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Supplementary File

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Title: In vitro and in vivo assessments of 3-(3,4-Dihydroxyphenyl)-2-propenoic acid bioconjugated gelatin based injectable hydrogel for biomedical applications

UV-visible spectrum

Fig. S1 depicts the UV-visible absorbance spectrum of the samples gelatin, caffeic acid and CBG respectively. It has been observed that the less absorbance value at 280 nm for gelatin reasoned to the presence of few aromatic amino acid residues. Caffeic acid displayed maximum absorbance at 290 and 315 nm similar to the observations made by Belay (2012). Caffeic acid conjugated gelatin displayed a new absorbance peak at 290 nm and a shoulder at 315 nm, which confirm the conjugation. In order to further confirm the conjugation, the samples were subjected to NMR analysis and the results were discussed in detail in the following paragraph.

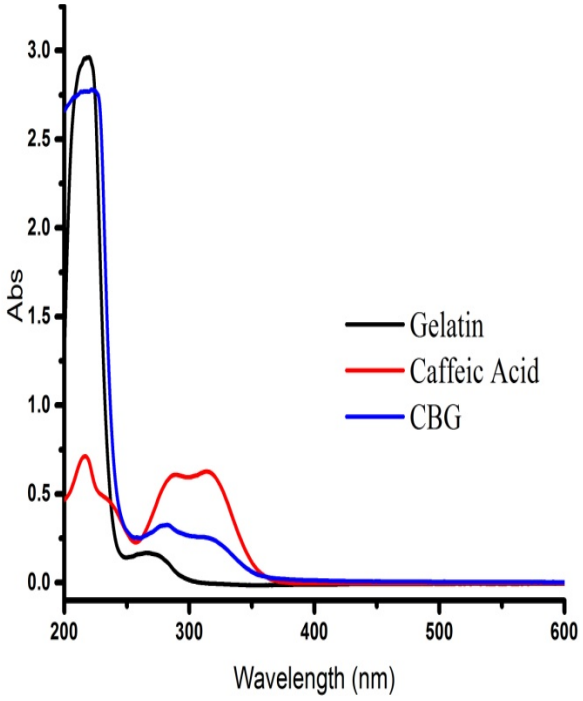

Fig. S1 UV–visible spectra of gelatin, caffeic acid, caffeic acid bioconjugated gelatin.

Proton NMR spectrum

Fig. S2 illustrates, ^{1}H NMR spectrum of caffeic acid, gelatin and CBG respectively. ^{1}H NMR spectrum of caffeic acid displayed chemical shifts at δ 6.34 (d, J = 16Hz, H7) and 7.42 (d, J= 16Hz, Hα) corresponds to α, β-unsaturation. The aromatic chemical shift values were given as, δ 6.65 (d, J=8 Hz, H5), δ 7.09 (dd, J = 2, J= 1.5Hz, H6), δ 7.17 (d, J= 1.5Hz, H2) (Anastasiadi et al., 2009). The ^{1}H NMR spectrum of gelatin and CBG displayed a broad chemical shifts from 7.2 to 7.4 corresponds to residual aromatic amino acid present in the protein backbone. However, CBG has significant chemical shift values at 6.3, 6.9 and 7.1 ppm similar to caffeic acid and confirms the conjugation.

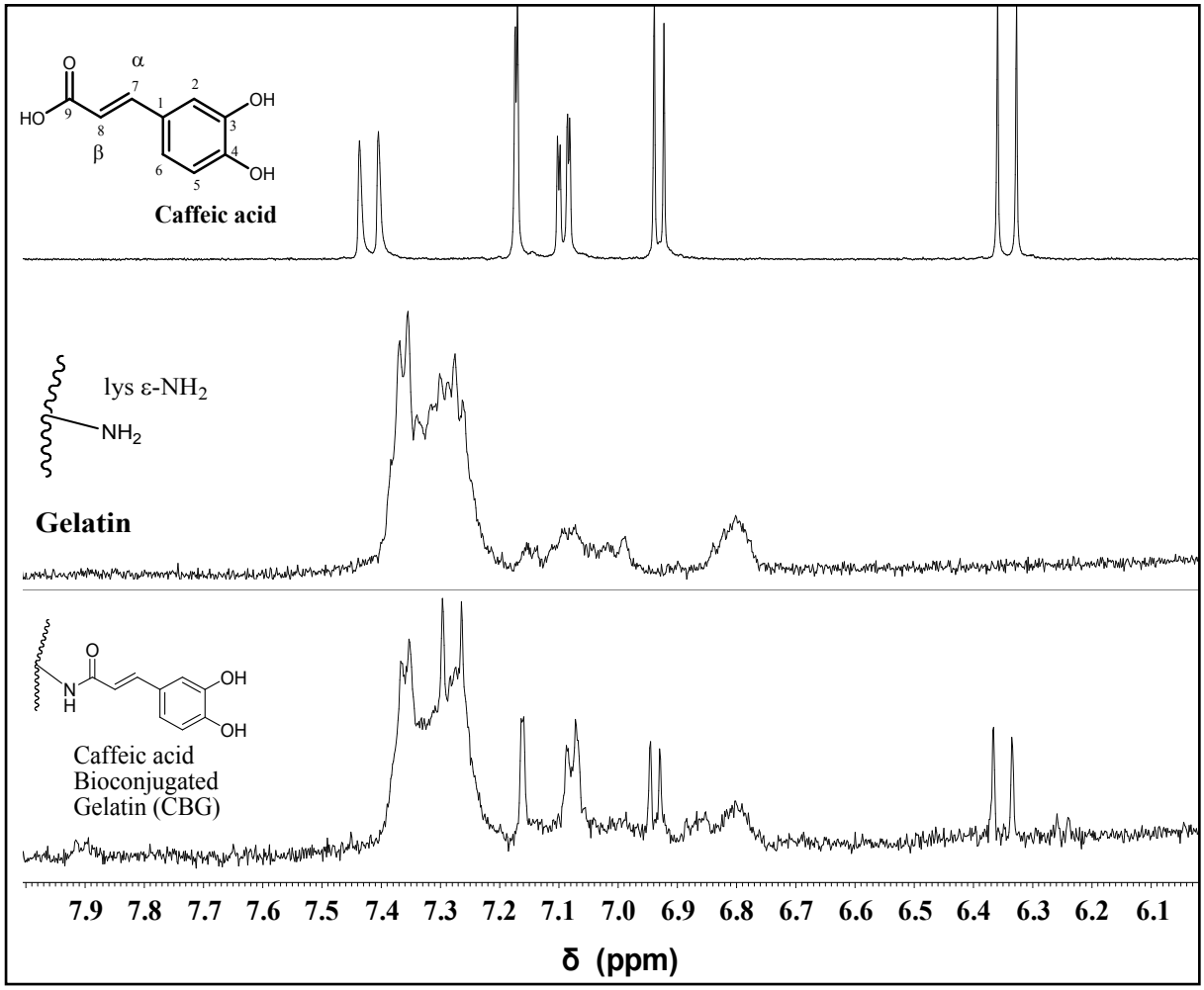

Fig. S2 ^{1}H NMR spectra of gelatin, caffeic acid, caffeic acid bioconjugated gelatin.

Contact angle

Contact angle measurements on thin film of gelatin and CBG samples displayed theta value of 66.2° ± 0.8 and 82.7° ± 1.2 as the angle of wettability respectively (Fig. S3). The significant increase in the contact angle of conjugated gelatin compared to gelatin could be due to the presence of alkyl chain and the aromaticity of the caffeic acid. The observed contact angle values less than 90° implied that good wettability of the surfaces and add value to the conjugated protein for its interaction with tissue surfaces when indented to the body system under live condition.

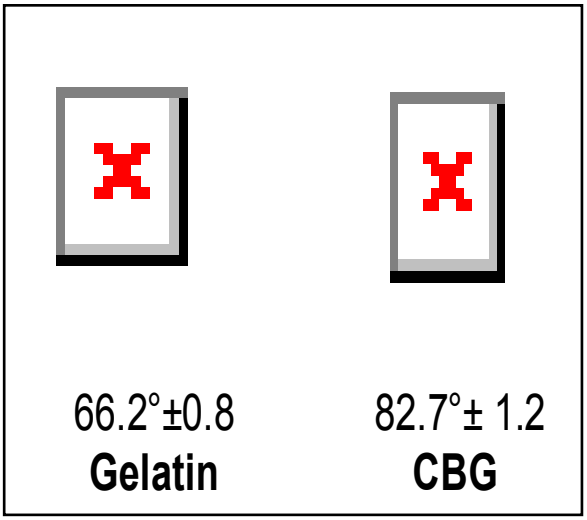

Fig. S3 Contact angle measurements for Gelatin and CBG samples

Antimicrobial property of CBG gel

Antimicrobial assessment of gelatin and CBG gel (Fig. S4) with selective Gram +ve and Gram-ve bacterial species suggested that the zone inhibition of 17 ± 0.8 mm was observed with Gram-ve bacteria (*E.coli*) and only less than 2 mm with Gram +ve bacteria (*B. subtilis*). Whereas native gelatin does not show antimicrobial property for the organism studied. Further, we found no growth on the surface of the gel and no recurrence. The

antimicrobial property exhibited by the CBG gel could be reasoned to caffeic acid and iodate ions in the gel.

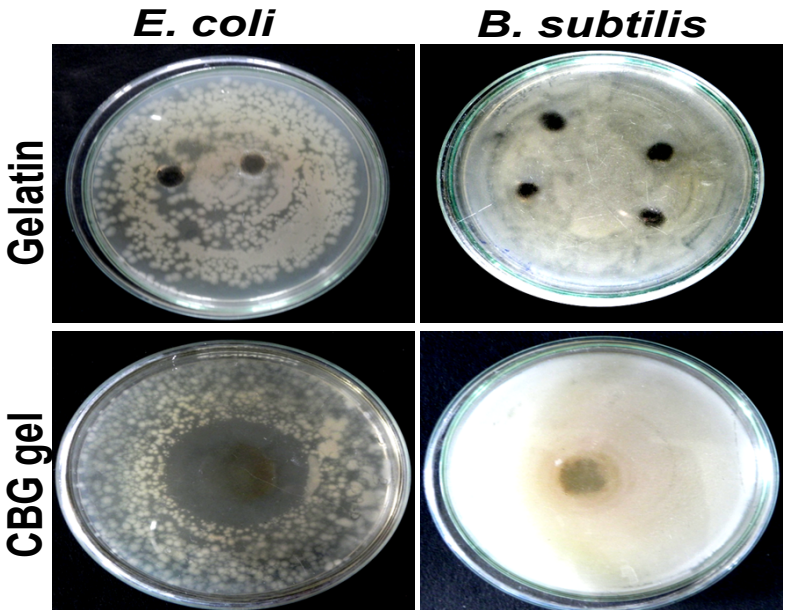

Fig. S4 Antimicrobial profile of CBG-gel in comparison with gelatin alone tested against *E.Coli* and *B. subtilis* (The clear zone exhibited by the sample has been considered as the zone of inhibition)

Radical scavenging activity of caffeic acid

Caffeic acid of various concentrations was subjected to assessment on radical scavenging efficacy using DPPH at room temperature under dark condition. Caffeic acid was dissolved in 99 % ethanol at a concentration (1 µg/ml), the concentrations were varied by taking 100 to 250 µl and made up to 1 ml using 95 % ethanol. To that 125 µl of 0.002 % of DPPH in 99.5 % of ethanol were added. After the scheduled time interval, a decrease in absorbance was measured at 517 nm. Tests were performed in triplicates and the scavenging effect was calculated as follows:

$$\text{Radical Scavenging Activity (RSA) \%} = (A - B)/B \times 100$$

where, A, B are absorbance of control and sample respectively. Ethanol without caffeic acid was taken as control and caffeic acid without DPPH was taken as blank.

Results obtained on the RSA of caffeic acid were plotted (Fig. S5), from that caffeic acid at 160 ng/ml was taken as IC_{50} value.

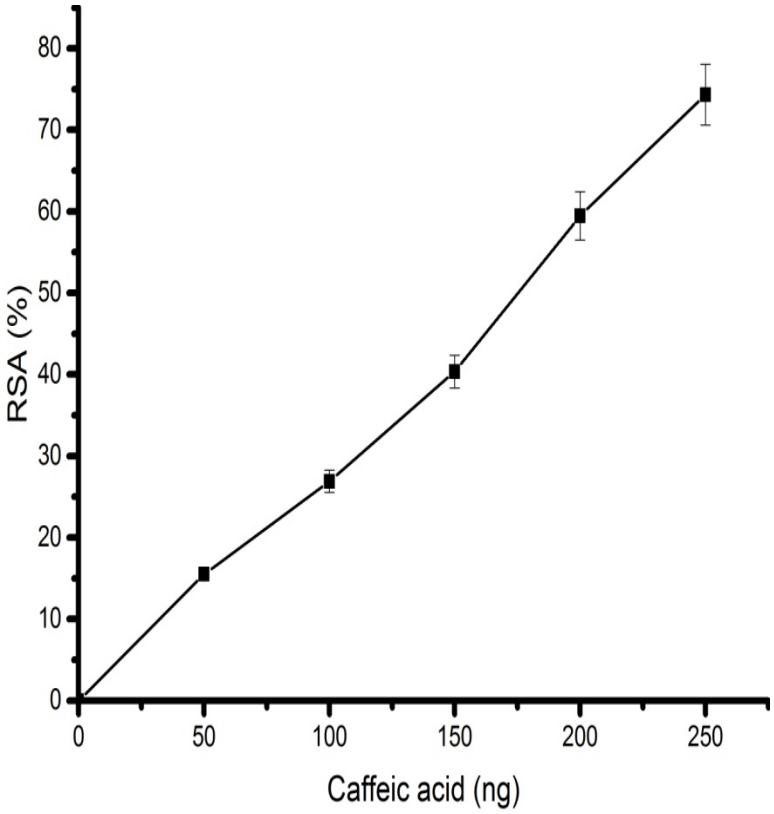

Fig. S5 : Free radical scavenging profile of caffeic acid at different concentrations (ng)

Fluid uptake ability of CBG gel at different pH environment

The swelling property of the CBG gel at different pH was studied by conventional gravimetric procedure. CBG gel (10%) of 2.5ml was prepared. The gel was pre weighed and immersed in different pH (3,6,7 and 9) and 0.1N HCl and 0.1N NaOH. Periodically the swollen gels were taken and the excess surface water were removed with the help of filter

paper and weighed. The percentage increase in the mass was calculated from the following equation, percentage increase in weight = (weight of swollen gel at given time – initial weight of the gel)/ initial weight of the gel X 100. Fig. S6 illustrates the swelling property of CBG gel.

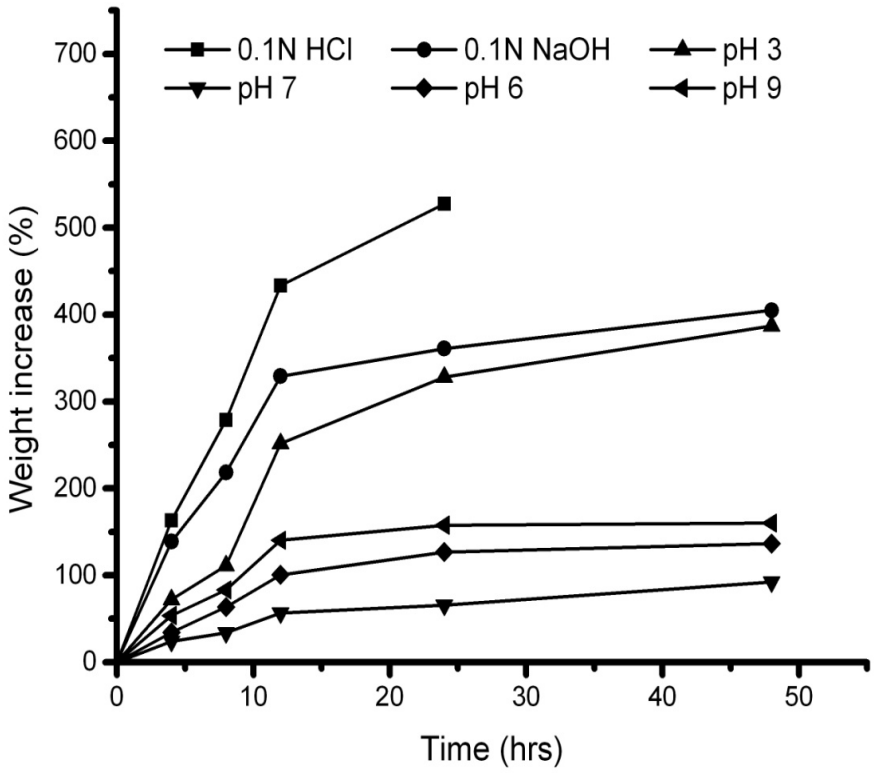

Fig. S6 Fluid uptake ability of CBG gel at different pH (3.0, 6.0, 7.0, 9.0, 0.1N HCl and 0.1N NaOH)