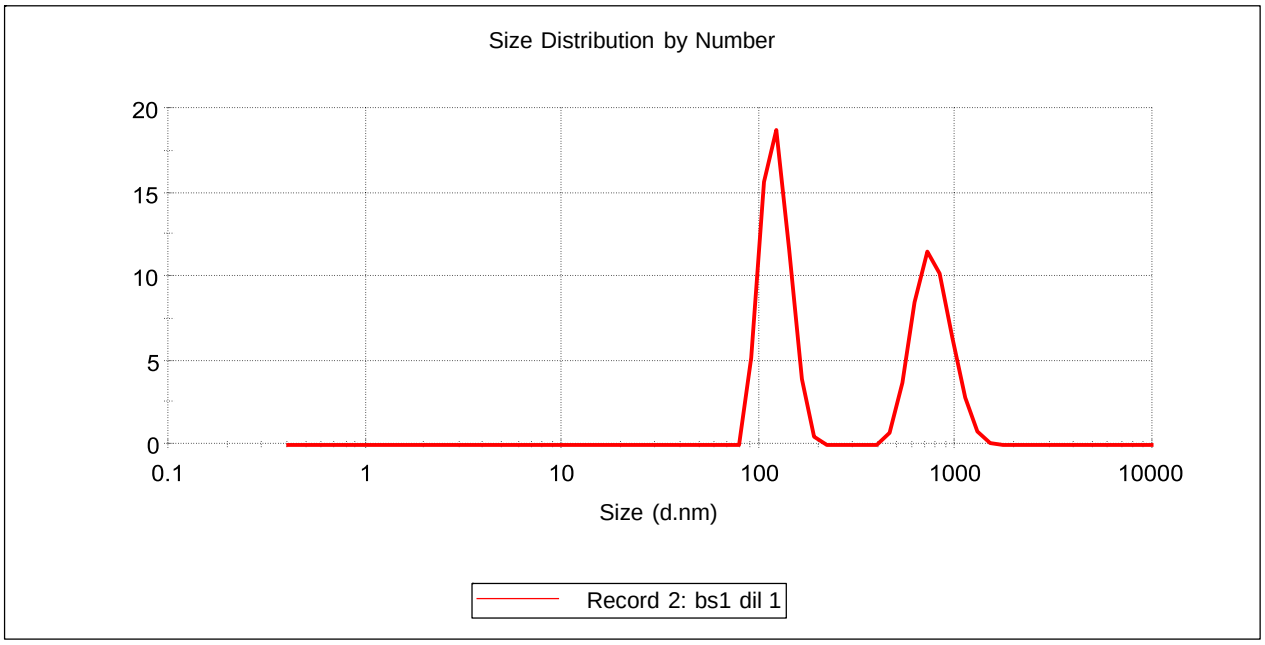
	Record Number:	2	Dispersant RI:	1.330
	Material RI:	0.20	Viscosity (cP):	0.8872
	Material Absorbtion:	3.320	Measurement Date and Time:	Monday, April 03, 2023 12:47:05 AM
System	Temperature (°C):	25.0	Duration Used (s):	50
	Count Rate (kcps):	104.0	Measurement Position (mm):	1.05
	Cell Description:	Disposable sizing cuvette	Attenuator:	5

Results						
				Size (d.nm):	% Number:	St Dev (d.nm):
	Z-Average (d.nm):	1530	Peak 1:	772.5	44.6	174.6
	PdI:	0.853	Peak 2:	122.4	55.4	20.26
	Intercept:	0.895	Peak 3:	0.000	0.0	0.000
	Result quality :	Refer to quality				

		report				



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Serial Number : MAL1022382
Record Number: 2

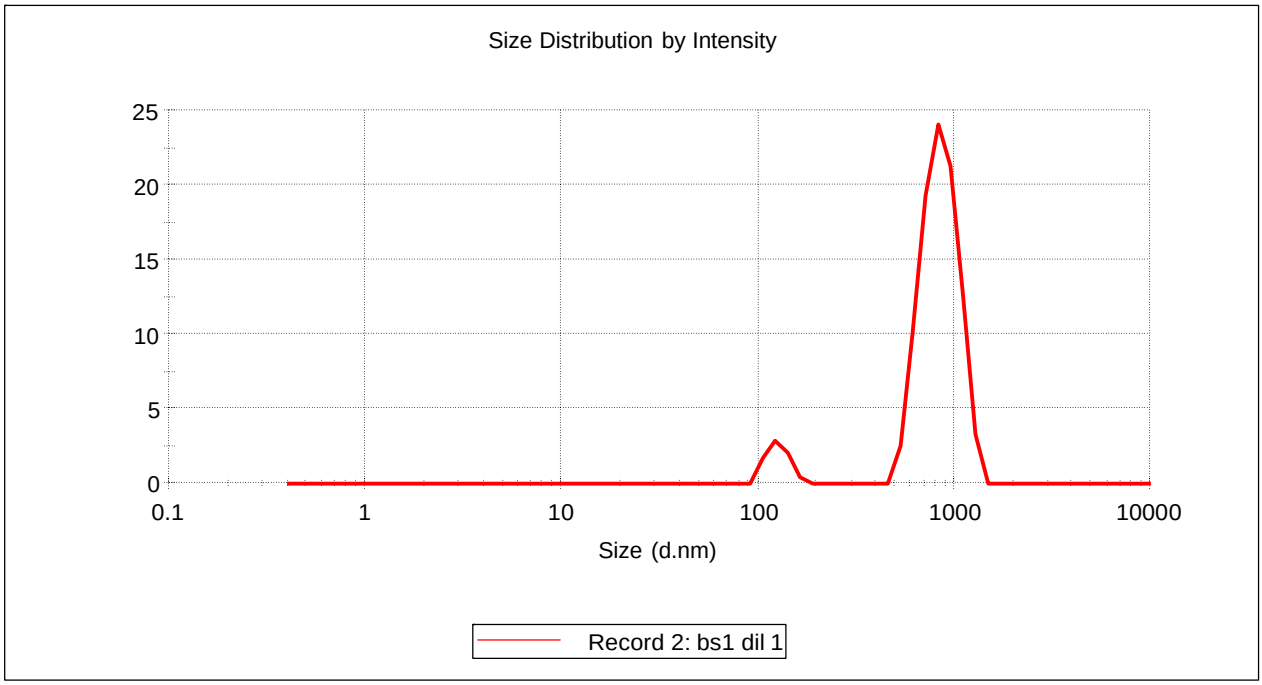
03 Apr 2023 12:57:28 AM

Size Distribution Report by Intensity
v2.2

Sample Details	Sample Name:	bs1 dil 1		
	SOP Name:	mansettings.nano		
	General Notes:			
	File Name:	03.04.23	Dispersant Name:	Water
	Record Number:	2	Dispersant RI:	1.330
	Material RI:	0.20	Viscosity (cP):	0.8872
	Material Absorbtion:	3.320	Measurement Date and Time:	Monday, April 03, 2023 12:47:05 AM
System	Temperature (°C):	25.0	Duration Used (s):	50
	Count Rate (kcps):	104.0	Measurement Position (mm):	1.05
	Cell Description:	Disposable sizing cuvette	Attenuator:	5
Results				

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 1530	Peak 1:	854.1	92.9	175.4
PdI: 0.853	Peak 2:	126.5	7.1	16.26
Intercept: 0.895	Peak 3:	0.000	0.0	0.000
Result quality : Refer to quality report				

Number (Percent)



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www.malvern.com
Serial Number : MAL1022382
Record Number: 2
03 Apr 2023 12:55:00 AM

Size Statistics Report by Number
v2.0

Sample Details

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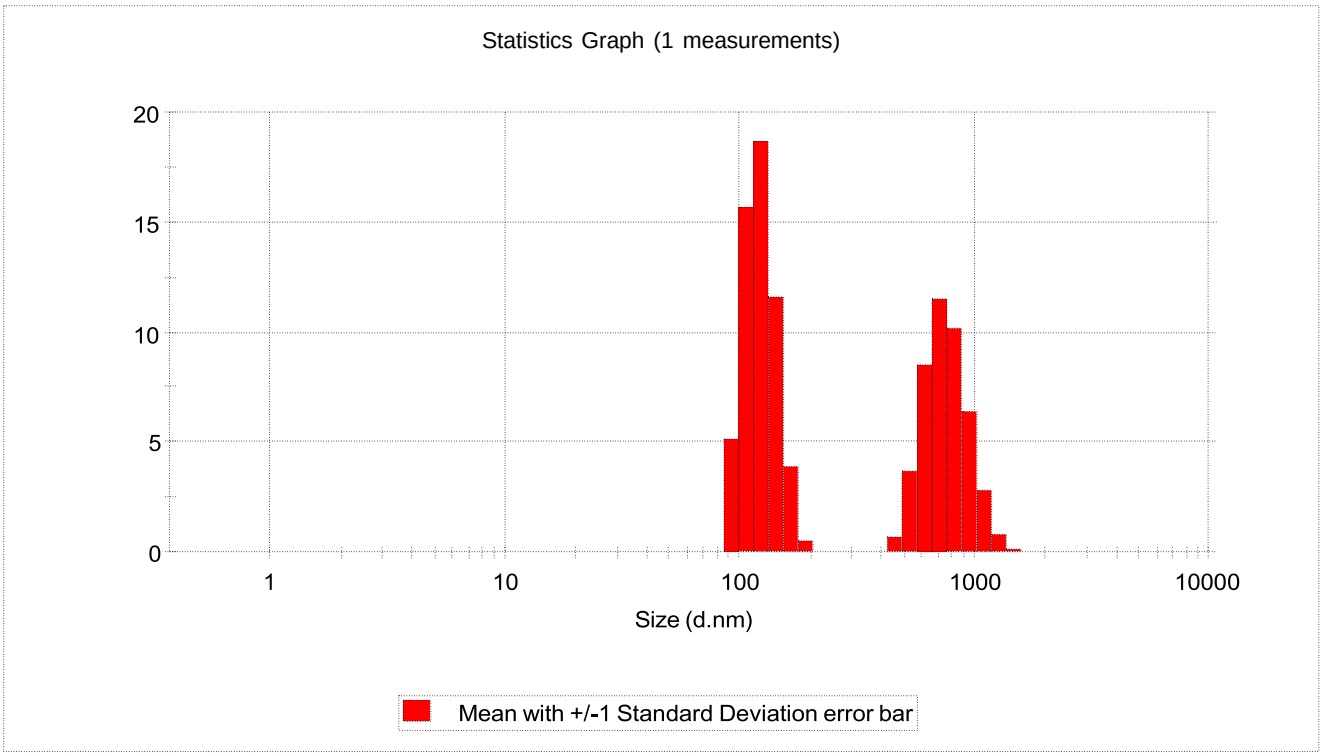
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Number Percent	Std Dev Number Percent
0.4000	0.0	
0.4632	0.0	
0.5365	0.0	
0.6213	0.0	
0.7195	0.0	
0.8332	0.0	
0.9649	0.0	
1.117	0.0	
1.294	0.0	
1.499	0.0	
1.736	0.0	
2.010	0.0	
2.328	0.0	
2.696	0.0	
3.122	0.0	
3.615	0.0	
4.187	0.0	
4.849	0.0	
Size d.nm	Mean Number Percent	Std Dev Number Percent
5.615	0.0	
6.503	0.0	
7.531	0.0	
8.721	0.0	
10.10	0.0	
11.70	0.0	
13.54	0.0	
15.69	0.0	
18.17	0.0	
21.04	0.0	
24.36	0.0	
28.21	0.0	
32.67	0.0	
37.84	0.0	
43.82	0.0	
50.75	0.0	
58.77	0.0	
68.06	0.0	



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EVALUATION STUDY

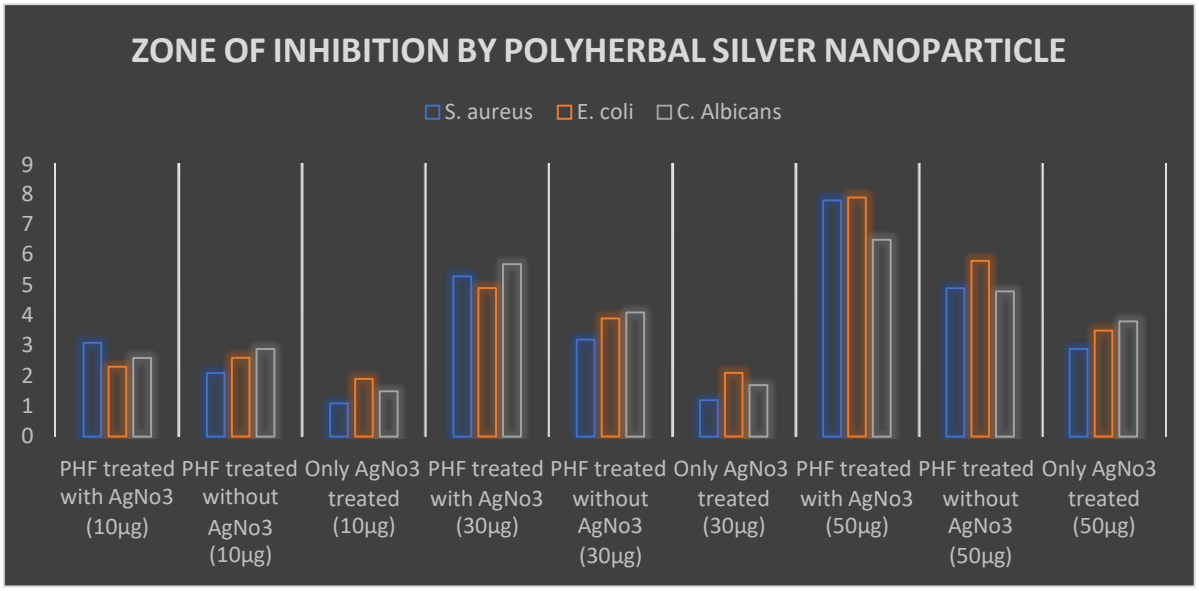
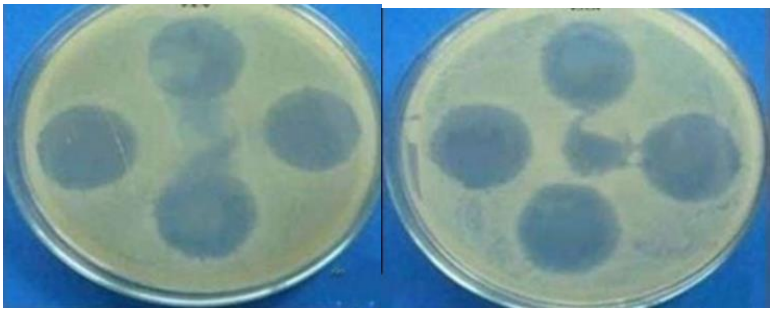


Chart : Anti-Microbial and Anti-Fungal Study of Poly-Herbal Nano-Particle

$AgNo_3$ Treated	Organism	Zone of Inhibition		
		10 µl	30 µl	50 µl
PHF treated with $AgNo_3$	S. aureus	3.1cm	5.3cm	7.8cm
	E. coli	2.3cm	4.9cm	7.9cm
	C. Albicans	2.6cm	5.7cm	6.5cm
PHF treated without $AgNo_3$	S. aureus	2.1cm	3.2cm	4.9cm
	E. coli	2.6cm	3.9cm	5.8cm
	C. Albicans	2.9cm	4.1cm	4.8cm
Only $AgNo_3$ treated	S. aureus	1.1cm	1.2cm	2.9cm
	E. coli	1.9cm	2.1cm	3.5cm
	C. Albicans	1.5cm	1.7cm	3.8cm

Table 5: zone of inhibition in different agar culture medial with different volume anddifferent combinations of samples.



CONCLUSION

From the above observation it can be concluded that, the bacterial growth is highest in samplecontaining both AgNo3 and herbal extract.

Gingerol is the Active constituent in Ginger, P-Coumaric Acid in Peas peel, Curcumin I, II, III is the active constituent in Turmeric are responsible for anti-microbial activity in Gram negative bacteria, antifungal activity fungus.

In all the sample both Gram-Negative and Gram-Positive type of bacteria are present in equalproportion.

Zone of inhibition of poly herbal silver nanoparticle is decreased serially followed by reduce in the concentration of poly herbal silver nano-particle.

If the volume is increased in the same concentration of poly herbal silver nanoparticle, the zone of inhibition is increased.

Therefore, to conclude, it is observed that herbal extract with AgNo3 has high potency to kill both the Gram-Positive and Gram-Negative bacteria, as well as it has potent antifungal activity.

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