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Article

Preparation and Characterization of a Novel Bioavailable Gingerol Formulation Using Polar−Nonpolar Sandwich Technology, Its Antioxidant Potential, and an *In Vitro* Release Study

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ABSTRACT: In this study, an innovative bioavailable gingerol formulation, Ginactiv, was prepared by using polar−nonpolar sandwich (PNS) technology with a full spectrum of ginger matrix (FSGM). SEM images showed that Ginactiv was almost spherical, was well dispersed, had a smooth morphology with a particle size range of 50−60 μm, and was divided by three layers of PNS formulation. IR, DSC, a stability study, an enhanced and sustained release profile, and the good antioxidant potential of Ginactiv furthermore confirmed the existence of gingerols with great stability in a bioavailable form in the PNS formulation. Ginactiv registered a higher moisture content (3.98 ± 1.17%), hygroscopicity (0.94 ± 0.23 g/g), and water solubility index (43.71 ± 3.42%) in comparison to the ginger oleoresin values of 1.96 ± 0.72%, 0.36 ± 0.13 g/g, and 9.62 ± 1.21%, respectively, due to the good encapsulation of gingerols by the PNS technology. The existence of the synergistic effects of FSGM in Ginactiv can be utilized as a natural dietary supplement for supporting gastrointestinal and gut health improvement and to maintain the gut−brain axis.

KEYWORDS: *bioavailable gingerols, Ginactiv, PNS technology, bioavailability, gastrointestinal health*

INTRODUCTION

Medicinal plants have a very extended history of utilization for the benefit of human beings and have been used as a source of components for the expansion of innovative medications. As per a World Health Organization report, more than 80% of the population around the world depends mainly on traditional therapies that involve the use of plant extracts or their phytochemicals containing biologically active components.[1] Recently, there has been increasing attention paid to the useful effects of these bioactive components and their effect on the prevention of disease and maintenance of good health.[2,3] Ginger (*Zingiber officinale* Roscoe) is one of the most prevalent spices utilized around the world because of its pungent taste and fresh aroma.[4] The main active components of ginger are gingerols, shogaols, polyphenols, flavonoids, essential oils, etc. Gingerols are the major components of the ginger responsible for the pungency, which are a collection of a sequence of structural analogues, particularly 6-gingerol, 8-gingerol, and 10-gingerol. Among them, 6-gingerol has been found to have the maximum biological activity.[5−9] Its dehydrated forms 6-shogaol and 8-shogaol also contribute to the pungency of ginger.[10] Some studies have reported that 6-shogaol is more active than 6-gingerol in various biological activities.[11]

Commercially, dried ginger is extensively used for the isolation of volatile oils, oleoresin (OR), and other bioactive components. The ginger oleoresin (ginger OR) contains pungent principles such as 6-gingerols, 8-gingerols, 10-gingerol, 6-shogaol, and 8-shogaol as the key active constituents, responsible for the distinctive pungent sensitivity. The volatile oil contributes to the distinctive aroma of ginger, which comprises β-sesquiphellandrene, α-zingiberene, Ar-curcumene, and β-bisabolene as the major bioactive components. Both extracts have an extensive series of applications as flavorings in the food and food product, beverage, fragrance, and nutraceutical industries.[12,13] Apart from this, the gingerols have also been established to convey a comprehensive series of therapeutic effects: particularly, anti-inflammatory, antioxidant, gastroprotective, antifungal, antibacterial, analgesic, and antipyretic activities.[14,15] Pharmacological research has also highlighted that the gingerols have anticancer, chemotherapeutic, and chemopreventive effects.[3,16]

However, the important function of gingerols in functional foods, drug therapy, and nutraceuticals is highly limited due to its poor water solubility because of its lipid nature, which severely decreases its bioavailability and bioefficacy, and its swift metabolism has hampered medical applications. To increase the stability, water solubility, and bioavailability of the bioactive compounds of ginger, particularly 6-gingerol, 8-gingerol, and 10-gingerol, different approaches have been proposed and investigated; some of them include complexation, solid dispersion, nanoparticles, micelles, micro- and

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nanoemulsions, etc.[17,18] Among them, most of the techniques have some limitations for utilization in functional foods, drugs, and nutraceutical applications due to the addition of excipients, the compounds might be degraded before reaching the target site, and the lack of techniques have restricted their use in powder form. It has been advised that innovative therapeutic methodologies should be focused on overcoming the problems regarding effective delivery at the target site, safety, toxicity, and industrial production with cost effectiveness.[19,20]

Polar−nonpolar-sandwich (PNS) technology is one of the most promising techniques among several methods utilized to expand the solubility of poorly soluble bioactive components, including gingerols, due to its cost-effectiveness and simple and commercially reliable nature for industrial manufacturing.[21,22] The PNS technology can be utilized to expand the stability of bioactive components, preserve functional properties, sustain the release of bioactive components at a specific time and a desired site, expand the bioavailability, and enhance health benefits. In this study, Aurea Biolabs Private Limited developed the bioavailable gingerol "Ginactiv", constructed by the recreation of the full spectrum of ginger matrix (FSGM) with active gingerols by a cutting-edge technique, PNS technology. Ginactiv was prepared in a powder form by good encapsulation of the gingerols sandwiched between the polar and nonpolar matrixes of ginger without any excipients. Ginactiv can be safely incorporated into food and food products, dietary supplements, and cosmetic preparations due to the bioavailable form of gingerols, which can have an effect on various biological activities, particularly gastrointestinal, digestivem and gut health. In this study, Ginactiv has been characterized by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), ultrahigh-performance liquid chromatography (UPLC), and differential scanning calorimetry (DSC). The functional properties and the bioavailability of Ginactiv were evaluated by *in vitro* dissolution; furthermore, the antioxidant potential of Ginactiv was also analyzed in this study.

MATERIALS AND METHODS

Materials. The ginger raw materials were acquired from the local market of Cochin, Kerala, India, which were subjected to quality examination and dried. Standards such as capsaicin, gallic acid, and quercetin, the HPLC-grade solvents acetonitrile and methanol, and also HPLC-grade phosphoric acid, bile salts, KH_2PO_4, and $NaNO_2$ were purchased from Merck, Mumbai, India. Other chemicals such as pepsin, pancreatin, HCl, NaOH, $AlCl_3$, and 1,1-diphenyl-2-picrylhydrazil (DPPH) were obtained from Sigma-Aldrich, Mumbai, India.

Preparation Method of Ginactiv. The unique product Ginactiv with bioavailable gingerols was created and established by Aurea Biolabs Private Limited, Cochin, India, to preserve the gingerols inside a full spectrum of ginger matrix (FSGM) by the recreation of three different entities: ginger OR containing total gingerols, which includes mainly 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and 8-shogaol (Figure 1), the ginger essential oil, and the aqueous extract of ginger. The dried ginger rhizomes were crushed and subjected to supercritical fluid extraction at a pressure of 300 bar to obtain an OR with high gingerol content, containing 35% of the major gingerols. After that, the spent residue was further subjected to aqueous extraction to obtain carbohydrates (~41%,) dietary fibers (~11%), and ginger protein (~6%). Ginger essential oils were separated by steam distillation from dried ginger rhizomes in the temperature range of 100−120 °C and with a pressure of 1 kg. These three constituents are merged in a fixed ratio (ginger OR containing total gingerols (40−45%), ginger essential oil (3−5%), and ginger aqueous extract (45−50%)) and organized by a distinctive process of polar−nonpolar-sandwich (PNS) technology, and the total gingerols are well preserved inside the FSGM. Because the oral consumption of bioactive molecules has to reach the internal intestinal barrier and must pass over the walls of the cell membrane, an earlier bioavailability in the bloodstream is beneficial for the sensed biological action. The molecules need to be soluble inside the gastrointestinal tract to attain the internal intestinal walls and penetrable via the lipid bilayer of the cell membrane. As molecules, gingerols are hydrophobic in nature; they cannot simply dissolve in the intestinal tract and similarly they cannot effortlessly pass over the cellular membrane due to their larger arrangement. The bioactive molecules other than gingerols present in Ginactiv perform a vital role in the bioavailability of gingerols rather than the gingerols themselves. The β-sesquiphellandrene, α-zingiberene, β-bisbolene, and Ar-curcumene existent in Ginactiv generate a nonpolar matrix, whereas the dietary fibers, water-soluble proteins, and carbohydrates create a polar matrix. Ginactiv similarly maintains improved systems of the preservation of drug molecules from deterioration in the body, sustained release of the drug, biocompatibility, enhanced physical stability, and research development to industrial scalability. The arrangement of Ginactiv has been carried out by a constant superiority regulator sequencer with constant monitoring to retain the superiority standards of the product. The PNS technology permits gingerols to be distributed to the intestinal barriers and pass over the cell membrane through simple diffusion by improved solubility and enhanced absorption. The graphic illustration of the PNS technology scheme for Ginactiv is shown in Figure 2.

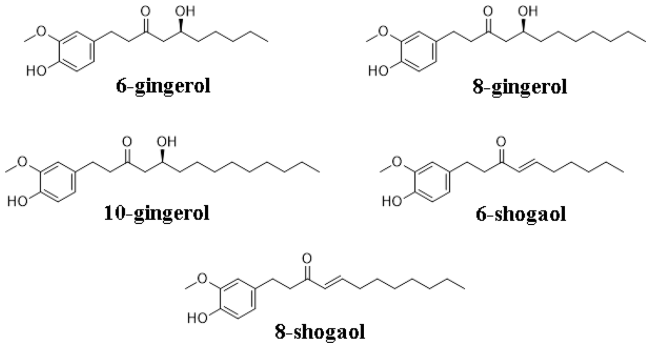

Figure 1. Chemical structures of the major gingerols.

Analysis and Quantification of Gingerols. An analysis of the content of major gingerols (6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and 8-shogaol) existing in the ginger OR and Ginactiv was executed by the method described by the United States Pharmacopeia (USP).[23] The analysis was carried out with a UPLC instrument (Shimadzu SP 20 AD, Nexera, Japan) by utilizing the column Xterra MS C18, 5 μm with volume 4.6 × 250 mm (Waters, USA). The mobile phase comprised a mixture of methanol, 0.1% phosphoric acid, and acetonitrile in a 1:44:55 ratio. The detection was carried out at a wavelength of 282 nm utilizing a photodiode array (PDA) detector.[24] The elution was carried out by an isocratic method with a flow rate of 1 mL/min. Ginactiv or ginger OR was weighed into a 100 mL volumetric flask and dissolved in methanol, and this solution was injected for the analysis of gingerols.

As per the USP method,[23] capsaicin was used as a reference standard for the quantification of total gingerols. The use of the USP system and method parameters ensures different ginger compounds appear at the subsequent retention times, relative to 1.0 for capsaicin: 0.8 for 6-gingerol, 1.5 for 8-gingerol A, 1.9 for 6-shogaol, 3.4 for 10-gingerol, 4.2 for 8-shogaol, and 5.8 for 10-shogaol. In accordance with the same system, the obtained UPLC chromatograms (Figure 3) confirmed the presence of the major gingerols with peak retention times at 5.32, 9.82, 11.9, 21.62, and 29.49 min for 6-gingerol, 8-gingerol, 6-shogaol, 10-gingerol, and 8-shogaol, respectively, whereas 10-shogaol was not detected in any of the samples.

According to the USP method, the total area corresponding to gingerols and shogaols can be compared with the area of capsaicin for

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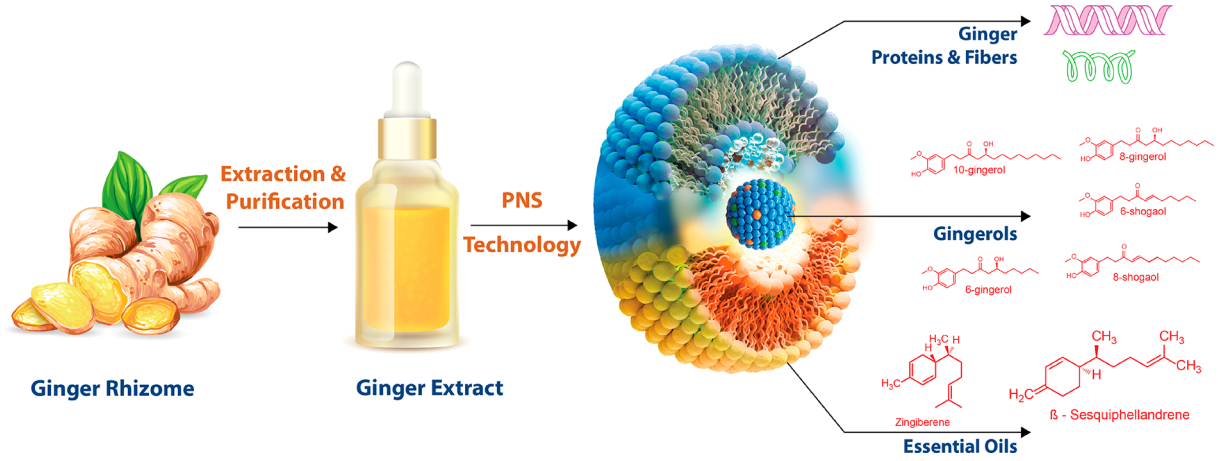

Figure 2. Graphical representation of the polar−nonpolar-sandwich (PNS) technology design of Ginactiv.

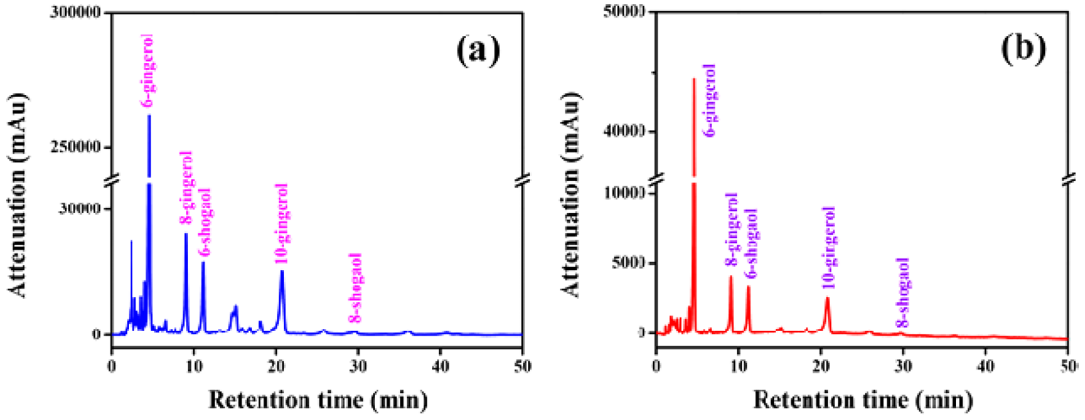

Figure 3. UPLC chromatogram of major gingerols in (a) ginger OR and (b) Ginactiv.

purity calculations, and the percentage of total gingerols can be expressed as

$$\text{total gingerols} = \left(\frac{r_T}{r_S}\right)\left(\frac{C_s}{W}\right) \times 10 \quad (1)$$

where r_T is the summation of peak responses from the sample solution, r_S is the peak response from the capsaicin standard solution, C_s is the concentration of the USP reference standard capsaicin (mg/mL), and W is the weight of sample taken (g).

Physical Parameters. Different physical characterizations such as moisture content, hygroscopicity, water solubility index, and bulk density were analyzed for Ginactiv by various adopted methods.

Moisture Content and Hygroscopicity. The moisture content was assessed by the well-recognized Association of Official Analytical Chemists (AOAC) method.[25] Triplicate samples of ginger OR and Ginactiv (20 mg) were weighed and then dried in a vacuum oven at 70 °C. The weighing and drying procedures were continued until a steady weight was reached. The hygroscopicity study of ginger OR and Ginactiv was assessed by dispersion of 1 g of the samples homogeneously on Petri dishes to allow for an elevated surface area for the humid air and sample. Each sample in the dishes was situated in desiccators under the conditions of 75% relative humidity and 23 °C utilizing nitric acid. The gain of weight of the samples was significantly lower after 90 min. Though hygroscopicity is established on the steadiness of moisture content, to relate hygroscopicities, the weight improvement per gram of the samples after being exposed to the environment with a relative humidity of 75% for 90 min was assessed.[26]

Water Solubility Index (WSI) and Bulk Density. The water solubility index of ginger OR and Ginactiv was assessed by utilizing the technique described by Amalraj et al.[22] Each sample (2.5 g) was thoroughly mixed with distilled water (30 mL) in a 100 mL centrifuge tube, incubated in a water bath at 37 °C for 30 min, and then centrifuged at 10000 rpm for 30 min. The supernatant was carefully collected in preweighed Petri dishes and dried in an oven at temperature of 103 ± 2 °C. The WSI (%) was assessed as the percentage of dried supernatant relative to the quantity of the original 2.5 g of ginger OR and Ginactiv.[22]

The bulk density (g/mL) was assessed through the placement of 10 g of Ginactiv into a blank 100 mL graduated cylinder, and the cylinder was placed on a ring stand. The ring stand was adjusted so that when the bottom of the cylinder was raised up to touch the ring, the base surface of the cylinder was close to 1 inch from the bottom of the ring stand. The ratio of the sample mass and the volume occupied in the cylinder determined the bulk density values.[22]

Chemical Composition Estimation. The chemical composition of Ginactiv, viz. total protein, dietary fiber, and total carbohydrates, was estimated using various standard methods. The total protein content of Ginactiv was estimated on the basis of the Kjeldahl method by calculating the total nitrogen content in the sample according to Indian Standard (IS) 7219.[27] The total dietary fiber content in Ginactiv was estimated using the principle of the enzymatic gravimetric method as per the AOAC 985.29 method.[28] The total carbohydrate content in Ginactiv was measured by a differential method based on the measurement of total solids, protein, fat, and ash content according to AOAC 986.25.[29]

Characterization of Ginactiv. *Fourier Transform Infrared Spectroscopy (FTIR).* FTIR spectra of ginger extracts and Ginactiv were recorded in the range of 400−4000 cm^{-1} by 32 scans for each sample utilizing a JASCO FT/IR-460 plus instrument; the samples were ground and mixed with KBr in a KBr/sample ratio of 4/1.

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Scanning Electron Microscopy (SEM). A SEM instrument (Vega3Tescan, Czech Republic) was employed to examine the organizational morphology of Ginactiv. The sample was scattered in aluminum stubs by dual-sided carbon tape, sputter-coated with a tiny covering of gold by a sputter gold coater, and scanned with an accelerating voltage of 20 kV.

Differential Scanning Calorimetry (DSC). The thermal stability performance of ginger OR and Ginactiv was investigated with a Q10 DSC differential scanning calorimeter (Mettler Toledo DSC822e, India). Approximately 5 mg of each sample was placed on a small pan. The empty pan was used as a reference. Each sample was heated from 0 to 400 °C at a heating rate of 10 °C under a nitrogen atmosphere.

Determination of Stability. The stability of Ginactiv was validated for 18 months at room temperature. At definite time intervals, the samples were taken and assessed for their physical look and gingerol content before and after storage. All determinations were executed in triplicate. The stability of gingerols was calculated using the formula

$$\text{stability of gingerols (\%)} = C_t/C_0 \times 100 \quad (2)$$

where C_0 is the initial concentration of gingerols and C_t is the concentration of gingerols at different time intervals.

***In Vitro* Release Profile of Gingerols from Ginger OR and Ginactiv.** An *in vitro* release study was executed in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) individually. The SGF and SIF were prepared as reported by Frenzel et al.[30] using suitable modifications. The SGF was produced by mixing 40 mL of a 0.65% pepsin solution (738 U/mg) with 1 L of distilled water with the pH value adjusted to 2 with hydrochloric acid. The SIF was prepared by mixing 250 mL of each of bile salts (5.16 g/L), pancreatin (4.76 g/L), sodium hydroxide (1.81 g/L), and potassium dihydrogen phosphate (8.09 g/L).

The *in vitro* release of gingerols from ginger OR and Ginactiv was assessed as per the modified method reported by Mukkavilli et al.[31] The samples were prepared in the SGF and SIF individually for the respective studies. Each sample (5 mL) was placed in a 12 kDa cutoff sized dialysis bag, and the bag was dipped in 100 mL of SGF and SIF, respectively, at a 37 °C thermostatic condition. The external fluid was stirred at 120 rpm. The samples (1 mL) were collected at specific time intervals, including 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 24, 36, and 48 h, and an equivalent amount of fresh medium was added to the flask to maintain the total volume. The samples were analyzed by a UPLC instrument as described earlier. The release rate was calculated by eq 3

$$\text{RR (\%)} = \frac{G_m}{G_0} \times 100 \quad (3)$$

where RR is the release rate of the major gingerols, G_m is the content of major gingerols at time t, and G_0 is the initial content of major gingerols.

Antioxidant Studies. *Total Phenolic Content.* The total phenolic content in Ginactiv was determined by the adopted Folin−Ciocalteu method defined by Rahman et al.[32] Gallic acid (standard) of different concentrations or Ginactiv (0.4 mL) was mixed with 2 mL of Folin−Ciocalteu reagent (1/10 v/v with distilled water). After 5 min 1.6 mL (75 g/L) of sodium carbonate solution was added. The tubes were vortexed for 15 s and permitted to stand for 30 min at 25 °C in the dark to develop the color. The absorbance was measured at 765 nm with a UV−vis spectrophotometer (Shimadzu UV-1800, Japan). A linearity curve was plotted with different concentrations of gallic acid against the absorbance of the corresponding concentration. From the curve, the total phenolic content in Ginactiv was calculated and expressed in mg/g gallic acid equivalents (GAE).

Total Flavonoid Content. The flavonoid content was determined by the aluminum chloride method using quercetin as a reference standard.[33] Ginactiv or different concentrations of standard (125 μL) were mixed with 75 μL of a 5% $NaNO_2$ solution. The mixture was permitted to stand for 10 min; after that 150 μL of 10% $AlCl_3$ was added and the sample was incubated for 5 min followed by the addition of 750 μL of 1 M NaOH. The final volume of the solution was made up to 2500 μL with distilled water. After 15 min of incubation the mixture turned pink and the absorbance was measured at 510 nm. A linearity curve was plotted using the absorbance of standards, from which the total flavonoid content of Ginactiv was calculated and expressed as mg/g quercetin equivalent (QE).

DPPH Radical Scavenging Assay. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity of Ginactiv was estimated as per the method described by Rahman et al.[32] Aliquots of different concentration of Ginactiv (5−250 μg/mL) in 1 mL of methanol were mixed with 2 mL of 0.1 mM DPPH in methanol solution and protected in a dark room for 30 min; after that the absorbances of the samples were measured at 517 nm. All of the experiments were done in triplicate. The DPPH reagent without a sample was used as a control, and ascorbic acid was utilized as a standard. The percentage radical scavenging activity was plotted against the resultant antioxidant substance concentration to obtain IC_{50} values.

RESULTS AND DISCUSSION

Physicochemical Parameters. The moisture contents of the ginger OR and Ginactiv were 1.96 ± 0.72% and 3.98 ± 1.17%, respectively (Table 1). Ginactiv showed a greater moisture content than the ginger OR due to the occurrence of polar fractions comprising dietary fibers, carbohydrates, and protein and also the occurrence of the hydroxyl groups which offer the active cores for moistness in Ginactiv.[21,22] The greater hygroscopicity outcomes of Ginactiv (0.94 ± 0.23 g/g) in comparison to ginger OR (0.36 ± 0.13 g/g) are also in acceptable agreement with the moisture content (Table 1).

Table 1. Physical Properties of Ginger OR and Ginactiv[a]

property	ginger OR	Ginactiv
moisture content (%)	1.96 ± 0.72	3.98 ± 1.17
hygroscopicity (g/g)	0.36 ± 0.13	0.94 ± 0.23
water solubility index (%)	9.62 ± 1.21	43.71 ± 3.42

[a]Values are means ± SD of three independent determinations.

The aqueous solubility of bioactive compounds is an essential physical parameter that influences their absorption and bioavailability. Consequently, the water solubility indexes of ginger OR and Ginactiv were assessed, and the values are given in Table 1. Ginactiv exhibited considerably greater solubility performance (43.71 ± 3.42%) in comparison to the ginger OR (9.62 ± 1.21%) in water, which may be due to the good encapsulation of gingerols between the polar and nonpolar ginger matrixes through PNS technology in this distinctive bioavailable invention. These outcomes are good agreement with the earlier PNS formulation of bioavailable curcuminoids and bioavailable bacosides.[21,22]

Ginactiv is a brown flowing powder with ∼15 g/100 g of major gingerols, which has registered 6.41 ± 1.04 g/100 g of total protein content, 11.58 ± 1.83 g/100 g of dietary fiber, 41.38 ± 2.47 g/100 g of total carbohydrates, and 0.42 ± 0.12 g/mL bulk density (Table 2). These outcomes confirmed that the Ginactiv-containing polar matrix has significant amounts of total protein, dietary fibers, and carbohydrates.

Characterization of Ginactiv. *FTIR Studies.* The FTIR spectra of the polar and nonpolar fractions of ginger, ginger OR, and Ginactiv are shown in Figure 4. The FTIR spectrum of the polar fraction of ginger is shown in Figure 4a. In the area of 3700−3000 cm^{-1}, the broad absorption band indicated the characteristic −OH stretching, which is a peak at 3286 cm^{-1}

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Table 2. Chemical Composition of Ginactiv[a]

constituent	composition
major gingerols (g/100 g)	15.02 ± 1.22
total protein (g/100 g)	6.41 ± 1.04
dietary fiber (g/100 g)	11.58 ± 1.83
total carbohydrate (g/100 g)	41.38 ± 2.47
bulk density (g/mL)	0.42 ± 0.12

[a]Values are means ± SD of three independent determinations.

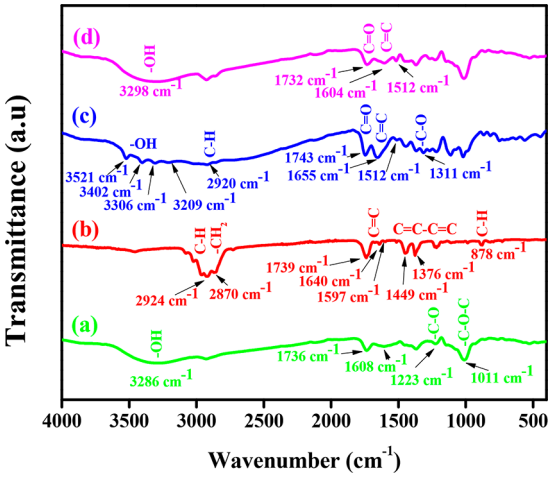

Figure 4. FTIR spectra of (a) polar and (b) nonpolar fractions of ginger, (c) ginger OR, and (d) Ginactiv.

related to the intramolecular hydrogen bonding in carbohydrates, protein, and cellulose.[34] A band at 1736 cm^{-1} is ascribed to the vibrations of the uronic ester and acetyl groups of hemicellulose or the ester linkage of the carboxylic group of the *p*-coumaric and ferulic acids of lignin or/and hemicellulose. A band at 1608 cm^{-1} characterizes the bending mode of moistness absorption. A band at 1223 cm^{-1} represents the aromatic C−O stretching, and an intense band at 1011 cm^{-1} is ascribed to the stretching vibration of the C−O−C skeleton in the pyranose ring, which indicates the occurrence of xylans, linked with cellulose and hemicellulose.[34]

The main components of the nonpolar fraction of ginger are ginger essential oils, which are mostly terpene compounds. A stretching vibration related to the C−H bond was observed at 2924 cm^{-1}, and a vibration mode linked to $-CH_2$ groups occurred in the nonpolar fraction at 2870 cm^{-1} (Figure 4b). The absorption bands of the nonpolar fraction of ginger (Figure 4b) at 1739, 1640, and 1597 cm^{-1} are related to C═C stretching and the bands at 1449 and 1376 cm^{-1} to the stretching vibration of C═C−C═C, and further C−H bond stretching is also detected at 878 cm^{-1}.[35,36]

In the FTIR spectrum of ginger OR (Figure 4c), the peaks at 3521, 3402, 3306, and 3209 cm^{-1} are ascribed to the O−H group stretching and 2920 cm^{-1} to C−H stretching, whereas the peak at 1743 cm^{-1} is related to the carbonyl group of gingerols and a peak at 1512 cm^{-1} is related to the aromatic molecules.[27,28] Intense absorption peaks detected at 1655 and 1311 cm^{-1} indicate C═C aromatic groups and C−O stretching groups of gingerols, respectively.

In the FTIR spectrum of Ginactiv, the possible interactions among the polar and nonpolar constituents with ginger OR were observed (Figure 4d). The characteristic −OH peaks of ginger OR, viz. 3521, 3402, and 3306 cm^{-1}, merged, became broader, and shifted to a peak at 3298 cm^{-1} in the spectrum of Ginactiv, which indicated that the band is not just due to the occurrence of an −OH group but also is due to the hydrogen bonding of gingerols between the polar and nonpolar matrixes. Moreover, the formulated Ginactiv displayed distinctive peaks such as 1732, 1604, and 1512 cm^{-1}, which were very near to the bands of gingerols (Figure 4c), indicating the existence of gingerols in the PNS technology with weak hydrogen bonding, and the slight shifts of the peaks may be due to the influence of other molecules available in the polar and nonpolar matrixes. Furthermore, the FTIR spectrum of Ginactiv shows the characteristic peaks of the ginger essential oil and the polar fraction of ginger with slight shifts, indicating that the gingerols were well preserved by the PNS technology in a bioavailable form through the interactions of polar and nonpolar fractions of ginger matrixes.

SEM Analysis. An SEM investigation was conducted to explore the morphology of Ginactiv, which is displayed in Figure 5. Ginactiv has a well dispersed, almost spherical shape

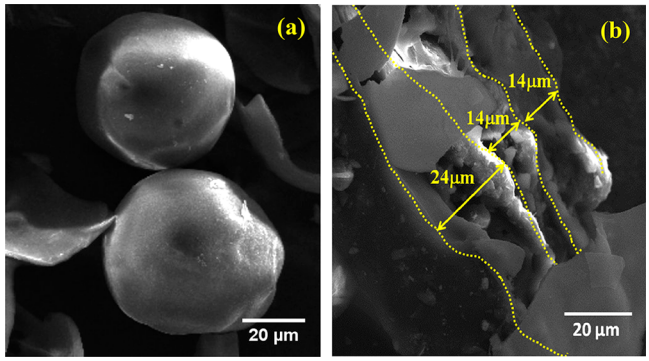

Figure 5. SEM images of Ginactiv showing (a) the well dispersed, almost spherical shape with a smooth surface and (b) a cross section of Ginactiv with three layers of polar−nonpolar-sandwich form.

with smooth morphology (Figure 5a), and the particle sizes range between 50 and 60 μm. Figure 5b evidenced the existence of three different layers with minor morphological variations, which might be the distinctive morphology of the polar−nonpolar-sandwich technology.[21]

DSC Analysis. The thermal stability of the total gingerols in the PNS formulation was assessed by a DSC analysis of ginger OR and Ginactiv, and the resultant thermograms are exhibited in Figure 6. Ginger OR exhibited three endothermic peaks at 90 °C, which revealed the characteristic peaks of other volatile compounds in the ginger OR, and the peaks at 240 and 313 °C were the characteristic peaks of gingerols and shogaols, respectively[37−40] (Figure 6a), whereas Ginactiv had a single broad endothermic peak at 238 °C and the absence of other endothermic peaks (Figure 6b), revealing the good preservation of total gingerols inside the PNS matrix with good stability.

Stability Studies. The stability of any product is one of the most significant aspects in analyzing its appropriateness for its application in different industries such as pharmaceuticals, functional foods, nutraceuticals, and food and beverages. Throughout the study period of 18 months at room temperature, no alternation was detected in the physical form or color of the Ginactiv. The major gingerols content in Ginactiv have been assessed at different time intervals and are shown in Figure 7. The outcomes demonstrated that there is

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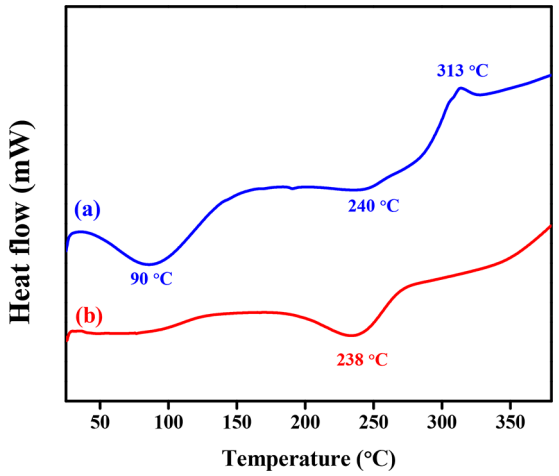

Figure 6. DSC thermogram of (a) ginger OR and (b) Ginactiv heated from 0 to 400 °C at a heating rate of 10 °C/min under a nitrogen atmosphere.

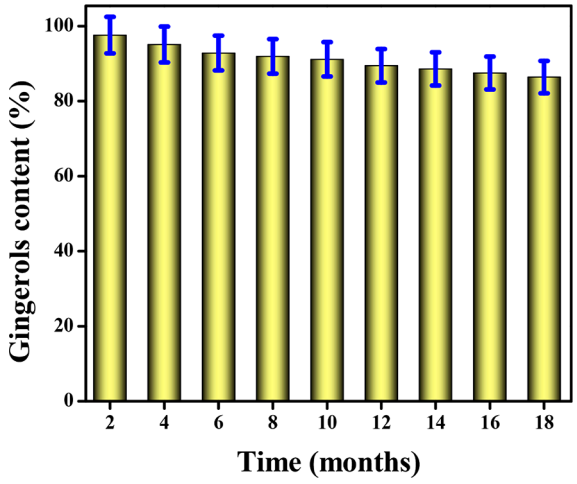

Figure 7. Stability study on Ginactiv at different time intervals of up to 18 months based on the major gingerols at room temperature.

no considerable change in the major gingerol content after 18 months, which discloses the good stability of Ginactiv due to the PNS technology.[21,22]

***In Vitro* Release Profile of Gingerols from Ginger OR and Ginactiv.** To understand the release profile of gingerols in the gastrointestinal tract, an *in vitro* study was conducted in SGF and SIF. From the results (Figure 8) it is clear that the gingerols in both ginger OR and Ginactiv were released at a lower rate in SIF than in SGF. After a 48 h incubation period in SIF, gingerol release rates were found to be 25.37% and 33.68% for ginger OR and Ginactiv, respectively (Figure 8a). However, after digestion in SGF, the gingerol release rate was considerably increased in comparison with SIF, even though the Ginactiv release rate (52.74%) was greater than that of the ginger OR (36.56%) (Figure 8b). The gingerol release rates of Ginactiv in both SGF and SIF were higher than those of ginger OR. After 24 h, gingerols exhibited a sustained release profile in both ginger OR and Ginactiv, irrespective of the media. Even after 48 h of this study the gingerols showed sustained release in SGF and SIF, confirming their existence in the system and suitability for oral dose administration.[31]

Antioxidant Studies. The total phenolic and flavonoid contents of Ginactiv were 29.13% GAE and 8.09% QE, respectively. The DPPH activity of Ginactiv increased in a concentration-dependent manner and gave a IC_{50} value of 43.04 ± 0.48 μg/mL (Figure 9), which showed that the

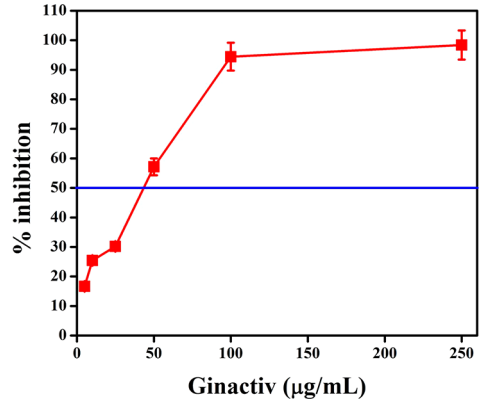

Figure 9. DPPH radical scavenging activity of Ginactiv on the basis of IC_{50} values.

effective free radical inhibition of Ginactiv is through hydrogen donation of higher phenolic and flavonoid contents. The

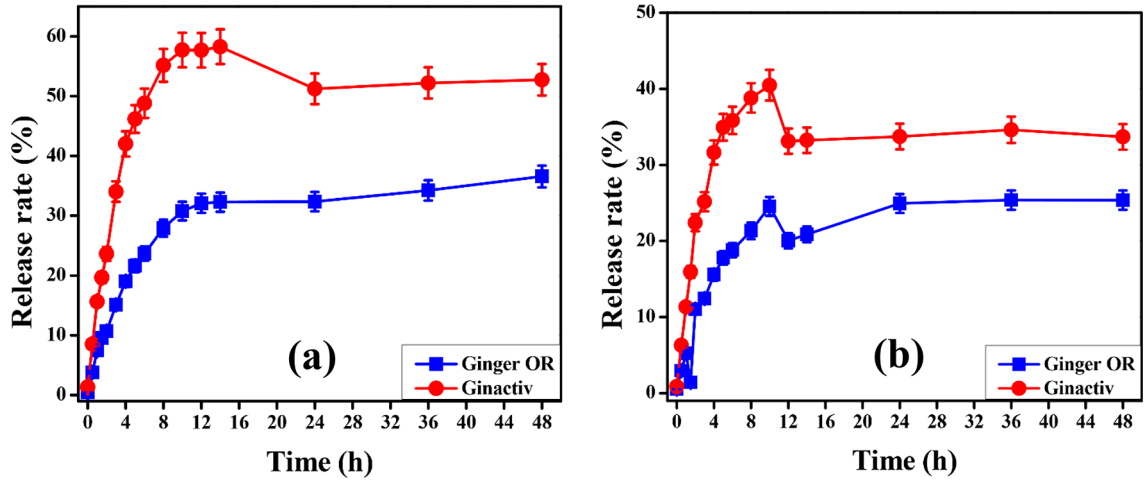

Figure 8. Gingerol release study of ginger OR and Ginactiv in (a) SGF and (b) SIF for a 48 h incubation period.

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results strongly suggest a high antioxidant capacity of Ginactiv even at lower concentration. Therefore, the existence of bioactive components in Ginactiv may be considered as an important natural source of highly bioaccessible and potentially bioavailable antioxidant molecules. The higher phenolic content and significant antioxidant activity are correlated with effective gastrointestinal digestion and gut health.[41]

Ginger is a common remedy utilized in traditional treatments or constituents of pharmacological preparations to cure digestive disorders.[41] The pharmacological benefits have been reported for ginger and its constituents such as gingerols, terpenes, lipids, polysaccharides, organic acids, and dietary fibers with their biological activities being anti-inflammatory, antioxidant, antiemetic, antidiabetic, antiarthritis, antimicrobial, antitumor, hypoglycemic, chemopreventive, hepatoprotective, gastorprotective, blood pressure and cholesterol lowering effects, and improvement in respiration.[42,43] These effects and activities are probably mediated by various molecular mechanisms such as the inhibition of cytokine expression, reduction of hormonal levels, regulation of cell signaling, and inhibition of reactive oxygen species functions.[43,44]

The mechanism of the digestive action of ginger has determined that ginger motivates production of bile acid via the liver and its secretion into bile, which plays a considerable role in the digestion and absorption of dietary fat.[43,45] The mechanism of action of ginger and its bioactive components, particularly gingerols, has been associated with various biological activities such as anti-inflammatory, antioxidant, improvement in gastrointestinal health, etc. Ginger has been shown to have a gastroprotective effect in different experimental models: for instance, ulceration induced in rats by NaCl, NaOH, HCl/ethanol, aspirin, reserpine, indomethacin, pylorus ligation, stress, and hypothermic restraint.[46,47] Gingerols are effective against HCl- and HCl/ethanol-induced ulceration.[47,48]

A diet containing ginger in a rat study enhanced the digestive enzyme activities such as sucrose, maltase, and intestinal lipase activities, stimulated the synthesis of chymotrypsin and trypsin, and also increased the activities of pancreatic lipase and amylase. The bioactive components of ginger stimulate the activity of brush border enzymes such as leucine amino peptidase, glycyl-glycine dipeptidase, and γ-glutamyl transpeptidase in the small intestinal mucosa.[49] Ginger decreases the level and severity of necrosis, inflammatory cell infiltration in the mucosa, desquamation, and edema. The administration of ginger reduced the levels of proinflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), prostaglandin E2 (PGE2), protein carbonyl content, colonic myeloperoxidase (MPO), and lipid peroxides and raised the expression of nuclear factor kappa B (NF-κB).[47,50]

The enteric nervous system is recognized as having a secondary memory due to its regulating characteristics on the gut.[51] The gut−brain axis activity normally reflects communications between the enteric nervous system and the central nervous system, which are mediated by bioactive peptides, metabolites, and gastrointestinal hormones.[52] Gut−brain peptides and gastrointestinal hormones regulate exocrine and endocrine roles, and bioactive peptides have an important mutual role in the peripheral organ systems and in the brain inside the regulatory system of the gut−brain axis.[53] Therefore, sustaining the gut−brain axis is very essential to enhancing neuroprotection, good mood, and cognition-enhancing capability, because the signals can be communicated among the digestive and the central nervous system via the gut−brain axis.[54] The intestinal microbiomes use ginger as a substrate that has a significant role in sophisticated intercellular and intracellular signaling arrangements that act as advisers in gut−brain communication by the stimulation of gut peptides such as motilin, glucagon like peptide-1, and ghrelin.[55,56] Accordingly, gingerols have curative effects against nervous system diseases such as psychiatric disorders, depression, stroke, neurosis, insomnia, dementia, and brain tumors.[57,58] Ginger influences the intestinal absorption of minerals and vitamins such as calcium, iron, zinc, and β-carotene due to its alteration ability of penetration characteristics presumably enhancing absorptive surfaces and thereby boosting the intestinal absorption of micronutrients.[41,59,60]

On the basis of the outcomes of this study the bioavailable form of gingerols, Ginactiv, is made up of the full spectrum of the ginger matrix by a cutting-edge PNS technology, giving it improved physicochemical properties, enhanced stability and antioxidant potential, and sustained release of gingerols due to the existence of the synergistic effects of the full spectrum of the ginger matrix. Furthermore, in agreement with the majority of studies, the bioavailable form of gingerols Ginactiv can be used in various nutraceuticals, functional foods and pharmaceutical formulations to preserve good health, particularly in gastrointestinal, digestive, and gut health, and can maintain the gut−brain axis.

To conclude, a novel bioavailable gingerol formulation, Ginactiv, was prepared by polar−nonpolar-sandwich (PNS) technology with the full spectrum of ginger matrix, which has been characterized and established by different instrumental techniques. SEM images clearly showed that Ginactiv was almost spherical in shape, with a well-dispersed, smooth morphology and a particle size ranging from 50 to 60 μm and possessing three layers of the PNS formulation. IR, DSC, stability, and antioxidant studies as well as the enhanced and sustained release profile of Ginactiv also confirmed the occurrence of gingerol with high stability in a bioavailable form in the PNS formulation. The existence of the synergistic effects of FSGM in Ginactiv indicate its use as a natural nutritional supplement to support gastrointestinal, digestive, and gut health improvement and to maintain the gut−brain axis.

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Notes

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