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Retention of antibacterial and antioxidant properties of lemongrass oil loaded on cellulose nanofibre-poly ethylene glycol composite

Disha Mishra[a], Puja Khare[a,*], Dhananjay. K. Singh[b], Suaib Luqman[b], P.V. Ajaya Kumar[c], Anju Yadav[c], T. Das[d], B.K. Saikia[d]

[a] Agronomy and Soil Science Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, 226015, Uttar Pradesh, India
[b] Molecular Bioprospection Department of Biotechnology Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, 226015, Uttar Pradesh, India
[c] Analytical Chemistry Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, 226015, Uttar Pradesh, India
[d] Polymer Petroleum and Coal Chemistry Group, Materials Sciences & Technology Division, CSIR-North East Institute of Science and Technology, Jorhat, 785006, Assam, India

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ABSTRACT

The lemongrass oil (LgEo) exhibits excellent antioxidant and antibacterial properties. However, low aqueous solubility and instability of its major constituents reduced the retention of these properties for the longer time. Hence, LgEo loaded composites of cellulose nanofibres (CNFs)- polyethylene glycol (PEG) were fabricated through melt and mixing process and sustainability of their antioxidant and antibacterial properties was assessed. The interaction of essential oil with composite systems was evaluated using Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscope (SEM), Transmission electron microscopy (TEM), and X-ray powder diffraction (XRD) and quantification of released major compounds up to 120 days was done by Solid phase micro extraction/Gas chromatography–mass spectroscopy (SPME/GC–MS) methods. Results suggested that composite systems were able to sustained major compounds of lemongrass essential oil (geranial, neral and geranyl acetate) up to 120 days and followed Pseudo Fickian diffusion of aroma molecules. *In vitro* study of total antioxidant capacity, total phenolic, free radical scavenging efficiency and antibacterial properties (against *S. aureus* and *E. coli*) of the composites were suggested that composite system retained the properties of the pure lemongrass oil. These results indicate that fabricated scented composites can be used further in various industrial applications such as indoor air quality improvement materials and food storage.

1. Introduction

Nowadays, the development of biodegradable polymer is gaining importance over the synthetic materials particularly encapsulation and/or coating of natural molecule. They can improve the quality and the shelf life of natural product (Bashir et al., 2017; Esfanjani and Jafari, 2016). They can also act as vehicles for different naturally occurring compounds for instance antioxidants or antimicrobial compounds, vitamins, flavor compounds, and colorants, (Aytac et al., 2016; Castro-Rosas et al., 2017; Cerqueira et al., 2016; Fidelis et al., 2015). The composite system of the biodegradable polymers and herbal products such as plant extracts and essential oils provides a better durability of desired properties such as increase its functionality, improve their appearance, and extended the shelf life through controlling the release (Echeverría et al., 2016; Oliveira et al., 2017; Sultanbawa, 2011). Cellulose, being most naturally abundant polymer has attracted attention for such type application. Cellulose nano-fibers (CNFs) obtained from the native cellulose persist remarkable excellent properties such as biocompatibility, nontoxicity, and biodegradability. The unique properties of CNFs widen the possible applications of this biopolymer such as in pharmaceutical, biomedical, electronic and textile, as well as in food packaging industries (Chauve and Bras, 2014; Lee et al., 2017). Recently, cellulosic fibers used for reinforcement due to its biodegradability, lower weigh, renewability, and molecular strength. These fibers are used to produced nano compounds with phenolic resin, styrene butyl acrylate, poly urethane, starch (Borah and Kim, 2017; Rojo et al., 2015). Similar to CNF, poly ethylene glycol (PEG) is also a biocompatible and nontoxic polymer composed of repeating ethylene glycol units. It is commonly used in designing of various successfully commercialized biomaterial in various applications, such as nonionic surfactants, lubricants, solvents, adhesives, cosmetics, and pharmaceutical formulations (Lou et al., 2017; Lu et al., 2017; Ravikumar et al., 2017; Wang et al., 2017; Zhang et al., 2017). It is reported that Polyethylene glycol (PEG) has excellent compatibility and affinity with CNF

* Corresponding author.
E-mail address: pujakhare@cimap.res.in (P. Khare).

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(Gu et al., 2016). Generally, hydroxyl groups of CNF act as coupling agents to promote the adhesion between the fiber surface and the polymer matrix. The CNFs isolated by TEMPO (2, 2, 6, 6-tetramethylpiperidine-1-oxyl radical)-oxidation method could also improve the coupling properties of CNF. The carboxyl functionalized CNFs by TEMPO-oxidation further enhanced the adhesion between the fiber and polymer matrix due to presence of higher anionic group (Nabeela et al., 2016). Hence, in present study, TEMPO oxidized CNF was used for reinforcement of the PEG polymeric matrix for development of the composite system.

Essential oils (EOs), extracts and spices pose little risk to human health and environment due to their natural origin and are considered as for their good antibacterial and antioxidant properties (Hashemi et al., 2016; Raeisi et al., 2016). The ingredients of the essential oils had comparable properties as reported chemical in preservatives (Burt, 2004). Essential oils derived from hydro-distillation of plants are rich in phenolic compounds, such as monoterpenes, flavonoids, and phenolic acids (Bouzenna et al., 2016) and have numerous pharmaceutical and medicinal applications (Iordache et al., 2015; Raut and Karuppayil, 2014). Recently, various essential oils have been intensively explored for the development of polymeric composite system for packaging films, air freshener, and wound healing due to their excellent antibacterial properties (Díez-Pascual and Díez-Vicente, 2015; Hosseini et al., 2016; Steinemann, 2017). Number of both biodegradable and non-biodegradable polymer films, such as chitosan, fish skin, whey protein isolate, low-density polyethylene, ethylene vinyl alcohol, poly(ethylene terephthalate), and polypropylene films have either coated or incorporated with Essential Oil and evaluated for their antibacterial effectiveness (Arfat et al., 2014; Bahram et al., 2014; Bedel et al., 2015; Licciardello et al., 2013; Ranjbar et al., 2017; Rieger and Schiffman, 2014; Valencia-Sullca et al., 2017). Current study has proposed the preparation of lemongrass (*Cymbopogon flexuosus* L.) essential oil (LgEo) loaded composite system of Pure CNF, composite of PEG reinforced with CNF and pure PEG loaded with oil were denoted as LG-A, LG-B and LG-C.

The LgEo has antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine, and sedative properties. Generally, it is used in remedy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia, and vascular disorders in traditional system (Raut and Karuppayil, 2014). Besides these promising properties, the major concern is about their volatility, poor water solubility, poor chemical physical stability, and aptitude for oxidation. Therefore, the labile content of these bioactive agents makes it unfeasible for practical usage for long duration (Tripathi et al., 2009). It is also reported that LgEo had a dual action as surfactant and stabiliser of cellulose acetate based natural polymer (Liakos et al., 2016b). Liakos et al. (2016a, 2016b, 2014), showed the incorporation of LgEo in natural polymers with their excellent antimicrobial properties of the composite materials (Liakos et al., 2014, 2016a). They demonstrated the formation of chemical binding with the cellulose acetate responsible for prolong stay in aquatic environment. It can be used for oral, ophthalmic, respirable, and other functions. Surprisingly, the study on the LgEo loaded CNF-PEG based composite system is limited. In addition, the information regarding the application of LgEo loaded CNF-PEG composites, especially for antioxidant and antibacterial properties is lacking. Also, most of the studies on lemongrass loaded composite films are limited to antibacterial properties with application in packaging of food (Arfat et al., 2015; Davoodi et al., 2017; Lee et al., 2016). The study on the sustainability of antioxidant properties of lemongrass loaded composite system with time is limited.

The present study was framed for the evaluation of sustained antibacterial and antioxidant capacity of LgEo based CNF-PEG composite system. These composite systems could be used in pharma and food industries due its inherent antibacterial and antioxidant properties with edible polymer matrix. The major questions to verify the hypothesis are, 1. What is the diffusion kinetics of major constituent of the LgEo up to 120 days? 2. What is the effectiveness of these oil loaded composite systems with time. Hence, the characteristics, release kinetics, antioxidant and antibacterial activities of composite systems were assessed with time. Antioxidants can reduce radicals primarily by two mechanisms: single electron transfer and hydrogen atom transfer. ABTS, FRAP, and DPPH are methods that measure the former, and ORAC and TRAP represent the latter. Hence, we have evaluated all these activities for the composite system. This preliminary study, which includes diffusion kinetics, antioxidant and antibacterial could be able to predict the industrial applications such as indoor air quality improvement materials and food storage of these composite systems.

2. Materials and method

2.1. Material

The lemongrass (*Cymbopogon flexuosus* L. cv. Krishna) were collected from the campus of (CSIR-CIMAP) Lucknow during the period of December–January and during cultivation the temperature and humidity varied according to north Indian plain's environment conditions. The volatile oil was extracted by hydro-distillation using Clevenger's apparatus (Tajidin et al., 2012). For this, 250 g freshly chopped leaves were mixed with 1 litter of distilled water in a 2 Litre round-bottom flask fitted with a condenser. The mixture was boiled for 1 h at 95–100 °C for 2 h and oil collected from the nozzle of the condenser. It was dried using sodium sulphate and stored at 4 °C, the oil was used for the further experiment. The purity of lemongrass oil was further evaluated by GC–MS (see Supplementary) before loading.

The distilled waste of citronella (*Cymbopogan winterius*) (DWC), for CNF isolation was taken from oil extraction unit of CSIR-CIMAP (Central Institute of Medicinal and Aromatic Plant), Lucknow. The chemical used in the study was toluene (Merck), ethanol (Merck), sodium hydroxide, hydrogen peroxide (Merck), sodium borate (Merck), nitric acid (Merck), acetic acid (Merck) and TEMPO (Sigma Aldrich) of analytical grade with purity of 98–99%. Polyethylene glycol PEG (Mw = 6000 Da) was purchased from Merck and used as received.

2.2. Isolation of cellulosic nanofibres (CNF)

Crystalline cellulose nanofibres (CNF) were isolated from distilled waste of citronella (DWC) by TEMPO (2, 2, 6, 6- tetramethylpiperidine-1-oxyl radical) oxidation followed by ultrasonication. Chopped and dried DWC was dewaxed and delignified by soxhlet extraction (24 h using toluene/ethanol in 2:1 v/v ratio). Prepared dewaxed fibers were filtered and washed with ethanol for 1 h and dried. The extractive free plant biomass was pre-treated with 0.1 M NaOH in 50% v/v ethanol for 3 h at 45 °C under continuous agitation; and then successive bleaching with hydrogen peroxide at pH = 11.5 (buffer solution) and 45 °C (a) 0.5% H_2O_2, (b) 1.0% H_2O_2 (c) 2.0% H_2O_2 and (d) 3.0% H_2O_2 was performed for 3 h each one under continuous agitation. After that, final treatment was done according to the methods described by Sun and Sun (2002) i.e. continuous agitation with 10% w/v NaOH—1% w/v $Na_2B_4O_7.10\ H_2O$ at 28 °C for 15 h. The chemically purified cellulose was characterized for cellulose content (Updegraff, 1969). Some amount of isolated cellulosic fibers was stored for analysis. The rest of the sample was freeze dried (Labconco freeze dryer-free zone 2.5 plus-7522800-USA).

The oxidation of chemically purified cellulose fibers was carried out using TEMPO coupled with ultra sonication with some modification (Saito et al., 2007). For this, the fibers (10 g) were soaked in water overnight and then homogenize to obtain a uniform suspension of fiber of 1% consistency. TEMPO solution was freshly prepared by using TEMPO ($0.11\ mmolg^{-1}$) and NaBr ($0.617\ g^{-1}$) in 50 mL deionized water and added to fibers solution. The pH of the fibers slurry was adjusted between 10 and 12 using 0.5 M NaOH. The oxidation was started by adding NaOCl (3.75 mMol) under ultra-sound at a frequency