

Research Article

Antibacterial Sodium Alginate Cosmetic Herbal Electrospun Nanofiber Mask

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Abstract | Sodium Alginate (SA) and polyvinyl alcohol (PVA) have earned industrial recognition due to its biocompatible nature. Traditionally, Musa Acuminata (MA) and Hibiscus Rosa Sinensis (HRS) leaves famous for its medicinal value as they contain phytochemicals such as triterpenoids, polyphenols, flavonoids, and alkaloids. Keeping in view and get benefit from their fruitful properties the aim of the study was to develop antibacterial dry sheet nanofiber mask loaded with leaf extracts of MA and HRS as antibacterial agents in SA/PVA nanofibers through electrospinning method, which was just wet before its application. Leaf extracts were used at three concentration levels (0.5, 1, and 1.5 %). Scanning Electron Microscope (SEM) images confirmed the formation of optimized nanofibers with diameter range from ± 49 to ± 545 nm. It was observed that addition of leaf extract increased the solution conductivity and decrease the surface tension and viscosity. Chemical reactions and presence of functional groups of SA, PVA, HRS and MA were studied by Fourier Transform Infrared Spectroscopy (FTIR). Crystallinity decreased as the SA and leaf extract loaded into the PVA nanofibers as studied through X-ray diffraction (XRD). SAMA nanofibers exhibited highest antibacterial properties against Staphylococcus aureus (gram positive bacteria) than *Escherichia coli* (gram negative bacteria), while SAHRS nanofibers showed moderate value against both bacteria. It was concluded that prepared antibacterial nanofiber cosmetic mask has an excellent application in medical cosmetic industry.

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Keywords | Electrospinning, Facial mask, Sodium alginate, Musa acuminata, Hibiscus rosa Sinesis



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1. Introduction

Various companies use different emblems for marketing their cosmetic products. These are

incorporation of antibacterial and more importantly ecofriendly agents in cosmetic products etc. Admiration from others and looking beautiful are important factors that builds self-confidence,

satisfaction ultimately increasing demand for high quality cosmetic products. Concerned with development, cosmetic industry is also producing high quality products with enhanced active ingredients for example nanoliposomes, nano pigments, nanoparticles with natural substances like vitamins and plant extracts (Taepaiboon *et al.*, 2007). This nanoscale size of the materials highlights the thermal, mechanical, electrical, structural, kinetical and thermo-dynamical properties (Agarwal *et al.*, 2016). The word nanofiber refers to the uniform, elongated and threadlike three-dimensional structures; length of it ranging from 1-500 nano meters in relation to its diameters. Nanofibers can be developed through a simple process called electrospinning (Bhardwaj and Kundu, 2010). More uniform nanofibers can be produced by optimizing the parameters such as viscosity, surface tension and conductivity, Voltage, flowrate, and distance from syringe needle to collector plate (Chaturvedi *et al.*, 2019; Haider *et al.*, 2018; Deitzel *et al.*, 2001). Electrospun polymer nanofibers have widely used in medical field such as in wound healing (Summa *et al.*, 2018; Kandhasamy *et al.*, 2017; Liakos *et al.*, 2014) and tissue engineering scaffolds (Ma *et al.*, 2012; Vasita and Katti, 2006). Polymers can be categorized into natural and synthetic polymers. Natural polymers possess properties of biodegradability and biocompatibility making it useful applicable in medical field (Kai *et al.*, 2015). Among all, Sodium alginate gets its importance because of its ecofriendly nature, biocompatible, nontoxic, and hydrophilic water-soluble natural polymer derived from marine brown algae. It can be stored for several months without any degradation at low temperature; it is less degradable in salt form than alginic acid form (Aprilliza, 2017). It is widely used in biomedical fields such as wound healing (Chaturvedi *et al.*, 2019; Summa *et al.*, 2018; Liakos *et al.*, 2014), nanofiber sponges (Singla *et al.*, 2019), tissue engineering scaffolds (Ma *et al.*, 2012). However, it is soluble in water but its solution viscosity and surface tension does not allow it to electro spin easily (Hu *et al.*, 2015). Polyvinyl alcohol (PVA), is well-known co-polymer due to its nontoxic, biocompatible, noncarcinogenic, more importantly easy to spinnable characteristics (Jiang *et al.*, 2011) which making it useful in developing nanofibers through electrospinning. Presence of hydroxyl group in PVA showed strong inter and intra hydrogen bonding, which confirmed the strong interaction of

SA and PVA. Both polymers SA and PVA are soluble in water so they formed homogeneous solution (Rafiq *et al.*, 2018).

Plants extracts are famous for usage in medical field like for treating wounds as well as in cosmetic products for treating skin problems such as acne, eczema, allergies and used as beauty enhancer. They contain phytochemicals like triterpenoids, polyphenols, flavonoids, and alkaloids possess antioxidant, antibacterial and anti-inflammatory properties. Bioactive plant leaf extracts such as Hibiscus rosa sinensis (HRS) is a flowering plant belong to Malvaceae family, is a versatile shrub, its leaf contained phytochemicals such as phenols, flavonoids, terpenoids, saponins and amino acid (Al-Snafi, 2018). It found useful application in pharmaceuticals and dermatological drugs as antioxidant, anti-inflammatory and antibacterial agent (Maraskolhe *et al.*, 2020; Summa *et al.*, 2018; Mondal *et al.*, 2016; Guddeti *et al.*, 2015). Similarly, Musa Acuminata (MA) famous for its excellent antibacterial, anti-inflammatory and antioxidant properties as it contains bioactive compounds like polyphenols, myricetin-3-O-rutinoside, naringenin glycosides, kaempferol-3-O-rutinoside, dopamine, N-acetyl serotonin, and rutin, (Nimalan *et al.*, 2019; Sonibare *et al.*, 2018; Bisht *et al.*, 2016). MA belongs to the Musaceae family also known as banana, is a perpetual tree-like herb developed in numerous tropical and subtropical districts (Mathew and Negi, 2017). Traditionally, its leaves were used for treating many skin conditions such as fine lines and wrinkles. Therefore, the main objective of the study was to develop antibacterial nanofiber cosmetic mask containing SA, PVA and leaf extracts (water) of HRS and MA through electrospinning. This facial mask is superior to other commercially available cosmetic mask in terms of, it is in dry form and natural substances are chemically bound which are released once the mask gets wet.

2. Materials and Methods

2.1 Material

SA was purchased from FMC Biopolymer, Norway, and PVA (C.A.S.: 9002-89-5; M.W., 72000) was purchased from VWR Chemicals industry and distilled water was purchased from Delta Lab Scientific Co. Lahore, Pakistan. Fresh leaves of MA, HRS were collected from botanical gardens of University of the Punjab (PU), Lahore, Pakistan.

2.2 Method

The procedure of the study was divided into three major parts. First part consisted of synthesis of plant leaf extract. Fresh leaves of HRS and MA were collected, washed, dried under shed (two months) and grinded into powdered form, then dipped in distilled water for seven days, filtered with Whatman Grade 1 filter paper, and dried on a rotary vacuum evaporator. The second part was based on the preparation of SA/PVA nanofiber mats through electrospinning process (Fludna Tek LE-10, Bioinicia, Spain) at National textile University, Faisalabad, Pakistan (machine consist of syringe 20ml, 21 gauge needle and a collector). Firstly, SA with 100% conc. was electro spun but unsuccessful, then PVA was used as copolymer with SA to improve its spinnability. Different ratios of SA/PVA such as 50:50, 40:60, 30:70, 20:80 were used to develop the nanofibers by changing process parameters (Table 1). Finally, the best possible ratio of SA and PVA was used to produce the leaf extract loaded nanofibers. Third part was based on the development of leaf extracts loaded SA/PVA nanofibers mats at various concentrations (0.5, 1 and 1.5%).

Table 1: Sodium alginate and plant extract nanofibers composition and process parameters.

Nanofiber samples	Polymer ratio SA:PVA	Plant leaf extract (w/v%)	Volt- age (KV)	Flow rate (μ l/h)	Dis- tance (cm)
SA	100%	-	25	200	15
SA	100%	-	20	100	10
SA/PVA5	50:50	-	18	100	10
SA/PVA5a	50:50	-	20	200	15
SA/PVA6	40:60	-	18	100	10
SA/PVA6a	40:60	-	20	200	15
SA/PVA7	30:70	-	18	100	10
SA/PVA7a	30:70	-	20	200	15
SA/PVA8	20:80	-	18	100	10
SA/PVA8a	20:80	-	20	200	15
SA/PVA8/HRS1	20:80	0.5	16	100	10
SA/PVA8/HRS2	20:80	1	16	100	10
SA/PVA8/HRS3	20:80	1.5	15	100	10
SA/PVA8/MA1	20:80	0.5	15	100	10
SA/PVA8/MA2	20:80	1	14	100	10
SA/PVA8/MA3	20:80	1.5	14	100	10

2.3 Characterizations

Presence of functional group of SA, PVA and leaf extracts in the nanofibers were identified through FTIR spectroscopy (Spectrum two, Perkin Elmer,

U.K.). The samples were scanned in a range of 400 cm^{-1} to 4000 cm^{-1} . Under a X-ray diffractometer (D8 Discover, Bruker, Germany) analysis to examine the crystallinity of the nanofibers, performed on a D8 Advance X-ray diffractometer equipped with a copper tube. The radiation was created at a rate of 0.154nm and at 40 KV. The diffractogram were found at 2θ angle between 10-70 degree at a step rate of 0.05 degree sec⁻¹. Morphology of nanofibers was examined through scanning electron microscope (FEI Nova Nano SEM). Fiber diameter of the nanofibers was measured by using Image J software. Antibacterial properties were examined through Agar disc diffusion assay.

2.4 Determination of antibacterial activity with agar disc diffusion assay

The antibacterial activity of electrospun nanofibers was tested against the *S. aureus* and *E. coli* through Agar disc diffusion assay AATCC 147, 1998. Nutrient broth medium was used to culture the bacteria. Both nanofiber mats (cut into disc shape) were placed on the agar plates. Penicillin was used as negative control. The plates were placed in the incubator at 37°C for 24 hours. Inhibition zone were measured and compare with the negative control.

3. Results and Discussion

3.1 Fourier transform infrared spectroscopy (FTIR)

SA and PVA are hydrophilic polymers as both contain hydroxyl group, due to this they were stable when they contact with water and water mixture. FTIR Spectrum of (a) pure PVA, SA and HRS leaf extract, (b) pure PVA SA/PVA8, SAHRS1, SAHRS2 and SAHRS3 nanofibers at different concentrations.

3.2 SAHRS nanofibers

SA/PVA8 blend can now serve as a base to interact with the additive named as HRS. The HRS extract showed in Figure 1a. The phytochemicals of HRS showed peaks at 3280-3500 cm^{-1} , 1690 cm^{-1} , 1081 cm^{-1} and 839 cm^{-1} . These peaks indicate the presence of OH group, CH₂ bond stretching and bending (Gomare and Mishra, 2018). Current analysis of phytochemical properties of HRS can be done using three different concentrations of HRS as 0.5%, 1.0% and 1.5%. FTIR spectra of different concentrations of plant extract on the SA/PVA8 base has been shown in Figure 1b. The spectra of SA/PVA8 to some broader peaks as compared to SA/

PVA8 spectrum. There was no change in spectrum with increase in concentration of HRS.

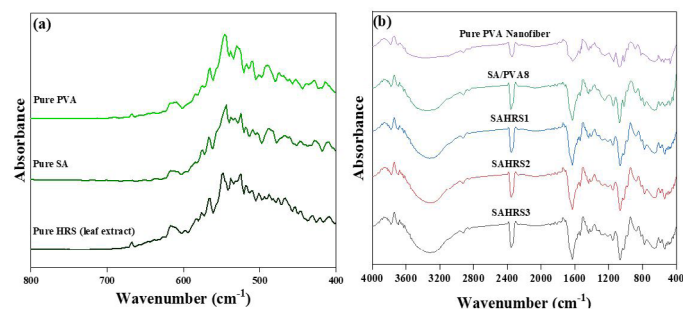


Figure 1: FTIR Spectrum of (a) pure PVA, SA and HRS leaf extract, (b) pure PVA SA/PVA8, SAHRS1, SAHRS2 and SAHRS3 nanofibers at different concentrations.

This consistency in the spectrum shows that once the interactions are developed with SA/PVA8 base then further increase in concentration does not increase the intermolecular interactions between the additive and base. This could be due to the stretching and bending of bonds because bonds don't stretch or bend beyond a certain level and increase in concentration does not increase the bending and stretching of bonds.

4.3 SAMA nanofibers

The FTIR spectra of isolated polyvinyl alcohol as well as in the presence of sodium alginate are shown in Figure 2a. They contained OH group at 3280–3500 cm^{-1} . This range showed that OH group in this region was available for the strong intermolecular interactions with other compounds. In addition, there were CH₂ bonds available for stretching and bending at 1680–835 cm^{-1} . These same functional groups were present in sodium alginate as the spectra was broader in the presence of SA (Safi *et al.*, 2007). This further broadening and sharpening of peaks were observed in the Figure 4 a in the presence of MA extract. The phytochemicals present in this plant were found to be phenols, flavonoids etc. The phytochemicals of MA have shown peaks at 3280–3500 cm^{-1} , 1690 cm^{-1} , 1081 cm^{-1} and 839 cm^{-1} as shown if Figure 2a. These peaks indicated the presence of OH group, CH₂ bond stretching and bending (Valsalam *et al.*, 2019). These groups were confirmed by FTIR analysis of MA with SA/PVA8 base. The peaks were broad and sharp, and this showed the molecular interactions with phytochemicals of plant extracts along with nanofibers. Once the intermolecular attractions developed between the polymer base and plant extract then there might be no sharpness in the peaks

with increasing concentration of the plant extract as shown in Figure 2b. Once the intermolecular attractions developed between the polymer base and plant extract then there might be no sharpness in the peaks with increasing concentration of the plant extract. The whole study had shown that there was a common traits between all the studied plant extracts and that was related to their phytochemicals as they contained somehow same phytochemicals such as phenols, flavonoids etc. (Hari and Nair, 2018). They have antibacterial, antioxidants and anti-inflammatory activities and that's why they had many applications in various antibacterial, antioxidants and anti-inflammatory reactions and this will contribute towards the green synthesis of various antibacterial, antioxidants and anti-inflammatory reactions.

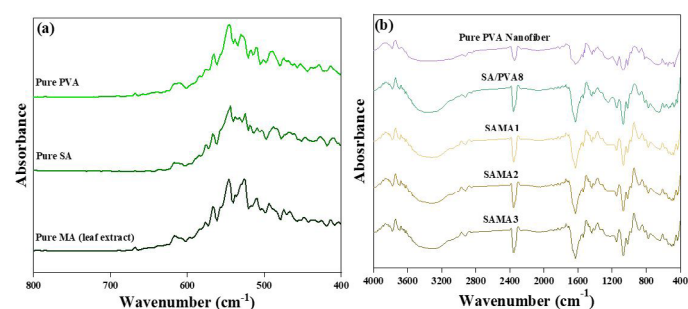


Figure 2: FTIR Spectrum of (a) pure PVA, SA and MA leaf extract, (b) pure PVA, SA/PVA8, SAMA1, SAMA2 and SAMA3 nanofibers at different concentrations.

4.4 Crystallinity of nanofibers

The XRD pattern of pure PVA and SA is shown in Figure 3a. The Figure 3a showed that both had high peaks at same places. It would indicate the presence of same structural properties. XRD pattern of PVA revealed a crystalline structure with $2\theta = 19.8$. This value of diffracting angle showed that PVA had a crystalline structure. The crystallinity of the polymer might be affected by the presence of the other compounds or leaf extracts. The SA XRD pattern showed that it had crystallinity at 35.53%. This amount showed that it had little crystallinity but mostly it is an amorphous species having irregular structural arrangements. The nanofibers of PVA, SA/PVA8 are shown in Figure 3b. The PVA and SA/PVA8 XRD pattern had shown sharp peaks which indicated PVA has more crystallinity with sodium alginate (Nasar *et al.*, 2009).

4.5 SAHRS nanofibers

As shown in Figure 3 the XRD pattern of PVA, SA

and HRS leaf extract. The XRD pattern of HRS had shown that it had a small value of 2θ that indicated that it had an amorphous structure. In Figure 4b, the SA/PVA8 combination would have served as a base for the different concentrations of plant extract. The graphs had shown that with increasing concentration of HRS, the crystallinity of SA/PVA8 base was decreased and there were more strong interactions between the SA/PVA8 base and plant extract. The structural changes indicated the presence of interactions between the extract and polymer nanofibers (Aprilliza, 2017).

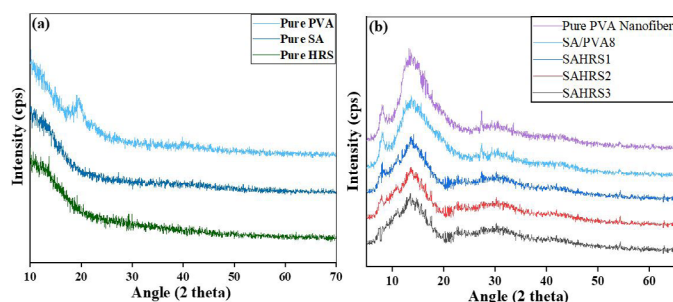


Figure 3: XRD pattern of (a) pure PVA, SA and HRS (b) pure PVA, SA/PVA8, SAHRS1, SAHRS2 and SAHRS3 Nanofibers.

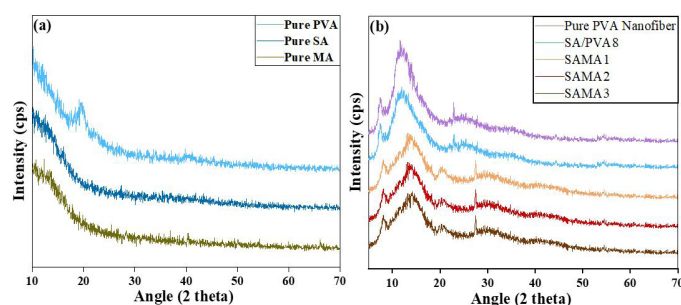


Figure 4: XRD pattern of (a) pure PVA, SA and MA (b) pure PVA, SA/PVA8, SAMA1, SAMA2 and SAMA3 Nanofibers.

4.6 SAMA nanofibers

Figure 4a showed the XRD pattern of PVA, SA and MA leaf extract. The XRD pattern of MA had shown that it had a small value of 2θ that indicated that it had an amorphous structure. In Figure 4b, the SA/PVA8 combination would have served as a base for the different concentrations of plant extract. The graphs had shown that with increasing concentration of MA, the crystallinity of SA/PVA8 base was decreased and there were more strong interactions between the SA/PVA8 base and plant extract. The structural changes indicated the presence of interactions between the extract and polymer nanofibers (Singh *et al.*, 2010). The structural changes indicated the presence

of interactions between the extract and polymer nanofibers (Aravind *et al.*, 2021).

4.7 Morphological properties

Figure 5 shows the SEM images of pure SA and SA/PVA nanofibers. It was observed that no formation of nanofibers was observed with the 100% sodium alginate, even when it combined with PVA at the ratios of 50:50, 40:60, 30:70, but 8% w/v of PVA with 2% w/v of SA (ratio=2:8) produced smooth and bead free nanofibers at the flow rate of 0.1ml, 18 KV Voltage and 10cm distance from needle to collector plate.

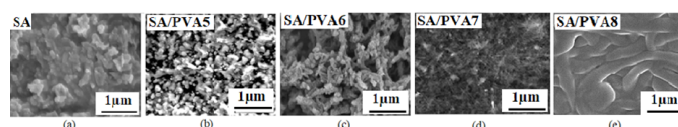


Figure 5: SEM images of nanofibers at different ratios of SA/PVA at magnification level of (a) 10000, (b) 5000, (c) 1000, (d) 5000, (e) 10,000.

As Figure 5 showed the SEM images of pure SA and SA/PVA nanofibers at different concentrations. SEM indicated that SA/PVA5 and SA/PVA6 did not show any formation of nanofibers, in fact SA and PVA precipitated at the surface, they did not chemically bound to form the polymers. Similarly, SA/PVA7 showed the mix matrix of nanofibers but they are not visible and fuse together. However, SA/PVA8 produced straight and bead free nanofibers with smooth surfaces.

4.8 Pure SA/PVA8 nanofibers

Smooth, thin and beadfree nanofibers were formed at the flow rate of 0.1ml, 18 KV Voltage and 10cm distance from needle to collector plate. Figure 6 showed the SEM images of SA/PVA8 nanofibers and graphical representation of fiber diameter using origin software. Diameter was measured through Image J software. It was observed that very thin, smooth nanofibers were developed with diameter ranging from ± 79 to ± 545 nm, this might be due to very low surface tension and high conductivity of the electrospinning solution.

4.9 SAHRS nanofibers

HRS leaf extract containing nanofibers produced smooth and beadfree nanofibers with different concentrations at 0.5, 1 and 1.5 % by varying process parameter of electrospinning. Figure 7 showed the SEM images of SAHRS1, SAHRS2, SAHRS3

nanofibers at 0.5, 1 and 1.5% concentrations respectively and graphical representation of its diameter. The result indicated that smooth and bead free nanofibers were formed with all concentrations of HRS extract. It was also observed that SAHRS1 nanofibers diameter ranging from ± 38 to ± 183 nm which is lessor than the diameter of pure SA/PVA8 nanofibers. This might be due to addition of leaf extract into the pure SA/PVA8 solution. Moreover, SAHRS2 and SAHRS3 nanofibers exhibited slight larger diameter than SAHRS1 nanofibers measured as ± 49 to ± 212 nm and ± 55 to ± 276 nm, respectively. This might be due to the concentration of HRS extract from 1 to 1.5%.

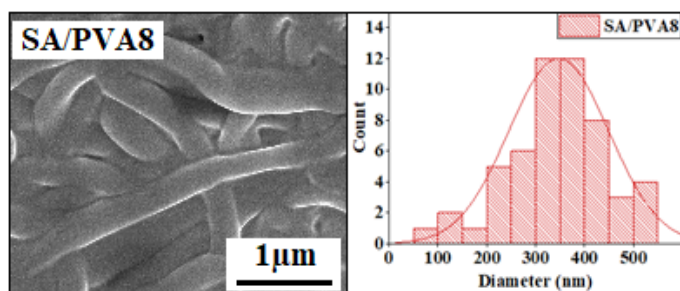


Figure 6: SEM images (magnification level of 30x) of SA/PVA8 nanofibers and Diameter distribution.

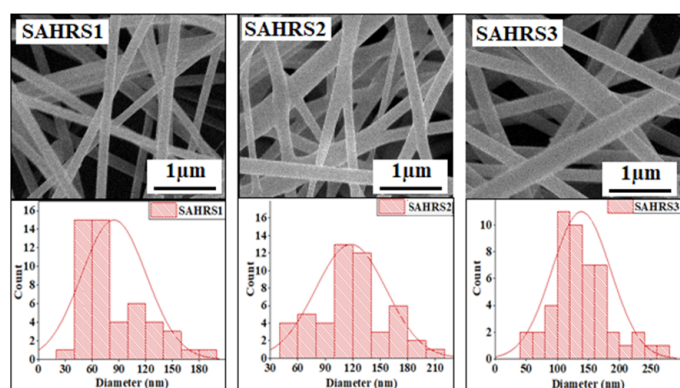


Figure 7: SEM images (magnification level of 30x) of SAHRS1, SAHRS2, SAHRS3 nanofibers at 0.5, 1 and 1.5% concentrations and Diameter distribution.

4.10 SAMA nanofibers

MA leaf extract containing nanofibers produced smooth and beadfree nanofibers with different concentrations at 0.5, 1 and 1.5 % by varying process parameter of electrospinning. Figure 8 showed the SEM images of SAMA1, SAMA2, SAMA3 nanofibers at 0.5, 1 and 1.5% concentrations respectively and graphical representation of its diameter. The result indicated that smooth and beadfree nanofibers were formed with all

concentrations of MA extract. It was also observed that SAMA1 nanofibers diameter ranging from ± 44 to ± 168 nm which is lessor than the diameter of pure SA/PVA8 nanofibers. This might be due to addition of leaf extract into the pure SA/PVA8 solution. Moreover, SAMA2 and SAMA3 nanofibers exhibited slight larger diameter than SAMA1 nanofibers measured as ± 57 to ± 172 nm and ± 86 to ± 179 nm respectively. This might be due to the increased concentration of MA extract from 1 to 1.5%.

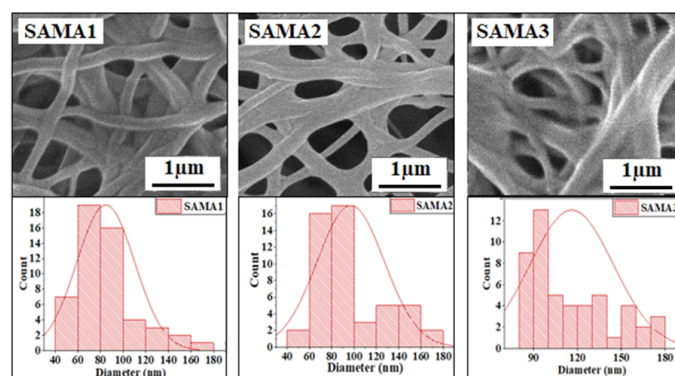


Figure 8: SEM images (magnification level of 30x) of SAMA1, SAMA2, SAMA3 nanofibers at 0.5, 1 and 1.5% concentrations and Diameter distribution

More importantly addition of plant extract in SA/PVA8 nanofibers increased the conductivity and lowers the viscosity and surface tention which resut into more well oriented, beadfree, smooth nanofibers (Figure 7 and 8).

4.11 Antibacterial activity

Antibacterial propeties of with and without leaf extract were checked through Agar disc diffusion method (Ali *et al.*, 2021; Hashmi *et al.*, 2020; Kim *et al.*, 2016) against Staphylococcus aureus (*S. aureus*) gram-psitive and *Escherchia coli* (*E. coli*) gram negative bacteria strains. It was observed that the pure SA/PVA8 nanofibers as shown in Figure 9 showed the lowest antibacterial properties against gram positive bacteria i.e., 16.02mm while it showed moderate inhibition zone of 18.2mm against gram negative bacteria which is higher than SAMA containing nanofibers. Antibacterial properties were significantly improved when leaf extract of MA and HRS were incorporated into the nanofibers of SA/PVA8 in case of *S. aureus* bacteria, and it became prominent as the proportion of the leaf extract increased in nanofibers (Kim *et al.*, 2016) as SAMA1, SAMA2, and SAMA3 nanofibers exhibited inhibition zone

19.5mm, 22.2mm and 28.7mm against *S. aureus* and showed minimum inhibition zone against *E. coli* such as SAMA1=15mm, SAMA2=15.4mm and SAMA3=16mm. This antibacterial property might be due to the presence of phytochemicals such as glycosides, alkaloids, tannis, flavinods, saponins and terpenoids in the leaf extract; presence of tannis compound inhibit the growth of bacteria which act as good antimicrobial agent against *S.aureus* bacteria (Nimalan *et al.*, 2019). As well as extract also contained the salvipisone and aethiopinone as many studies proved that which is the type of diterpenoids act as anti-biofilm against gram-negative bacteria's (Barbieri *et al.*, 2017; Nimalan *et al.*, 2019). These attack on the cell wall of bacteria (Urzúa *et al.*, 2008) and inhibit its growth (Sivasamugham *et al.*, 2021).

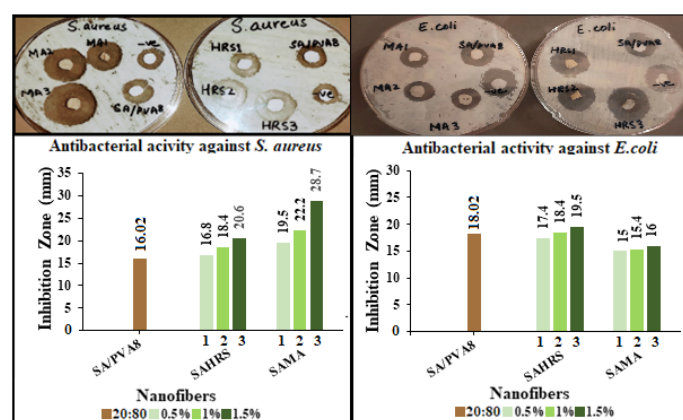


Figure 9: Antibacterial activity of with and without laef extract nanofibers against *S. aureus* and *E. coli*.

Many studies showed that presence of flavonoids in MA extract such as kaempferol, myricetin and flavones inhibits the growth of *S. aureus* (Barbieri *et al.*, 2017). It was also observed that the alkaloids oraganic compounds such as tannis, alkaloids and triterpenoids (Maraskolhe *et al.*, 2020) present in HRS containg nanofibers found to be more effective against gram positive bacteria than gram negative bacteria (Al-Snafi, 2018), as inhibition zone of SAHRS1, SAHRS2 and SAHRS3 nanofibers were recorded as 16.8mm, 18.4mm and 20.6mm against *S. aureus* and SAHRS1=17.4mm, SAHRS2=18.4mm and SAHRS3=19.5mm against *E. coli* which is less than pure SA/PVA8 nanofibers.

SA and PVA are the biocompatible polymers which serve as excellent carrier for antibacterial agents for medical cosmetic industry, which is one of the main reasons that leaf extract of MA and HRS in SA/PVA8 nanofibers exhibited highest antibacterial properties

due to high surface area in relation to its diameter of developed electro spun nanofibers.

Conclusions and Recommendations

In the present study antibacterial nanofibers were developed with the diameter range from ± 21 to ± 540 nm. Successful incorporation of leaf extract through electrospinning into Sodium alginate nanofibers not only increased the antibacterial resistance but also impact the nanofiber morphology by increasing the solution conductivity and lowers the surface tension and viscosity which lead to the production of smooth, bead free nanofibers. MA containing nanofibers exhibited excellent antibacterial activity against *S. aureus* than *E. coli* while HRS nanofibers showed moderate antibacterial activity against *S. aureus* and *E. coli*. More importantly, crystallinity of nanofibers decreased as conc. of leaf extract increased in the nanofibers. Whereas antibacterial properties increased as the conc. of leaf extract increased. So, it is a good sustainable replacement of commercially available medicated facial mask because of its excellent antibacterial property against gram positive and gram-negative bacteria.

Novelty Statement

The novelty of the research lies in the fact that the developed antibacterial cosmetic nanofiber mask is a good sustainable replacement of commercially available medicated facial mask because of its excellent antibacterial property due to the presence of active ingredients i.e. Musa Acuminata, Hibiscus Rosa Sinensis along with natural polymers (Sodium Alginate).

Author's Contribution

Ayesha Saeed: Conceived idea, designed and performed the experiment, **Farzana Kishwar:** Critical review and editing **Ahsan Nazir:** Critical review and contribute Reagents, Materials, **Sharjeel Abid:** Experimental guidance **Muhammad Abiodullah:** Data analysis **Danial Ali:** Anti-bacterial Testing.

Conflict of interest

The authors have declared no conflict of interest.

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