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Development of Silver Nanoparticles/PEG/Glycerine Composite for Antibacterial Effect using Leaf Extract of *Ocimum sanctum* and *Ocimum basilicum*

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ABSTRACT

The main purpose of the experiment is to use green synthesis method for silver nanoparticles (SNP) fabrication using phytochemical and functional groups inherent in aqueous leaf extract of *Ocimum sanctum* and *Ocimum basilicum* for formulation of polyethylene glycol (PEG)/ Glycerine film. The SNP synthesis reaction is performed under sun condition and change in colour from light brown to dark brown was the initial indication, observed for nanoparticles synthesis. The 95 mL of 0.001 M $AgNO_3$ is mixed with 5 mL of leaf extract and reaction performed under Sun light at alkaline pH 8 was found efficient to produced stable NP. The synthesized SNP are mixed with (10%, 50%, 100%, 150%, 200% and 250%), polyethylene glycol (PEG):glycerine (G) in 1:1 ratio to form a film. The UV-spectroscopic analysis confirms absorption at 420-430 nm for synthesized SNP. The FTIR characterization determines alkynes (terminal), 1°, 2° amines, amides, nitriles, alkynes, alkyl halides functional group from *O. sanctum* (OS) leaf extract and aldehydes, alkynes (terminal), alkyne, alkene, from *O. basilicum* (OB) leaf extract responsible for reducing and capping silver nitrate to form nanoparticles. The SEM analysis verify that the *O. sanctum* based nanoparticles are spherical in shape although *O. basilicum* based nanoparticles have bright contrast coral reef like morphology. The average zeta potential of silver nanoparticles was found to be 27.74 mV and 23.50 mV that are embedded in *Ocimum sanctum*-SNP/PEG and *Ocimum basilicum*-SNP/PEG films. Also, the average diameters of SNP in *Ocimum sanctum*-SNP/PEG and in *Ocimum basilicum*-SNP/PEG was found to be 463.2 nm and 43.0 nm. These Sun light mediated SNP shows antimicrobial activity against *E. coli* and *S. aureous* pathogens.

1. Introduction

Ocimum is a genus of aromatic annual and perennial herbs, shrubs in the family Lamiaceae, native to the tropical and sub temperate part of globe, with the large number of species found in Africa and few species of Basil are native to India. The word *Ocimum* is derived from the Greek word "ozo" meaning smell and is called as "king of herbs" due to its immense use in traditional system of medicine, perfumery and pharmaceutical industry [1]. The diversity of *Ocimum* is geographical distributed in three main regions Tropical and subtropical regions of Africa, Tropical Asia and Tropical parts of America (Brazil) up to an altitude of about 1800 m from the mean sea level [1]. In India, so far about nine species of *Ocimum* have been reported including three exotic species namely *O. americanum* L., *O. minimum* L., and *O. africanum* Lour [1]. The *Ocimum sanctum* also called as *Ocimum tenuiflorum* (holybasilor tulsi) is native to the Indian subcontinent and cultivated for religious and traditional medicine purposes of its essential oil. Similarly, *Ocimum basilicum* (sweet basil) is economic plant grown in India, Iran, and in few warm regions of Africa [2].

The chemical composition of *Ocimum* species shows clear variation with respect to seasons. The major oil constituents from different *Ocimum* plant species include 1, 8-cineol, linalool, pinene, eugenol, camphor, methyl chavicol, ocimene, terpinene, and limonene [3]. *Ocimum* species is widely used in medicine for treating digestive disorders, such as stomach ache, diarrhoea, kidney complaints and infections, whooping cough and various types of fever. Moreover, they are use in curing or show antibacterial, ameliorative, antidiabetic, genoprotective, analgesic, antistress, hepatoprotective, memory booster, antipyretic, radioprotective, effect on testicular function, anticancer and anti-cataract effect. It is also effective in Alzheimer's disease, oral submucous fibrosis [4], anti-inflammatory and anti-nociceptive [5, 6].

The medicinal plant extracts are used in nanoscience for developments of formulation for varied application in textile, mobile phones, geo sensing technology, paper, food, fertilizers, pesticides and agrochemical industries. The silver (Ag) is the most compatible with biological system, living organisms and medicine which make ideal candidate to form silver nanoparticles AgNPs (<100 nm) for wound healing, burns and other medical purposes [7]. Recently, the plant mediated silver nanoparticles are complexed with potential, biodegradable polymers to enhance better releasing and effective antimicrobial activity. The one of the important polymer is polyethylene glycol (PEG) which is known as an osmotic-type laxative [8] referred as polyethylene oxide (PEO), that could be cross linked into networks, can hold high water content and forms hydrogels. PEG is a condensation polymer of ethylene oxide and water. Polyethylene glycols do not hydrolyze upon storage, inhibit growth of molds and beneficial for biological, chemical and pharmaceutical applications [9].

Therefore, in present study an attempt have been made to formulate silver nanoparticles using plant extract of *O. sanctum* and *O. basilicum* for film/composite development using blend of polyethylene glycols and glycerine as a polymer to give assistance for film formation and to enhance its releasing properties. The characterization and measurements are performed to depict the importance of formulated SNP/PEG/G composite in antibacterial application.

2. Experimental Methods

2.1 General Reagents and Solutions

The 250 mL conical flask, 100 mL measuring cylinder, mortar, pestle, cover slip, various organic solvents and doubled distilled water (Millipore Milli-Q ultrapure water system). The 1 mM sodium hydroxide (NaOH), hydrochloric acid (HCl), silver nitrate ($AgNO_3$), glycerine (G) polyethylene glycol (PEG) is purchased from Sigma-Aldrich. The plant samples of *O. sanctum* and *O. basilicum* have been collected from predefined places mentioned in Table 1 and their leaf extract were used for experimentation.

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Table 1 Display the picture, location and possible antibacterial compounds present in *Ocimum sanctum* and *Ocimum basillicum*

S. No.	Plant species	Location	Possible Antibacterial Compounds
1	*Oscimum sanctum*	Latitude N2⁰ 8' 57 Longitude E 78⁰ 59' 27	Monoterpenes, camphor, cineole, estragol,eugenol, α-elemene, bornyl acetate, α-, β-pinenes, sesquiterpenes, camphene, methyl eugenol germacrene, campesterol, caryophyllene, bisabolene, methyl chavicol and eucalyptol [10].
2	*Ocimum basillicum*	Latitude N2⁰ 8' 57 Longitude E 78⁰ 59' 27	Methyl eugenol,methyl chavicol, terpinolene, eugenol, cubenol, linalool, methyl cinnamate-rich [11], alkaloids, tannins, flavonoids, cholesterol, terpernoids, glycosides, phenols, cardiac glycosides, carbohydrates, and phlobatannins [12].

2.2 Instruments and Bacterial Strains

The UV spectrophotometry (Shimadzu Dual Beam spectrometer, ModelUV-1650 PC), Zeiss light microscope of Stemi DV4 (made in Germany), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopic (SEM), water bath, microwave oven and weighing balance were used in this experiment. Bacterial strains used in present antimicrobial study are *Escherichia coli* (ATCC no.11105) and *Staphylococcus aureus* (ATCC no. 6538).

2.3 Leaf Extracts Preparation

The 10 g leaf of *O. sanctum and O. basilicum* washed with tap water, dried at room temperature and macerated using mortar and pestlein 75 mL autoclaved double distilled water for five min. The extract was filtered using double coated cotton muslin cloth and the filtrate was set for centrifugation at 4000 rpm or 1792 relative centrifugal force (RCF) or G force for 10 min. The 65 mL of supernatant was stored in refrigerator for further fabrication of SNP.

2.4 Silver Nanoparticle Synthesis

The 95 mL of 0.001 M silver nitrate ($AgNO_3$) is mixed with the 5 mL leaf extract of *O. sanctum* and *O. basilicum* independently. The reaction was performed in Sun light (June 2018; temperature 35-45 °C) and after 5 min 0.0001 M, NaOH solution was added to mixture solution to maintain alkaline pH 8 (Fig. 1). The reaction mixture of silver nitrate and leaf extract was observed through visual change in colour [7].

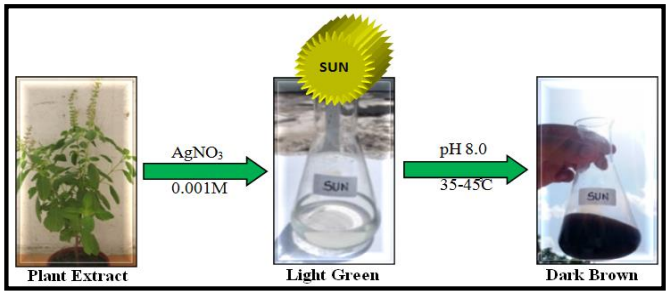

Fig. 1 A diagrammatic representation of silver nanoparticles synthesis, using leaf extract of *O. sanctum* under specific conditions

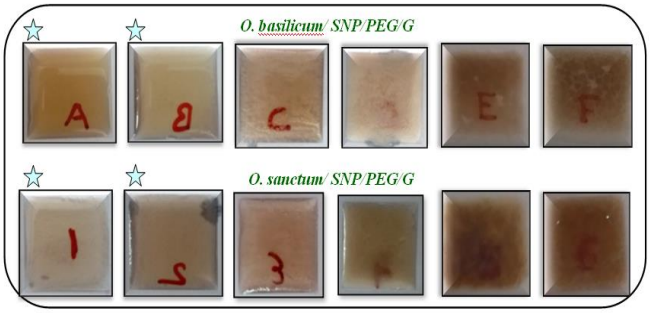

Figure 2. Shows the synthesized films of *O. basilicum/ SNP/PEG/G*, **A:** (100%PEG+100%G), **B:** (150%PEG+150%G), **C:** (200%PEG+200%G), **D:** (250%PEG+250%G), **E:** (10%PEG+10%G), **F:** (50%PEG+50%G), and *O. sanctum/SNP/PEG/G*, **1:** (100%PEG+100%G), **2:** (150%PEG+150%G), **3:** (200%PEG+200%G), **4:** (250%PEG+250%G), **5:** (10%PEG+10%G) and **6:** (50%PEG+50%G). **Note:** indicates best film in term of formation, texture, quality, transparency and dries at RT

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2.5 Preparation of SNP/PEG/G Biopolymer

The polyethylene glycol 10%, 50%, 100%, 150%, 200% and 250% mixed with 1 mL of synthesized SNP solution and glycerine was added (1:1) ratio with respect to PEG. The SNP/PEG/G biopolymer complexes solution was then kept in water bath at 70 °C for 30 min. Finally, the SNP/PEG/G biopolymer was set for microwave oven heat burst of 30 sec for five times. The pale yellow colour and homogenous solution of SNP/PEG/G biopolymer complexes are allowed to cool down and poured over cover slip (22 mm size) (Fig. 2).

2.6 Characterization of Silver Nanoparticles

The *O. sanctum* (OS) and *O. basilicum* (OB) leaf extract, *Ocimum sanctum*-nanoparticles (OS-SNP) and *Ocimum basilicum*-nanoparticles (OB-SNP) and film (OS-SNP/PEG/G), (OB-SNP/PEG/G) fetched for characterization, measurements and application activities.

2.7 UV-Visible Spectroscopy

The instrument was set for base line correction and auto zero using ddH_2O and 1 µL of extracts, SNP, films OS-SNP/PEG/G and OB-SNP/PEG/G were placed on the central top of the cuvette of nanophotometer for measuring UV-Vis spectra at a resolution of 200-800 nm.

2.8 FTIR Spectroscopy

The *O. sanctum* and *O. basilicum* leaf extract and their silver nanoparticles (OS-SNP) and (OB-SNP) were characterised using FTIR in 4000 to 450 cm^{-1} at a resolution of 4 cm^{-1}.

2.9 Solubility Test

The solubility test of the prepared films (OS-SNP/PEG/G, OB-SNP/PEG/G) has done by adding distilled water over film in petri dishes. The time to dissolve film, photographs and videos have recorded during the test. Furthermore, the individual solubility of PEG at RT and boiling at 70 °C for 20 min and glycerine at RT was done in various solvents to check solubility potential.

2.10 SEM Analysis

The OS-SNP, OB-SNP, OS-SNP/PEG and OB-SNP/PEG films without glycerine were used for scanning electron microscopic analysis using Carl Zeiss SEM-EVO18 with accelerated voltage of 130 KV. The morphology and size of silver nanoparticles and SNP/PEG-films were investigated using SEM specific software. The magnification was set at 4.85 KX for (OS), 9.83 KX (OB), 4.00 KX for OS/SNP/PEG/G and 3.39 KX for OB/SNP/PEG/G.

2.11 DLS Size and Zeta Potential Analysis

The synthesis OS-SNP/PEG and OB-SNP/PEG films were fetched for determination of size analysis and to identify zeta potential surface charge over nanoparticles in films. The nanoparticles are subjected to Brownian motions due to thermal energy that produces Rayleigh scattering. The size of nanoparticles in film are determined by using Particle Sizing Systems Inc. Santa Barbara, Calif., USA at CICR-CIRCOT Mumbai, India and the Number-Weighted Gaussian distribution analysis (solid particle), Intensity-wt, volume-wt NICOMP distribution and zeta potential in (mV) were calculated.

2.12 Antimicrobial Test

Antimicrobial properties of *O. sanctum* and *O. basilicum* leaf extract, OS-SNP and OB-SNP, OS-SNP/PEG/G and OB-SNP/PEG/G film against *Escherichia coli* and *Staphylococcus aureus* is done as per the protocol followed by [10]. The extract, SNP and their films are placed on culture of *E. coli* and *S. aureus* spreaded agar plates. The inhibition of bacteria has visualized after 24 h of incubation at 37 °C and the zone of inhibition was measured.

3. Results and Discussion

3.1 Visual Conformation

The change in colour from light brown to dark brown colour was visualised by naked eyes and addition of 1 mM NaOH to the reaction mixture to maintain pH 8 make the solutions' colour more dark (Fig. 1).

3.2 UV-Visible Analysis

The UV-visible analysis has been done for leaf extract of *O. sanctum* and *O. basilicum*, their silver nanoparticles (OS-SNP and OB-SNP) and films OS-

SNP/PEG/G and OB-SNP/PEG/G (Fig. 3). The synthesized SNP exhibited a well-defined absorption peak at 430 nm for *O. basilicum* and 420 nm for *O. sanctum*.

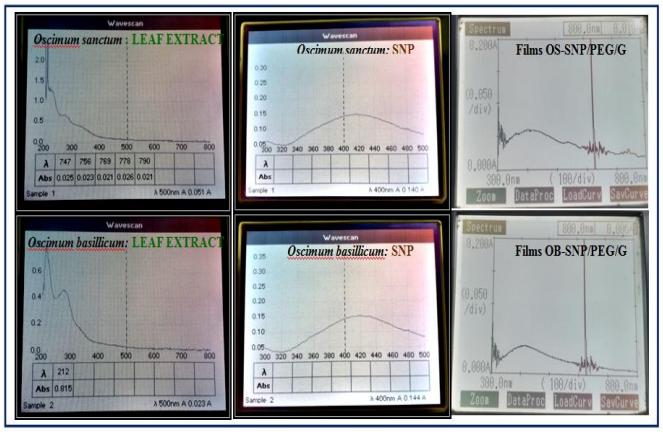

Fig.3 UV spectrophotometer analysis of leaf extract of *Oscimum sanctum, Oscimum basillicum,* SNP and films OS-SNP/PEG/G, OB-SNP/PEG/G formed by utilizing the leaf extract of *O. sanctum and O. basillicum* as capping agent

3.3 Nanoparticles Concentration

The concentration parameters in nanophotometer was set as wavelength 420 nm, units picomole/µL, pathlength 10 mm and factor 50.0. The concentration parameters of *O. sanctum*-NP at wavelength 410 nm were found to be 7.0 picomole/µL and absorbance 0.140 Å. Likewise, the concentration parameter of *O. basillicum*-NP at wavelength 420 nm was found to be 7.500 picomole/µL with absorbance 0.150 Å.

3.4 FTIR Analysis

The FTIR was used to identify the potential functional group, chemical compounds and biomolecules present in the leaf extract of *O. sanctum* and *O. basilicum* that are involved in capping and reducing Ag^+ ions to Ag^0. The Fig. 4 and Table 2 shows the FTIR spectra of leaf extract of O. sanctum and *O. basilicum* and their SNPs.

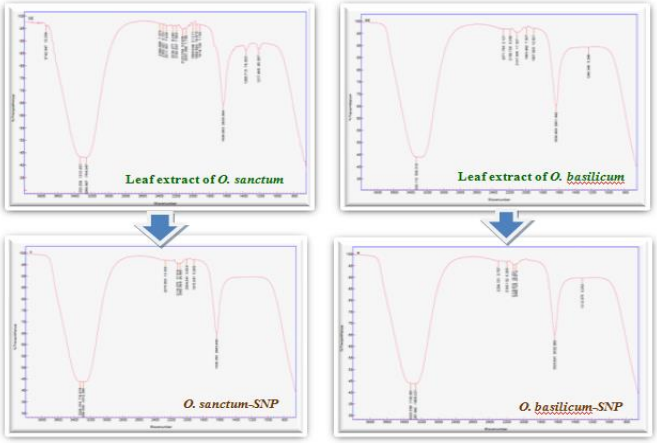

Fig. 4 FTIR spectrum of *O. sanctum, O. basillicum* leaf extract, *O. basillicum*-SNP and *O. sanctum*-SNP

Table 2 Functional group detected on the basis of FTIR spectra for *O. sanctum* (OS) *and O. basilicum* (OB) leaf extract, OS-SNP and OB-SNP with its absorption range, absorption frequency strength and characteristic bond

Peaks	Absorption	Characteristic bond	Functional group	Peaks	Absorption	Characteristic bond	Functional group
Ocimum sanctum (OS) leaf extract				*Ocimum sanctum*-Nanoparticles (OS-SNP)			
1	3333.204 1310.461	–C≡C–H: C–H stretch	alkynes (terminal)	1	3328.169 716.474	–C≡C–H: C–H stretch	alkynes (terminal)
2	3260.907 -784.047	N–H stretch	1°, 2° amines, amides	2	3289.560 -3012.368	–C≡C–H: C–H stretch	alkynes (terminal)
3	2236.277 8.240	C≡N stretch	Nitriles	3	2270.803 12.333	H–C=O: C–H stretch	aldehydes
4	2193.567 7.590	–C≡C– stretch	Alkynes	4	2118.075 17.519	–C≡C– stretch	alkynes
5	2120.254 32.908	–C≡C– stretch	Alkynes	5	1636.350 2845.490	–C=C– stretch	alkenes
6	1638.683 2903.064	N–H bend	1° amines	-	-	-	-
7	1369.715 74.300	C–H rock	Alkanes	-	-	-	-
8	1217.445 95.397	C–H wag (–CH2X)	alkyl halides	-	-	-	-
Ocimumbasilicum (OB) leaf extract				*Ocimumbasilicum*-Nanoparticles (OB-SNP)			
1	3328.169 716.474	–C≡C–H: C–H stretch	alkynes (terminal)	1	3323.586 1182.891	O–H stretch, H–bonded	alcohols, phenols
2	3289.560 -3012.368	–C≡C–H: C–H stretch	alkynes (terminal)	2	3267.842 -1906.321	N–H stretch	1°, 2° amines, amides
3	2270.803 12.333	H–C=O: C–H stretch	aldehydes	3	2119.810 16.221	C≡N stretch	nitriles
4	2118.075 17.519	–C≡C– stretch	Alkynes	4	1635.991 3032.993	N–H bend	1° amines
5	1636.350 2845.490	–C=C– stretch	Alkenes	5	1312.075 3.253	C–O stretch	alcohols, ethers carboxylic acids, esters,

3.5 Light Microscopic Analysis

The best formulated films using *O. sanctum* and *O. basilicum* are observed under light microscope in 10X focus for observation of SNP in PEG/G composite (Fig. 5).

3.6 Solubility Check

The OS-SNP/PEG/G and OB-SNP/PEG/G films were used to check solubility in double distilled water (Fig. 6). From the tested samples of concentration 10%, 50%, 100%, 150%, 200% and 250% glycerine and PEG (1:1) ratio, the sample B and 2 (150%) have optimized for giving best texture, film forming capability and solubility in water. Besides, the solubility of glycerine has performed in different solvents. However, various concentrations of PEG were used to check the solubility potential and dissolve time at room temperature (RT), at 70 °C (Table 3).

3.7 Antimicrobial Potential of SNP/ PEG/G Film

Antimicrobial potential of OS-SNP/PEG/G and OB-SNP/PEG/G film in (150%PEG+150%G) concentration were tested against *Escherichia coli* and *Staphylococcus aureus* lawn (Fig. 7). A small inhibition could be seen in *O. sanctum/SNP/PEG/G* (150%PEG+150%G) films however it is not prominent. The other combination of SNP and (PEG+G) shows no inhibition.

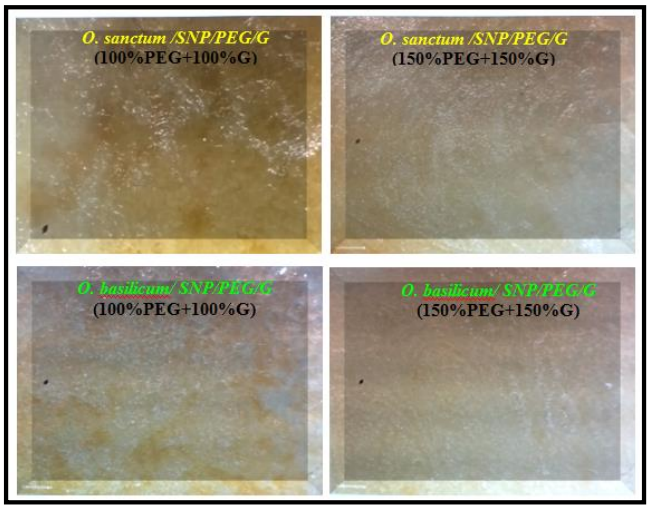

Fig. 5 Represents the light microscopic structure of SNP/films samples *of O. basilicum* and *O. sanctum* synthesized using different concentration of PEG and glycerine (100%PEG+100%G; 150%PEG+150%G)

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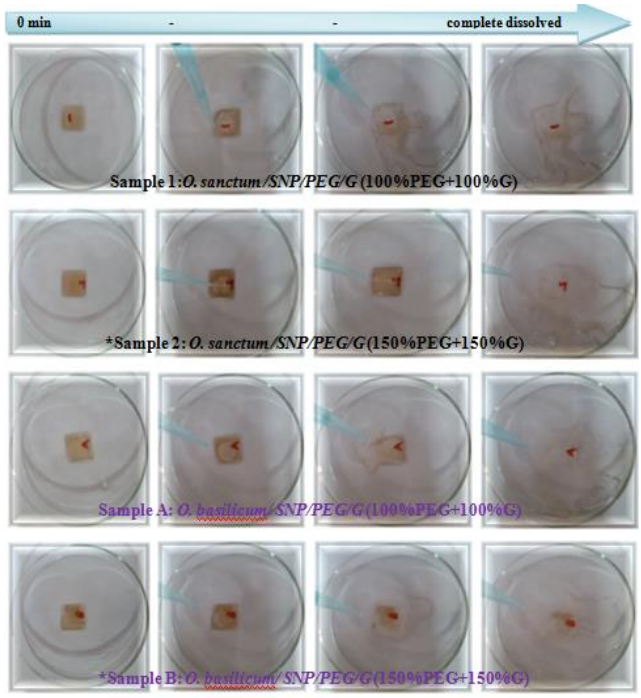

Fig. 6 Solubility behaviour of *O. basilicum/SNP/PEG/G* and *O. sanctum /SNP/PEG/G* succeeding very few seconds of water exposure

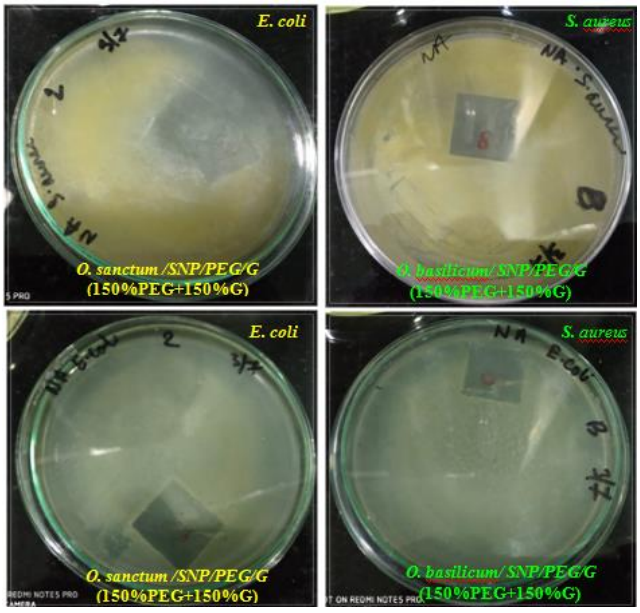

Fig. 7 Antimicrobial potential of OB-SNP/PEG/G and OS-SNP/PEG/G composite formed by (150%PEG+150%G) concentration against *E. coli* and *S. aureus*

Table 3 Solubility potential of glycerine and PEG at room temperature (RT)

S.No	Solvent system	Samples	Cond.	Dissolved time
1	Water*	Glycerine	RT	2 sec*
2	Methanol	Glycerine	RT	4 sec
3	Ethanol*	Glycerine	RT	1 sec*
4	Chloroform	Glycerine	RT	immiscible
5	Toluene	Glycerine	RT	Immiscible (bubbles at bottom)
6	Ethyl acetate	Glycerine	RT	immiscible
7	Glacial acetic acid	Glycerine	RT	10 sec
8	Formic acid	Glycerine	RT	2 sec
9	Butyl alcohol	Glycerine	RT	6 sec
10	Benzene	Glycerine	RT	Immiscible (bubbles at bottom)
11	Petroleum ether	Glycerine	RT	Immiscible
12	Water*	PEG	RT	23 sec*
13	Methanol	PEG	RT	2 min 30 sec
14	Ethanol	PEG	RT	2 min
15	Chloroform	PEG	RT	20 sec (white ppt)
16	Toluene	PEG	RT	Immiscible
17	Ethyl acetate	PEG	RT	Immiscible
18	Glacial acetic acid	PEG	RT	2 min 30 sec
19	Formic acid	PEG	RT	30 sec
20	Butyl alcohol	PEG	RT	Immiscible
21	Benzene	PEG	RT	50 sec
22	Petroleum ether	PEG	RT	Immiscible
23	-	10% PEG	RT	1 min
24	-	25% PEG	RT	3 min
25	-	50% PEG	RT	8 min
26	-	100% PEG	RT	26 min
27	-	10% PEG	Boiled at 70 °C	20 sec
28	-	25% PEG	Boiled at 70 °C	35 sec
29	-	50% PEG	Boiled at 70 °C	45 sec
30	-	100% PEG	Boiled at 70 °C	1 min 40 sec

*Note: *represent quick solubility*

3.8 SEM Analysis

The SEM analysis of OS-SNP, OB-SNP and the films that are synthesized by leaf extracts of *Ocimum* species labelled OS/SNP/PEG and OB/SNP/PEG have done. The Fig. 8 shows the SEM analysis of the SNP and film, yet the film or the composite were devoid of glycerine since the dry sample is required for SEM characterization, thus glycerine was not added in film for investigation of SEM characters. The shape of OS-SNP is spherical, OB-SNP is like bright contrast coral reef, OS/SNP/PEG composite shape is kind of rosette shape and OB/SNP/PEG is somewhat irregular in shape.

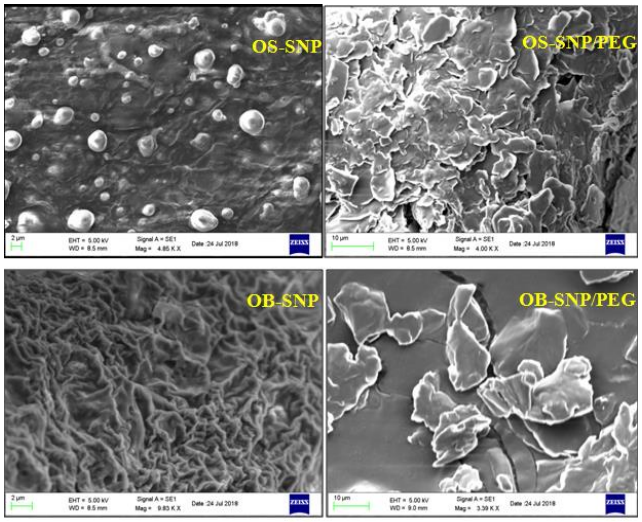

Fig. 8 SEM images of Ocimum sanctum-SNP, Ocimumbasilicum-SNP, Ocimum sanctum-SNP/PEG and Ocimum basilicum-SNP/PEG

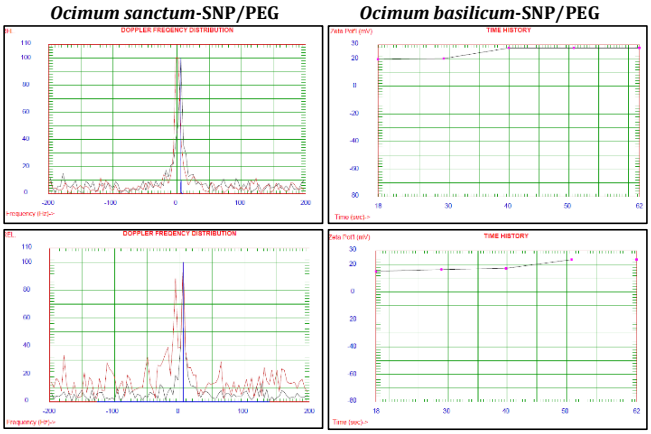

Fig. 9 Zeta potential of silver nanoparticles in Ocimum sanctum-SNP/PEG and *Ocimum basilicum*-SNP/PEG

3.9 DLS Size and Zeta Potential Analysis

The average zeta potential was found to be 27.74 mV and 23.50 mV for with average mobility is 2.07 M.U. and 1.75 M.U for silver nanoparticles integrated in *Ocimum sanctum*-SNP/PEG and *Ocimum basilicum*-SNP/PEG film respectively. The doppler frequency distribution to identify zeta potential and time history for 1 min are represented in Fig. 9. The size and intensities of silver nanoparticles in composite *Ocimum sanctum*-SNP/PEG and *Ocimum basilicum*-SNP/PEG is shown in Fig. 10.

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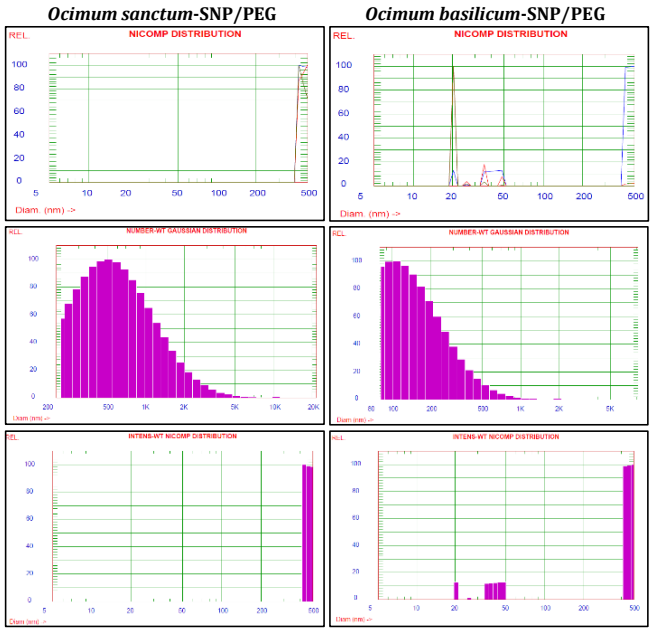

Fig. 10 Dynamic light scattering (DLS) characterization of silver nanoparticles in form of size, distribution and intensities in Ocimum sanctum-SNP/PEG and Ocimum basilicum-SNP/PEG film

In present study, the silver nanoparticles from the leaf extract of *Ocimum sanctum* (OS) and *Ocimum basilicum* (OB) have synthesized successfully and exhibited absorption peak at 420 nm for *O. basilicum* while 410 nm for *O. sanctum*. Similarly, the research of Malapermal [13] shows the absorption peak at 438 nm from *O. basilicum* and 439 nm for *O. sanctum*. The difference in SNP absorption peak may be due to the involvement of different functional group and the method adopted for leaf extract preparation. The films containing nanoparticles such as OS-SNP/PEG/G, OB-SNP/PEG/G have been evaluated for presence of silver nanoparticles using UV spectroscopic analysis in range 300 nm – 800 nm and a sharp peak around 400 – 4500 nm confirms silver nanoparticles incorporated in polymers, besides it was observed that the nanoparticles were stable for 45 days (Fig. 3). The wider range of chemical groups present in aqueous extract of *Ocimum sanctum* are alkynes (terminal), 1°, 2° amines, amides, nitriles, alkynes, alkanes and alkyl halides. Similarly, alkynes (terminal), aldehydes, alkynes and alkenes are found in *Ocimum basilicum* are mainly responsible for reduction of Ag^+ ions to zero valent Ag^0.

The FTIR spectra of the crude aqueous extract and SNPs derived from *O. sanctum* showed a decrease in stretching frequency from 3333.204 to 3328.169 cm^{-1} suggesting an alkynes functional group. In the same way, Malapermal [13] suggested the range of 3250-3400 cm^{-1} for amide region. Furthermore a shift from 1638.683 to 1636.350 cm^{-1} is assigned to the N–H bend and alkenes, whereas peaks at 1217.445, 95.397 cm^{-1} can be attributed to alkyl halides. The other groups that found are 1°, 2° amines, amides, nitriles, and alkyl halides. In contrast, *O. basilicum* consist of aldehydes, which shift from 2270.803 to 2119.810cm^{-1}. Overall, the FTIR analysis predicts that SNP have formed using *O. sanctum* and *O. basilicum* extracts confirming the involvement of –C≡C–H: C–H stretch and H–C=O: C–H stretch respectively (Table 2).

The nature of *Oscimum sanctum-NP* and *Oscimum basillicum-NP* are colloidal and are capped using respective phyto chemical present in extract of *Oscimum species*. The reference was set to zero using both plant extract as well as water, they have generated the concentration of *Oscimum sanctum-NP, Oscimum basillicum-NP* using nanophotometer as 0.900 picomole/µL and 3.500 picomole/µL respectively.

The film of composite of *O. sanctum-NP* and *O. basillicum-NP* was formulated which was used to depict the solubility of in water. Beside, individual PEG and glycerine was also used to check the solubility in different solvents (Table 3). The polymer PEG is dissolved in water by two methods: room temperature and boiling at 70 °C for 20 min. It was observed that the boiling of PEG makes it to dissolve quickly in water when compare with room temperature method. The 10% PEG dissolved in 1 min at room temperature whereas; at 70 °C it was dissolved in 20 sec. Also, 100% PEG in room temperature requires 26 min, however on boiling at 70 °C takes less time of 1 min 40 sec. The boiling process for film formation was found more efficient than room temperature method. There are various papers on silver nanoparticles synthesis using PEG with combination of other polymer such as glucose [14] however none is there with glycerine.

The texture and solubility of synthesized films of *O. basilicum/ SNP/PEG/G* and *O. sanctum/SNP/PEG/G* with different concentration of PEG and G are discussed. The film texture of sample A, B, 1, 2 are observed as to be good, transparent and dries at RT in more time as compare to C, D,E, F, 3, 4, 5 and 6 that shows crystals formation and dried quickly. The *O. basilicum/ SNP/PEG/G* (A, B) and *O. sanctum /SNP/PEG/G* (1, 2) dissolved and mixed in 15 min at 70 °C whereas, sample *O. basilicum/ SNP/PEG/G* (C, D) and *O. sanctum* /SNP/PEG/G (3,4) were dissolved and mixed in 30 min at 70 °C. The samples 1, 2 and A, B forms films effectively, from these sample 2 and B (150%PEG+150%G) are finalized. However, samples D and 4 are inefficient to form films. In sample C and 3 crystals formation was observed during film formation. In comparison, D and 4 sample films are not formed properly and large amounts of crystals formed during air drying.

The solubility of polymer and release of silver nanoparticles from composite was studied by applying distilled water over film and evaluating the time and quantity of water requires for dissolving it. The *O. basilicum/SNP/PEG/G* samples A: (100%PEG+100%G), B: (150%PEG+150%G) are soluble in 1 mL and 2 mL of water that requires about 1 min 2 sec and 2 min 6 sec to get dissolved respectively. In contrast, the samples E: (10%PEG+10%G), F: (50%PEG+50%G) are soluble in 5 mL and 10 mL of water that requires about 1 min 30 sec and 2 min 10 sec to get dissolved respectively. Similarly the *O. sanctum/SNP/PEG/G* samples1: (100%PEG+100%G), 2: (150%PEG+150%G) are soluble in 2 mL and 3 mL of water that requires about 1 min 8 sec and 1 min to get dissolved respectively. However, the sample 5: (10%PEG+10%G), 6: (50%PEG+50%G) are soluble in 6 mL and 12 mL of water that requires about 1 min 30 sec and 2 min 50 sec to dissolve respectively. The film formed with the PEG and G (100%:100% and 150%:150%) in 1:1 ratio were dissolved quickly as compare to other concentration combinations. The light microscopic structure of films for sample A, B, 1 and 2 are shown in Fig. 5. It can be observed that in sample 2 and B, there is uniform distribution of silver nanoparticles in PEG and glycerine (1:1) film of 150% concentration and it allows slow liquefy of polymer and efficient release of silver nanoparticles, furthermore the texture and dissolved ability is very good as compare to samples 1 and A. The SEM analysis confirms the spherical shape of OS/SNP and bright contrast coral reef morphology for OB/SNP at 2 µm scale similarly, OS/SNP/PEG and OB/SNP/PEG composite show rosette and irregular shape morphology respectively in 10 µm scale.

The zeta potential of silver nanoparticles integrated in *Ocimum sanctum*-SNP/PEG were calculated in range of 5.20 to 6.48 Freq. Shift(Hz) and found to be 24.62 (mV) to 30.66 (mV). In same fashion the zeta potential of silver nanoparticles in *Ocimum basilicum*-SNP/PEG film were -27.61 to 36.72 Freq. Shift(Hz) and found to be -115.99 and 154.27 (mV). The silver nanoparticles mean diameter (nm) in term of intensity weighting, volume weighting and number weighting in composite *Ocimum sanctum*-SNP/PEG was found to be 463.2, 465.1 and 459.5 nm. Beside cumulative result based on number-weighted Gaussian distribution confirms the presence of SNP show that the SNP in same composite have 25% of distribution < 402.8 nm, 50% of distribution < 606.6 nm, 75% of distribution < 972.9 nm, 90% of distribution < 1527.9 nm, 99% of distribution < 3409.4 nm and 80% of distribution < 1099.7 nm. However, mean diameter (nm) in term of intensity weighting and volume weighting in composite *Ocimum basilicum*-SNP/PEG was found to be 43.0 and 465.4 nm. Additionally, the cumulative result based on number-weighted Gaussian distribution confirms the presence of SNP, 25% of distribution < 122.8 nm, 50% of distribution < 163.9 nm, 75% of distribution < 239.6 nm, 90% of distribution < 354.2 nm, 99% of distribution < 735.0 nm and 80% of distribution < 265.9 nm. Therefore the composite of SNP of average diameters 463.2 nm in *Ocimum sanctum*-SNP/PEG and 43.0 nm in *Ocimum basilicum*-SNP/PEG could inhibit pathogens. However we could not observed such activity that may be due to some unknown reasons which is still unclear. Pinzaru [15] used PEG-coated SNPs for in vitro and in vivo studies in mice for a toxicological studies.

4. Conclusion

In conclusion, this study shows that the aqueous soluble compounds presents in leaf extract of *O. sanctum* and *O. basilicum* are efficient for fabrication of silver nanoparticles, in addition biopolymer composite formulation is possible using PEG and glycerine. The SNP are synthesized in presence of sun light, at alkaline pH 8 and change in solution from light green to dark brown was observed. The characterization done successfully and UV- spectroscopic analysis confirms at 410 nm to 420 nm for *O. sanctum/SNP, O. basilicum/SNP* and a sharp peak around 400-4500 nm confirms silver nanoparticles incorporated in polymers composite OS-SNP/PEG/G, OB-SNP/PEG/G. The FTIR determines the involvement of alkanes, alkyne, amines, amides, nitriles and alkyl halides in aqueous

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extract of *O. sanctum* and alkynes, aldehydes and alkenes in *O. basilicum* as sole stabilizing agents for silver ions. The optimized film *O. sanctum /SNP/PEG/G* and *O. sanctum /SNP/PEG/G*(150%PEG+150%G) are best in texture, film forming ability, quickly dissolve in water and slow release of SNP. The SEM characterization confirms the spherical shape and bright contrast coral reef for OS-SNP and OB-SNP respectively; although the SNP incorporated film of *O. sanctum* OS/SNP/PEG has rosette shape while *O. basilicum* based OB/SNP/PEG film is irregular in shape. The average zeta potential was found to be 27.74 mV and 23.50 mV for with average mobility is 2.07 MU and 1.75 MU for silver nanoparticles integrated in *Ocimum sanctum-SNP/PEG* and *Ocimum basilicum-SNP/PEGfilm* respectively. The *Ocimum basilicum*-SNP/PEG have much stable and SNP below 100 nm ie., 43.0 nm as compare to *Ocimum sanctum*-SNP/PEG which have SNP of average diameters 463.2 nm and could demonstrate study for its antimicrobial potential against *E. coli* and *S. aureous* pathogens which could be possible for development of products helpful in medical application.

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