

Influence Of *Nardostachys jadamansi* DC. Into Transparent Polymer Biocomposites for In-Vitro Anti Fungal Efficacy

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Abstract: The herbal plants are predominately used in the field of bio medicine. *Nardostachys jadamansi* DC. is a perennial herb, belongs to the family of valerianaceae. The jadamansi plant roots have high potential anti-fungal, anti-microbial activity and rich antioxidant properties. The purified root product can be used for the treatment of cancer due to its tumor suppressive effect. Further jadamansi biopolymer has been synthesized from monomer of methyl methacrylate by radical polymerization technique. The biopolymer products are characterized through NMR, ATR-IR, UV-vis and Fluorescence measurement. The transparent polymer biocomposites product has high economic value and it taken short period of time for synthesized process. This biocomposites exhibited excellent anti-fungal activities in various fungal species. However, the final product of polymer biocomposites may be used in the treatment of several cancers.

Keywords: *Nardostachys jadamansi* DC., Poly methyl methacrylate (PMMA), Biocomposites, Nuclear magnetic resonance (NMR).

I. Introduction

The *Nardostachys jadamansi* DC. is a well known medicinal aromatic plant, which is belongs to the family valerianaceae commonly grown in Himalayan hills region also known as Muskroot, spikenard. The rhizome of aromatic plant of *Nardostachys jadamansi* DC. acquires to cure severe nervous headache, hypertension, epilepsy, depressive illness. The rhizomes of herb could exploit due to their excellent therapeutic and anti-stress application. The major components of this plant are alkaloids jadamansone, nardostachone, jadamansic acid, coumarin, lignin, neolignan and sesquiterpenes [1]. The rhizome can be used as an aromatic addition in the synthesis of medicinal oils to endorse hair growth [2].

However, this kind of herbal plant is used as effective antimicrobial activity against pathogenic bacteria and exhibited persuasive antifungal resistance against human and plant fungal pathogens [3]. The rhizome can also be used as an alternative for valerian and whole plant has lingering smell and it has remove the impurities from blood. Since, the rhizome can be used in jaundice, kidney stones, flatulence, typhoid, gastric disorder, seminal debility and also used as hair oil to promote hair growth, it has been choose for investigation and there is no side effect have been noticed so far. Sarbhoy et al., thoroughly investigated Jadamansi oil on different fungal species competence against *Aspergillus flavus*, *A. fumigatus*, *A. sulphureus*, *Mucorfragilis* and *Rhizopus stolonifer* and excellent fungistatic or fungicidal effect were reported [4]. Parveen et al., reported that the Jadamansi roots was conducted the antibacterial activities against both gram positive and gram negative bacteria's such as *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescence* and *Enterobacter aerogenes* [14] by using the disc diffusion method [5]. Bhagat et al., reported, rhizomes of *Nardostachys jadamansi* DC. was used for antitumor or against sarcoma-180 solid tumor model and enhance excellent anticancer treatment. Further this plant can be widely exploited for cancer drug development [6]. However, Poly methyl methacrylate (PMMA) has better optical clarity, high mechanical strength and good weather ability, and it can be used as a transparent engineering material [7]. Kong et al., reported that poly (methyl methacrylate) Nano fibers containing silver nanoparticles has enhanced better antibacterial efficacy in both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria using minimum inhibitory concentration [8]. Dizman et al., reported that the microbial/fungal infection can be controlled, due to the incorporated antimicrobial agent with polymer matrix, which was achieved by coating the drug, covalent and non-covalent method [9]. There are several polymerization techniques have been conducted on the monomer of methacrylic acid, methyl methacrylate, poly (triethylene glycol dimethacrylate) and 2-hydroxyethyl methacrylate [10]. Among them, we have choose in the methyl methacrylate is a special monomer to synthesis of poly (methyl methacrylate) by free radical polymerization technique. In the present work involved, a novel transparent polymer biocomposites were synthesized for effective antifungal activities.

II. Experimental Section

2.1 Materials

The monomer of methyl methacrylate (MMA) was purchased from Aldrich and passed through an Al_2O_3 column (for removal of inhibitor) before use. Benzyl peroxide was purchased from Sigma-Aldrich, USA. Chloroform and methanol were distilled and dried out as per standard protocols before use. *Nardostachys jadamansi* DC., root were collected from kollimalai hills of Namakkal district of Tamilnadu, India.

2.2 Methods

The 1H NMR spectra were recorded on a Bruker Advance 400 spectrometer (400 MHz). All the Spectra in solution were recorded in $CDCl_3$, at room temperature. UV-visible absorption spectra were measured on a JASCO UV-visible 530 spectrometer. FT-IR spectroscopy was performed using ABB MB3000 and Fluorescence spectra were recorded on a Varian Carey Eclipse under the following conditions: excitation wavelength 274 nm, emission wavelength of 338 nm and 423 nm, the optical polarizing microscope was performing by Olympus BX50.

2.3 Synthesis of transparent polymer biocomposites

The monomer of methyl methacrylate was taken in a triple neck bottom flask fitted with nitrogen gas purged for 30 minutes to remove oxygen from the system. Radical initiator of benzyl peroxide was dissolved in distilled chloroform and added slowly to the reactor and stirring for 2 h at 70 °C. In addition *Nardostachys jadamansi* DC. rhizomes was extracted from methanol and dried under vacuum at 50 °C. This rhizome extract further dissolved in different concentration (1, 2.6 and 3.6 %) in chloroform and added to the polymerization system stir for 30 min at ambient temperature and cast as transparent films.

III. Results And Discussion

3.1 ATR-IR spectroscopy study

The ATR-IR spectra of the PMMA, *Nardostachys jadamansi* DC., 1, 2.6, 3.6 % of polymer biocomposites as shown in Fig.1. We observed a new peak at 974 cm^{-1} for *Nardostachys jadamansi* DC. extract, corresponds to aromatic -C-H stretching vibration frequency, which was absent at different percentage of polymer biocomposites, which indicates that the aromatic plant extract could interact with polymer matrix.

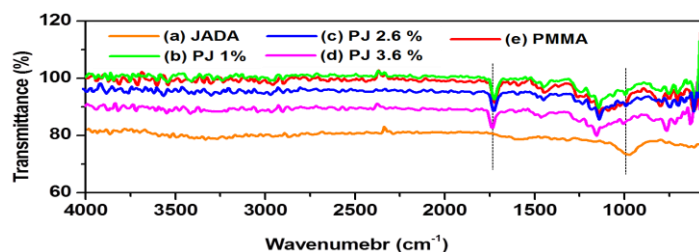


Fig. 1 ATR-IR spectra for (a) *Nardostachys jadamansi* DC. (b) PJ 1% (c) PJ 2.6% (d) PJ 3.6% and (e) PMMA.

However, 1722, 1724 and 1726 cm^{-1} for 1, 2.6 and 3.6 % biocomposites assigned to carboxylic group (-C=O), which indicates that the jadamansi acid present in the compounds, also some shift was noticed, while increasing the concentration. Similarly, the characteristic vibration bands are observed, ART-IR (cm^{-1}): 1725 (-C=O); 1454(-C-H); 1151(-C-N); $974(=C-H)$; 587(C-H) gave additional evidence for the formation of composites.

3.2 UV-vis absorption study

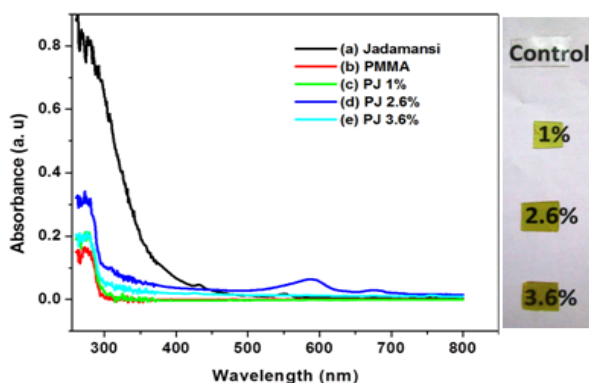


Fig. 2 UV-vis spectra of (a) *Nardostachys jadamansi* DC. (b) PMMA (c) PJ 1% (d) PJ 2.6% and (e) PJ 3.6% with photo shot images of transparent polymer biocomposites (right side).

The UV-visible spectrophotometer is one of the most often used techniques to find out the maximum wavelength region. It is used to measure ultraviolet or visible radiation absorbed by material in solution state [11]. The wavelength of λ_{\max} exited at 279 nm corresponds to *Nardostachys jadamansi* DC. as shown in Fig. 2. Similarly, λ_{\max} values were slightly shifted from 274 to 279 nm, which is good agreement for the formation of polymer biocomposites. Additionally, the absorption spectra exhibited the characteristic peaks for polymer biocomposite (1, 2.6 and 3.6%) λ_{\max} at 272 nm, which is better concord with polymer and plant extract.

3.3 Fluorescence measurement study

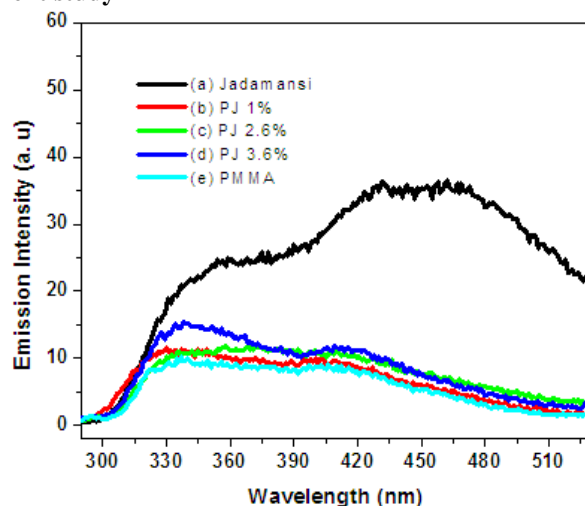


Fig. 3 Fluorescence emission spectra of (a) *Nardostachys jadamansi* DC (b) PJ 1% (c) PJ 2.6% (d) PJ 3.6%. and (e) PMMA

The fluorescence emission spectra of the compounds used (*Nardostachys jadamansi* DC., PMMA, PJ 1, PJ 2.6 % and PJ 3.6 %.) were recorded in chloroform, at the concentration of 10^{-5} mol/litre, as shown in Figure 3. The emission spectrum of *Nardostachys jadamansi* DC. was very wide and shows two emissions, maximum at 338 nm and 423 nm, which was mainly attributed to the fluorescence active plant extract. When the percentage of concentration has been increased (1 to 3.6 %) some shift was absorbed, In Fig 3, the obtained results clearly shows that the interaction between *Nardostachys jadamansi* DC. and PMMA.

3.4 Nuclear magnetic resonance study

The ^1H NMR spectra (500 MHz, CDCl_3 , δ , ppm) of the *Nardostachys jadamansi* DC., polymer biocomposites (1, 2.6, and 3.6%) are shown in Fig. 4. The ^1H NMR spectrum of the *Nardostachys jadamansi* DC.in CDCl_3 shows peaks at 7.2 and 7.3 ppm integrating CDCl_3 and overlapped aromatic proton respectively. These peaks were correspond to aromatic protons from (-Ar-H) groups and confirm the aromatic structure present in the plant extract as shown in Fig. 4(a) taken in CDCl_3 , shows peaks at 3.4, 1.6 and 0.8-1.3 ppm were correspond to protons from the $-\text{OCH}_3$, $-\text{CH}_2$ and $-\text{CH}_3$ groups confirm the structure of PMMA respectively. These peaks clearly corroborate the growth of PMMA on the rhizome of *Nardostachys jadamansi* DC. as shown Fig. 4 (b-d).

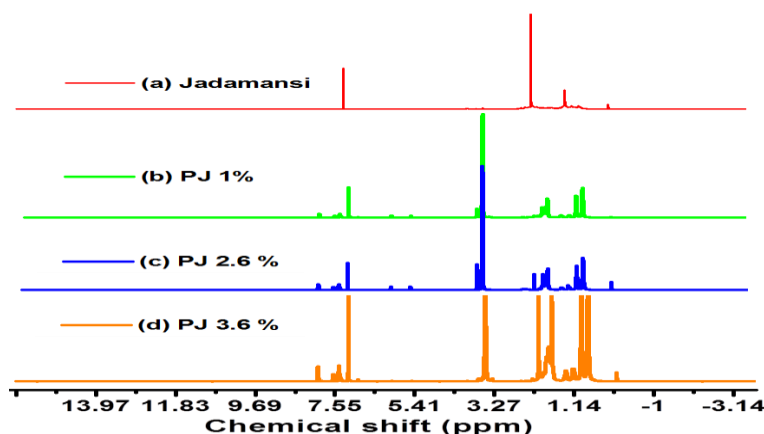


Fig. 4 ^1H NMR spectra of (a) *Nardostachys jadamansi* DC (b) PJ 1 % (c) PJ 2.6 % and (d) PJ 3.6 %.

3.5 Morphological study

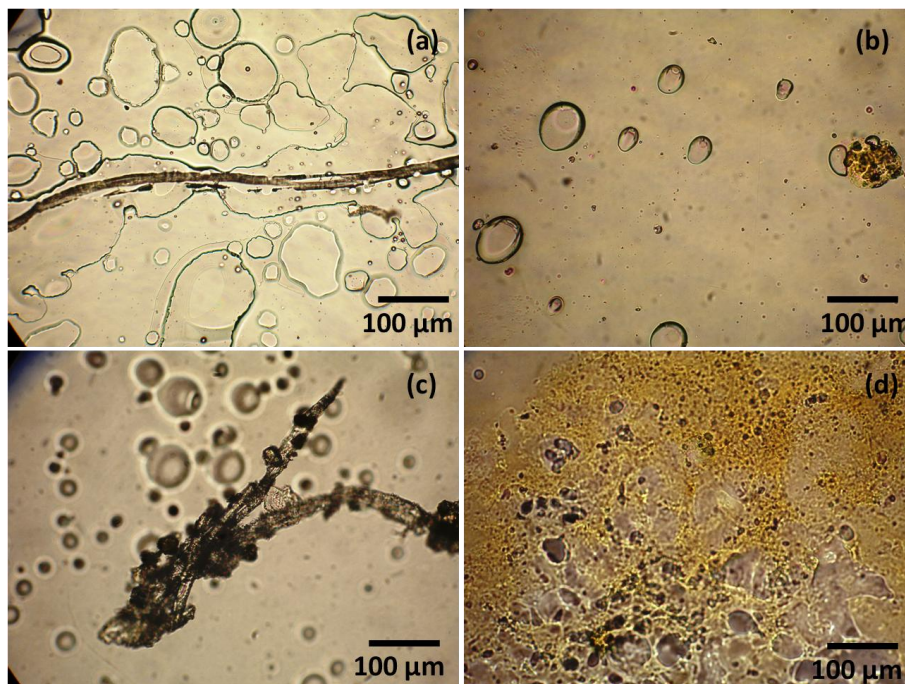


Fig. 5 Optical microscopic images of (a) PMMA (b) PJ 1% (c) PJ 2.6% and (d) PJ 3.6%.

The optical images observed for PMMA and its corresponding polymer biocomposites at different concentrations (1, 2.6 and 3.6 %) were shown in Fig. 5. It shows variable morphology, which surrounded by fine tubular structure which was less when compared to the previous one due to bundle architecture. The PMMA shows separated morphologies, which contain spherical structure and the polymers were present on the surface of *Nardostachys jadamansi DC.* rhizome, is an evident by seeing the images in Fig. 5 (b-d). It may be concluded that *Nardostachys jadamansi DC.* could well interact with the polymer.

3.6 Anti-fungal study

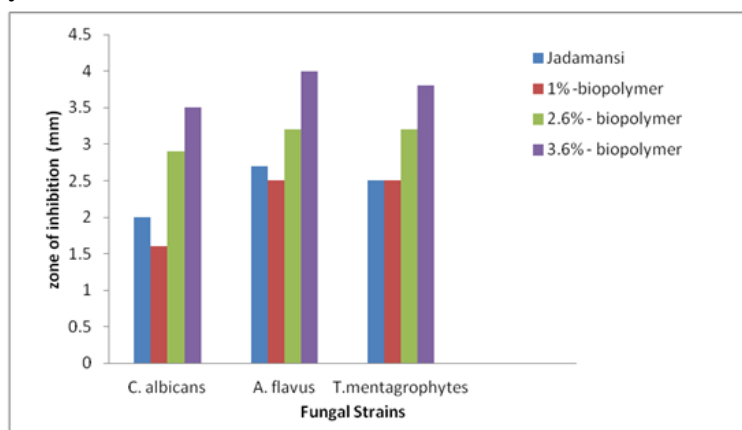


Fig.6 Anti-fungal activity of *N. jadamansi DC.* and polymer biocomposite at different concentrations.

The *Nardostachys jadamansi DC.* oil was toxic to some of the fungal species such as *A. jumigatus*, *A. sulphureus* and *M. fragilis*. These findings represent, *N. jadamansi DC.* oil can be controlling diseases from crops and plants. *N. jadamansi DC.* can act as fungicidal effect to all the fungal species [12]. Ahmad *et al.*, reported that *N. jadamansi DC.* is a traditional ornamental plant for precise therapeutic effect [13]. The plant extract and polymer biocomposites were tested on pathogenic fungi of *Candida albicans*, *Aspergillus flavus*, *Trichophyton mentagrophytes* by using disk diffusion method. The potato dextrose broths were prepared for test fungal broths incubating at 37 °C for 72 h. The potato dextrose broths along with fungal culture were placed in PDA Petri plates. Blank disk impregnated with water was used as control. After 72 h of incubation (37 °C), antifungal activity zone in mm was determined by Hi-Media zone reader and it shows that diameter of inhibition zone was increased with increasing percentages of plant extract with polymer as shown in Figure. 6. It may conclude that this polymer biocomposites could be act as excellent antifungal agent.

IV. Conclusion

In the present report, the effect of *Nardostachys jadamansi* DC. on polymerization of PMMA was successfully demonstrated. The presence of polymer chains on the plant extracts are clearly confirmed through ATR-IR, ¹H-NMR, UV-vis, Fluorescence spectra, which show the corresponding peaks in the region of 1725(-C=O); 1454(-C-H); 1151(-C-N); 974(=C-H); 587(C-H) in ATR-IR. In the meantime, peaks at 3.4, 1.6 and 0.8-1.3 ppm correspond to protons from the -OCH₃, -CH₂ and -CH₃ groups confirm the structure of PMMA on plant extract. NMR analyses provide supportive evidence for the formation of polymer biocomposites. In the optical microscopic images various morphological structures were observed in both PMMA and composites. Anti-fungal activities are conducted in various fungal species such as *Candida albicans*, *Aspergillus flavus*, *Trichophyton mentagrophytes* and it enhances to act as excellent anti-fungal agent. We report the simple and cast effective biocomposites synthesized from *Nardostachys jadamansi* DC. and Poly (methyl methacrylate) (PMMA), which was achieved by free radical polymerization technique using radical initiator of benzyl peroxide for effective antifungal activities conducted in various fungal species to prevent fungal infection. This research work further extends its application for anticancer and other biological process.

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