Polyacrylamide grafted *Eucalyptus camaldulensis* (EC-g-PAM) gum as an efficient binding agent in drug formulations

Zeeshan Ali¹, Fatima Qureshi¹, Dilshad Hussain¹,², Hira Munir¹ and Muhammad Sajid¹

¹ Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan
² HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

E-mail: dr.msajid@bzu.edu.pk

Keywords: *Eucalyptus camaldulensis*, drug formulations, modified gums, polyacrylamide, microwave irradiations

Abstract

The use of plant based gums in pharmaceutical sphere is desirable binding agents during pharmaceutical formulations. In this research, *Eucalyptus camaldulensis* gum is modified by microwave irradiation in order to estimate its binding characteristics for the fabrication of varied pharmaceutical formulations. Gum is analyzed in three forms; native, purified and grafted EC-g-PAM. The distinctive chemical assays for the characterization of carbohydrates indicated the existence of reducing sugars in all three types native, purified and grafted EC (EC-g-PAM) gum extracts. The relatively high phenolic contents i.e. 0.159 μg ml⁻¹ GAE of grafted EC extract indicate considerable antioxidant potential worthy of further investigations. In case of antimicrobial assay, grafted gum proved to be highly effective and produced a wider ring of no bacterial growth with *E. coli* while showed comparatively lesser change in the surrounding *S. aureus* concentration. Owing to its non-toxicity, it is incorporated into the paracetamol and it revealed excessive controlled drug-release profiles. Grafted gum possessed significantly controlled drug release profile, thus, the drug formulations based on the proposed gum, could be more beneficial site-specific oral drug carrier system.

1. Introduction

A major challenge associated with the development of robust and optimized therapeutic formulations is the advancement of drug carrier technologies that may facilitate innocuous distribution of therapeutics within the patient’s body and may customize their release profiles, assimilation, dissemination and elimination as required to procure their desired therapeutic efficacy coupled with patients’ compliance and convenience [1–3]. Advanced drug formulation strategies empower the pharmaceutical sphere with all the realistic tools required to develop and formulate up-to-date drug systems day-by-day with a view to comply with the upsurging therapeutic demands [4, 5]. At the moment, nearly all drug manufacturing approaches predominantly incorporate inert pharmaceutical excipients mainly as binders, in conjunction with pharmacologically active ingredients to achieve extended release of therapeutic agents in finalized dosage designs [6, 7]. For the most part, binders are employed in formulations to impart adequate mechanical characteristics by enhancing the adhesion and cohesion existing between individual components of powdered mixtures, as a consequence promoting the toughness of the finalized formulations produced [8, 9].

Binders are distinguished as familiar inactive additive substances to be incorporated in the formulations, along with the active pharmaceutical ingredients (APIs) to ensure appropriate tensile strength and to compensate granulate compaction by offering requisite cohesiveness to the fine powders, thus permitting the formulations to disintegrate and disseminate upon ingestion providing the APIs for assimilation [10, 11]. At the moment, development of plant-derived natural polysaccharides as binders has evoked considerable attention in biochemistry and biopharmaceutics on account of their availability, biodegradability, biosafety, renewability,
stability and sustainability [12–14]. Numerous native, synthetic and semi-synthetic polysaccharides such as celluloses, starches and plant-derived natural gums are the examples of substances employed in pharmaceutical solid dose formulations mainly as binders and directly compressible excipients [15, 16]. Plant-derived natural polysaccharides serve as more advantageous pharmaceutical excipients on account of their convenient accessibility, economical availability, non-toxicity, renewability and stability and are, therefore, utilized as matrix formers within controlled-release dosage forms on a large scale [17, 18]. Native and unmodified gums are also modified through chemical or biochemical means to have tailor-made substances and can, therefore, keep pace with other commercially accessible synthetic excipients for drug carrier systems [19, 20].

Gums, more or less, are the natural exudations of individual plant species that are produced from the degeneration of inner plant tissues, mostly from the cellulose disintegration during gummosis [21]. The gums acquired from plants are compact solid masses comprising of composite mixtures of the most abundant, most diverse and versatile class of naturally existing organic compounds—the polysaccharides (carbohydrates) [22]. Numerous shortcomings such as free flowing characteristics, uncontrollable rates of hydration and thickening, sizeable microstructure formation, inadequate clarity and microbial contaminations also exist related to the industrial exploitation of miscellaneous crude and unmodified gums. Thus, gums are rarely utilized in the native forms, due to their reduced stability and sustainability [15, 23]. Such limitations can be minimized by surface modifications (i.e. graft copolymerization). These gums involve certain specified chemical mechanisms including carboxymethylation, graft-copolymerization, oxidation and thiolation. Carboxymethylation is preferred due to its convenient handling, low cost and versatility [24, 25]. The grafted gums predominantly serve as an outstanding media for the sustained release of formulations [26]. Hence, these composite polysaccharides can conveniently be utilized as flocculants, viscosifiers and matrices for sustained-release [27]. Number of modification methods has been utilized but graft copolymerization has advantage of radical initiators to generate free radicals on gums’ backbone. However, to overcome certain complications experienced during the modifications, microwave irradiation is contemplated as an efficient technique to introduce enviable characteristics into the macromolecules [28].Grafting of polyacrylamide (PAM) with gum offer significant advantages in pharmaceutical formulations [29]. These advantages include better flocculating efficiency [30], water swelling properties [31] and drug loading [32, 33].

_Eucalyptus camaldulensis_, also known as long beak eucalyptus, river red gum or Murray red river gum, is a large, fast-growing evergreen tree of the genus _Eucalyptus_ [34]. The gum produced by this plant is brittle, has refreshing odor and can be easily powdered. In this research, gum obtained from _Eucalyptus camaldulensis_ is modified via microwave assisted method for the synthesis of polyacrylamide grafted _Eucalyptus camaldulensis_ (EC-g-PAM) gum which serves as efficient binder for drug formulation. Binding characteristics, biocompatibility and loading release ability are also evaluated as a proof of concept.

### 2. Experimental

#### 2.1. Chemicals and reagents

All of the analytical grade chemicals and reagents utilized in this research were either purchased from E. Merck (Darmstadt, Germany) or Sigma-Aldrich Chemical Company (St. Louis, MO) unless otherwise observed. Acetone, Barfoed’s reagent, Benedict’s reagent, Ciprofloxacain, Crystal violet stain, DMSO solution (Dimethyl sulfoxide), Distilled Water, DPPH Solution 1,1-Diphenyl-2-Picrylhydrazyl, Ethanol, Fehling Reagent A and B, Folin-Ciocalteau reagent, Gallic acid, Glacial acetic acid, Folin-Ciocalteau reagent Hydrochloric acid, Magnesium stearate, Maize starch, Methanol, Methylene blue solution, α-Naphthol, PBS (Phosphate buffer saline), Polyacrylamide, Sodium carbonate, Sodium hydroxide, Sulfuric acid, Triton X-100. The crude _Eucalyptus camaldulensis_ gum (kino) was procured in the form of dried exudations from the local market of district Multan situated in Punjab, Pakistan. The gum was well-pulverized to a fine powder prior to utilization for the research.

#### 2.2. Instrumentation

Analytical balance (Shimadzu AW-220 Kyoto, Japan), Centrifuge machine (Kokusan H-103N Tokyo, Japan), Elementer analyzer (Model Vario EL III Elementer, Germany), FT-IR spectrophotometer (Bruker Impact 400 IR, Germany), Haemacytometer (Marienfeld-Superior, Germany), Light microscope (Leica Microsystems, Germany), Microplate reader (Biotek, USA) Microwave oven (Dawlance DW-20M, Pakistan), Scanning electron microscope (Model JSM-5910 Jeol, Japan), Thermo-gravimetric and differential thermal analyzer (TG/DTA Perkin Elmer, USA), UV/Visible spectrophotometer (Jenway-6405), United States Pharmacopeia (USP) dissolution apparatus.
2.3. Procedure for the preparation of gum
The estimated amount of gum powder was soaked over-night in sufficient distilled water which swelled up to produce a gel-like mass. That concentrated viscous solution was further treated for purification by means of washing with excess of absolute ethanol along with continuous stirring. The precipitated gum formed as a reddish mass was obtained and dried out within hot-air oven at 40 to 50 °C. The obtained dried gum product was pulverized and powdered, passed through sieve and eventually stored in an air-tight container for further study.

As an alternative to the free radical initiator approach, microwave irradiation mechanism was employed exclusively to create free-radical regions on the polymer backbone. The general scheme of the methodologies employed for grafting of gum was as described elsewhere [28]. 1 g of E. camaldulensis gum powder was dissolved in 40 ml of distilled water. In 10 ml of distilled water, estimated quantity of acrylamide was dissolved and then mixed with the E. camaldulensis gum solution. The two solutions were transferred to a 1000 ml reaction vessel that was eventually kept onto the turn-table microwave oven (Dawlance DW-20M) for irradiation at power of 900 W for requisite duration of time varying from 2 to 4 min. The leftover gel-like mass was allowed to cool and washed with acetone. The precipitated polyacrylamide grafted polymer (EC-g-PAM) was obtained and dried out within hot-air oven. Subsequent to that, it was well-pulverized and sieved.

2.4. Physicochemical characterization of E. camaldulensis gum
FTIR analysis of native, purified and modified E. camaldulensis gum was carried out in the range of 4000 to 500 cm\(^{-1}\) for functional group analysis during chemical and biochemical graft-modification processes. The elemental investigation was done by Elemental Analyzer. The estimation of eight discrete elements Carbon, Oxygen, Magnesium, Silicon, Sulphur, Potassium, Aluminium and Calcium was undertaken. Scanning electron microscope (SEM) (Model: JSM 5910) was used to analyze the external morphology of native, purified and grafted specimens of gum. Thermo-gravimetric (TGA) instrument (Perkin Elmer, USA) was employed for the evaluation of time-based temperature dependence with reference to modified and unmodified gum specimens. The temperature range of 30 °C to 1200 °C was set with the constant heating rate of 10 °C per minute in nitrogen atmosphere.

2.5. Quantitative analysis of carbohydrates, antimicrobial assay, bacterial assay and biofilm inhibition analysis
Experimental detail of these tests is given in supporting information.

2.6. In vitro toxicity analysis by hemolytic activity

*In vitro* hemolytic capabilities of crude, purified and processed gum extracts were estimated. The extracts were prepared with varied concentrations i.e. 2%, 4%, 6%, 8% and 10% of gum. Hemolytic activity of the samples was conducted by following the procedure mentioned as follows.

2.6.1. Collection and count of erythrocytes
About 3 ml of fresh human blood was obtained in heparinized tubes, mixed gently and then poured into 15 ml sterile falcon tubes followed by centrifugation at 850 × g for around 5 min. The supernatant was decanted off and three times rinsing of RBCs was done by using 5 ml of isotonic sterile phosphate buffer saline (PBS) solution chilled at 4 °C and pH adjusted at 7.4. The washed red blood cells were suspended within 20 ml volume of chilled PBS. Counting of erythrocytes was performed on Hemocytometer.

2.6.2. Collection of supernatant for hemolytic activity
20 μl of gum extract sample was taken in Eppendorf tube and 180 μl diluted blood cell suspension was added and further incubated for half an hour at 37 °C along with agitation. Then, these Eppendorf were positioned on ice for 5 min and centrifuged for other 5 min at 1310 × g. 100 μl of supernatant was withdrawn from the tubes and diluted by adding 900 μl of chilled PBS. Then, all the Eppendorf tubes were sustained on ice. The supernatant was used to measure the absorbance of extracted hemoglobin from erythrocytes at 576 nm.

2.6.3. Analysis of hemolytic activity (estimation of released hemoglobin)
200 μl of the prepared mixture was placed into 96 well-plates. 0.1% triton X-100 was referred to as positive control while phosphate buffer saline solution (PBS) was referred in every analysis as a negative control. At 576 nm, absorbance was recorded that showed the amount of hemoglobin within the extracellular environment. Every assay was executed in triplicate. The %age hemolysis was calculated by using the following formula:
to graft copolymerization and stimulates the generation of additional homo-polymers. On exposure to for extended time-durations induces degeneration of the polysaccharide backbone. This serves as an alternative generation, and as a consequence, homo-polymerization is reduced to minimal. Besides, microwave irradiation as a speci
alteration of native gum utilizing polyacrylamide under time-speci

2.7. Preparation of tablets
Direct compression methodology was employed for the formation of tablets. The active pharmaceutical ingredient was paracetamol. Magnesium stearate (2.5% w/w) was used as lubricant and maize starch (5% w/w) was employed as the dis-integrate. All material was processed in a climate-controlled room at 21 °C temperature.

3. Results and discussion

3.1. Polyacrylamide grafted Eucalyptus camaldulensis (EC-g-PAM) gum synthesis
The synthesis of polyacrylamide grafted Eucalyptus camaldulensis (EC-g-PAM) gum is carried out through microwave irradiation that assisted the creation of radicals on the gums’ polymeric backbone. Therefore, for the most part, synthesis is based on free radical generation [35]. The synthesized polymer are obtained in the utmost proportion at an irradiation time-span of 4 min. However, by means of incremental time duration, additional modification percentage can be attained as well. The contemporary investigations revealed the alteration of native gum utilizing polyacrylamide under time-specific irradiation. Graft copolymerization serves as a specified modification technique by which grafting mechanism is propagated through radical sites generation, and as a consequence, homo-polymerization is reduced to minimal. Besides, microwave irradiation for extended time-durations induces degeneration of the polysaccharide backbone. This serves as an alternative to graft copolymerization and stimulates the generation of additional homo-polymers. On exposure to microwave radiations, hydroxyl (–OH) groups possessing polar-nature assimilate microwaves, as a result, distinctive immobilized and localized rotations cleave the polar bonds and generate free radical sites within the E. camaldulensis molecules. Meanwhile, absorption of microwaves occurs at certain infinitesimal polar molecules such as water (H₂O) as well, yet, free radicals are never generated and only thermogenesis along with rotations be the ultimate outcomes of that absorbed heat. As polyacrylamide has been a well-known responsive towards grafting reactions, so appropriate substitutions carried out on the exteriors of polysaccharide can intensify hydrophobic character. Owing to these substitutions, modified EC-g-PAM gum demonstrate enhanced hydrophobicity and diminished aqueous viscosity. Moreover, bio-compatibility and water consistency are the two additional indispensable characteristics possessed by the Eucalyptus gums.

3.2. Characterization of EC-g-PAM gum
A comparison of FTIR analyses is done for crude, purified and synthesized E. camaldulensis (EG-g-PAM) gum that presented diverse changes in their chemical organizations. Figure S1 is available online at stacks.iop.org/ MRX/7/045307/1media shows the FT-IR spectrum of native E. camaldulensis gum. In FTIR spectrum, the fundamental vibrations observed within the 4000–2500 cm⁻¹ region are attributed to –OH, CH and –NH stretching, which are abundantly present in gum. Moreover, C–H stretching vibrations of methylene (–CH₂) groups on the gum’s polymeric backbone, less intense peak appeared at 1200 cm⁻¹. Similarly, peaks appearing in the 2000–1500 cm⁻¹ region correspond to carbonyl and C–C stretching, the most intense band in the 1830–1650 cm⁻¹ region of the spectrum is due carbonyl groups. FT-IR spectral observations of purified E. camaldulensis gum is also shown in figure S2. A broader peak at 3350 cm⁻¹ is due to hydroxyl group stretching vibrations which also exhibited hydrogen bonding with water molecules. One other at 2916 cm⁻¹ corresponds C–H stretching vibrations of methylene (–CH₂) group. IR band appearing at 1740 cm⁻¹ and 1200 cm⁻¹ correspond to carbonyl group and C–C stretching vibrations. Distinct peaks between 1200 cm⁻¹ and 800 cm⁻¹ for glycoside linkages represent highly coupled C–C–O, C–O–C and C–O–H stretching modes. In case of EG-g-PAM, IR spectrum is different from those of crude and purified gums. FTIR spectrum of EC-g-PAM (figure S3) the peak at 3409 cm⁻¹ show overlapping of –OH stretching band of EG and N–H stretching band of amide (–NH₂) group. A clear peak at 3000 cm⁻¹ in EG-g-PAM is observed due to C–H stretching vibrations, while the detection of other bands at 1740 cm⁻¹, 1676 cm⁻¹ and 1410 cm⁻¹ are predicted as C–O and C–N stretching vibration bands. During graft-copolymer synthesis of E. camaldulensis gum along with polyacrylamide, a distinct peak at 1289 cm⁻¹ is characteristic for C–N stretching of secondary amide groups and hence authorized grafting. These additional bands within grafted EC as compared with crude EC confirmed the grafting of polyacrylamide chains onto the polymeric backbone of EG.

Scanning electron microscope of native, purified as well as modified EC-g-PAM gum provides practical comprehension with reference to graft copolymerization. A comparative analysis of the SEM micrographs of crude EC gum and EC-g-PAM gum is shown in figure 1. SEM image show the profound morphological modifications, evidenced in the form of transition from granular to fibrillar arrangement, which is due to the

\[
\text{Lysis of RBCs(\%age)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of triton X – 100}} \times 100
\]
grafting of polyacrylamide (PAM) chains onto EC gum’s polymeric backbone. Prior to modification, particulates of the native gum exhibited rough and irregular external morphologies (figure 1(A)). On the other hand, SEM micrographs of purified gum revealed roughness, yet to a lower extent, as compared to the crude gum (figure 1(B)) and lesser topographical irregularities are observed in case of purified gum. It is, therefore, evident that granular morphology of EC is lost as a consequence of grafting and got transformed into fibrillar form and an even, smooth and non-porous texture is visible for grafted product (figure 1(C)). From these results, two specifications could be conferred: at first, when dissolved in water, the native *E. camaldulensis* gum converted into viscous solution, primarily due to the hydrogen bonding. Secondly, the synthesis of *E. camaldulensis* gum utilizing polyacrylamide imparted softness to the micelles’ particulates that organized themselves homogeneous in all respects, along with even and non-porous textures and reduced viscosities for aqueous solutions. So, the grafting mechanism can be authenticated by SEM showing difference is surface of *E. camaldulensis* gum which changed into fibrillar form, from irregular surface morphology.

Energy dispersive x-ray spectroscopy (EDS) analysis support our observation (figure 2). The percentage of each element in crude, purified and EC-g-PAM gum are mentioned in tables S2–S4 (supporting information).
respectively. The elemental analysis (atomic percent as well as mass percent) of native gum samples indicate the highest concentrations for element carbon i.e. 51.81% and 59.30% respectively where oxygen exhibited second highest fractions by weight (46.71%) followed by potassium (0.72%) and calcium (0.41%). Four elements including potassium, calcium, magnesium and sulphur are also present in trace concentrations, not exceeding 1%. In case of purified EC gum, the sequential order of percentage composition by weight of the elements as carbon, oxygen, potassium, calcium, magnesium, silicon, sulphur and aluminium with 52.81%, 45.09%, 0.63%, 0.44%, 0.38%, 0.32%, 0.23% and 0.12% respectively. The atomic fractions provide a similar sequence for these elements except for magnesium (0.21%) that show slightly higher concentration in comparison with potassium (0.15%). However, the enhanced percentages of carbon and some other elements in correspondence to the native EC gum can be attributed to removal of impurities from the gum. For polyacrylamide grafted Eucalyptus camaldulensis gum (EC-g-PAM), carbon maintain its utmost bulk proportions i.e. 53.24% followed by oxygen with 45.37% weight composition. Other elements such as potassium, calcium, silicon, sulphur and magnesium are present in minute mass concentrations viz. 0.59%, 0.34%, 0.19%, 0.15% and 0.12% respectively (table S4). Similarly, the atomic fractions are observed to be 60.65%, 38.80%, 0.21%, 0.12%, 0.09% for carbon, oxygen, potassium, calcium, silicon respectively, however, sulphur and magnesium show the least and comparable atomic proportions of 0.07%.

Thermo-gravimetric analysis (TGA) is carried out to access the changes in masses of the unmodified, purified and modified samples of E. camaldulensis gum with respect to temperature. Four distinctive zones are

![Figure 2. Energy dispersive x-ray spectroscopy analysis of (A) crude EC gum (B) purified EC gum and (C) EC-g-PAM gum.](image-url)
observed in the TGA curve of native EC gum, characteristic of the percentage reduction in weight. Figure S4 (supporting information) is the TGA for native E. camaldulensis gum and which reveal that initial decomposition set about at 49.66 °C after a time-span of 2.50 min with a correspondent 8.42% loss in mass that could be assigned to the removal of moistness in the EC gum sample. The succeeding zone of weight loss within the range of 87.87 °C to 241.61 °C showing 5.70% additional reduction in weight which occurs due to thermal decomposition of lower molecular compounds within the polysaccharide chains. The significant change is observed in the range of 241.61 °C to 395.64 °C and percentage reduction in weight is approximately 20.74% which may be due to partial decomposition of higher molecular weight compounds. A weight loss of 12.85% is recorded within the temperature range of 395.64 °C to 618.40 °C that characterize the decomposition of the polymeric backbone incorporating primary alcoholic functionalities. A time-lapse of about half an hour, resulted in an approximate reduction of 50% in mass at 618.40 °C. Similarly, TGA curve of purified gum also exhibit similar four distinctive thermal peaks, comparable to that of the crude gum on the whole, along with the similar % age mass reduction (figure S5). After initial 9% loss, all the subsequent degradation peaks are sharper, in comparison to crude EC gum, particularly attributed to high purity of the gum. Figure S6 show the TGA curves of EC-g-PAM where weight loss is distinctive and steeper, in comparison to native and purified gums. Thermal decomposition at 87.58 °C show the thermal stability of the graft copolymer, in comparison to crude gum that formerly exhibit the initial decomposition at 49.65 °C. Thus, the earliest weight loss from 87.58 °C to 245.16 °C is observed to be 7.05%. Then, weight loss from 87.58 °C to 245.16 °C indicate the degradation of the simpler, low molecular weight compounds existing within the polysaccharide chains. Third zone start from 245.16 °C to 313.89 °C is due thermal decomposition of somewhat more complex compounds such as secondary alcoholic groups (–CHOH). Fourth region of degradation from 313.89 °C to 485.69 °C represent the degradation of remaining polymeric backbone i.e. –CHOH functionalities. One more distinctive zone is evident between 485.69 °C and 614.84 °C, which is due to the grafted amide group (–CONH2) decomposition on the polysaccharide chain of EC gum. This distinct curve is due to amide group which confirms the graft modification process.

3.3. Qualitative analysis of carbohydrates
Carbohydrates present in native, purified and grafted EC gum extracts is done by distinctive chemical assays including Molisch’s test, Benedict’s test, Fehling’s test, Methylene blue test and Barfoed’s test, respectively. The violet-colored ring that appear at the juncture signifies the presence of carbohydrates via Molish’s test. The formation of orange red colored precipitates indicate the presence of reducing sugars in Benedict’s test. In Fehling’s test, red-colored precipitates also confirmed reducing sugars. While methylene blue solution turned colorless which indicate exposure to the reducing sugars. The generation of brick red colored precipitates indicate positive result for reducing sugars in Barfoed’s test. Detail of these tests is given in table S5.

3.4. Antioxidant analysis
Phenolic and poly-phenolic compounds are the main class of natural antioxidants that strengthen the oxidative stability of biological systems due to their redox properties. These compounds play significant roles in neutralizing free radicals, quenching singlet oxygen, decomposing hydro-peroxides generated during normal metabolism and energy production within the body. The study allowed the evaluation of the antioxidant potentials of three extracts with different phenolic contents and as up to now no literature is reported about phenolic contents of the gum in this plant. All three extracts have comparable phenolic contents of 0.106, 0.129 and 0.159 μg mL⁻¹ gallic acid equivalents (GAE) for crude, purified and grafted gum respectively (table S6). However, the results show strong graft-dependent increase in total phenolic contents. High phenolic contents of grafted extract indicate high antioxidant potential, attributed to varying hydrogen and electron-donating capacities of those phenolic compounds and suggested considerable antioxidant compounds worthy of further investigations. The standardized graph curve of gallic acid for the determination of whole phenolic contents is shown in the figure 3.

DPPH assay is among the most popular spectrophotometric methods for the determination of antioxidants in plant extracts and estimate the antioxidant activity of compounds. Detail of DPPH radical scavenging of crude, purified and grafted EC gum extracts is presented in table S7 (supporting information). The obtained results suggest that all three extracts are apparently good free radical scavengers and probably have the ability to inhibit autoxidation of lipids. Thus, these extracts can be beneficial for the treatment of various diseases where lipid peroxidation serves as an important mechanism for pathogenesis. The results indicate that crude gum samples exhibit maximum activity (79%) while grafted EC gum show minimum (64%) and the purified samples have an intermediate antioxidant scavenging activity (70%) which further prove the potential antioxidant effectiveness of E. camaldulensis gum.
3.5. Antimicrobial assay

Antimicrobial test is carried out to determine the effectiveness of the *E. camaldulensis* gum against two resistant pathogens - *E. coli* and *S. aureus*. For that purpose, inhibition produced by crude, purified and amended gum samples are compared to establish their appropriate antimicrobial roles (table S8). Since the amount of space around each plate - zone of inhibition -indicate the lethality of that gum extract against the *E. coli* and *S. aureus* concentration, highly effective grafted gum samples produce a wider ring of no bacterial growth i.e. 16 mm wide as compared to crude and purified gum. These results are recorded with reference to *E. coli* which demonstrate comparatively lesser change in the surrounding *E. coli* concentration. Minimum zone of inhibition is observed in case of crude gum samples i.e. 12 mm wide ring whereas purified gum samples exhibit a moderate zone of inhibition i.e. 14 mm wide ring. In case of *S. aureus* pathogen, the effectiveness of gum decrease with grafting, exhibiting lesser bacterial sensitivity i.e. 13 mm wide region of growth inhibition as compared to crude and purified gum extracts that show maximum—14 mm wide and intermediate—12 mm wide zones of inhibition, respectively. Overall results show slightly more antimicrobial effect of gum extracts against *E. coli* than *S. aureus*. The obtained results are in agreement with the antimicrobial capability of *Eucalyptus camaldulensis* leaf extracts against *Bacillus subtilis* (Gram-positive), *Klebsiella spp.* (Gram-negative), *Pseudomonas aeruginosa* (Gram-negative), *Salmonella typhi* (Gram-negative), *Staphylococcus aureus* (Gram-positive) and *Yersinia enterocolitica* (Gram-negative) strains. The results further demonstrate broad spectrum action of dichloromethane fraction, methanol extracts and residues against these test organisms altogether except petroleum ether fraction that do not possess activity at all. Furthermore, the phytochemical analysis of this plant show the existence of cardiac glycosides, saponins and tannins. Thus our findings predict the use of *Eucalyptus camaldulensis* gum as an antimicrobial agent.

3.6. Biofilm inhibition

Bacteria are more resistant to most of antimicrobial agents within a biofilm and can withstand critical environments and escape the hosts’ immune systems. In vivo studies have shown the potential of EC gum extracts to reduce the virulence of *Bacillus subtilis* and *Escherichia coli* and predicted that they can constructively influence the forthcoming human medicine either by facilitating the dispersion of pre-fashioned biofilms or inhibiting the generation of innovative biofilms. Our biofilm inhibition assay results show that biofilm formation can be prevented up to 50% by using crude gum (table S9). However, grafting reduce the biofilm dispersion to some extent but it is still very effective. Previously, no reports concerning the biofilms’ inhibition capability of EC gum are available in literature. The evaluation of crude, purified and grafted EC gum samples is carried out by a rapid assay against two bacterial strains - *B. subtilis* and *E. coli*. All three gum extracts possess significant anti-biofilm potentials. Crude gum extracts demonstrate the highest proportions of biofilms dispersion i.e. 49.70% against *B. subtilis*, followed by 45.50% inhibition against *E. coli*. On the other hand, purified gum extracts also show remarkable activity against both bacterial strains. In case of *E. coli*, an effective anti–biofilm activity of 40.41% is evaluated that is lesser in comparison to *B. subtilis* (43.78%). The grafted gum extracts exhibit the best anti–biofilm activity against *B. subtilis* and limit the production of biofilms up to 39.12%. However, a lesser dispersion percentage of 37.23% is observed with *E. coli* (figure 4).
In vitro toxicity by hemolytic activity

Erythrocytes membranes are affected by the consumption of natural bioactive compounds originating from ethnobotanical plants and their products (including gums). There exists negligible previous toxicological evidences with regards to E. camaldulensis gum within the established literature. The native, purified and synthesized E. camaldulensis gum samples underwent screening analyses utilizing a fast assay on human erythrocytes. The percentage lysis increased with increasing concentrations of the gum samples (table 1). Very low toxicity is observed by purified gum but somewhat comparable hemolytic capability is recorded for crude and grafted samples at elevated concentrations. The results indicate that there exists prohibitive correlation between the hemolytic activity and concentration of gum. At the same concentrations, crude extracts exhibit considerably higher hemolytic activity in comparison with other gum extracts and resulted in elevated total percentage lysis of 3.23%, 3.85%, 3.97%, 4.27% and 4.5% at 2%, 4%, 6%, 8% and 10% gum concentrations, respectively. In case of grafted gum extracts, the results demonstrate comparatively lower lysis percentage than crude extracts yet higher than that of purified gum extracts. Similarly, at gum concentrations of 2%, 4%, 6%, 8% and 10% the observed lytic percentages is 2.56%, 2.97%, 3.18%, 3.72% and 4.59%. However, at higher concentration i.e. 10%, no significant change in activity between the crude and grafted extracts is observed. Purified gum extracts on the whole exhibit lower toxicological activity i.e. 2.11%, 2.45%, 2.93%, 3.69% and 3.88% respectively at 2%, 4%, 6%, 8% and 10% gum concentrations.

Table 1. Hemolytic analysis of crude EC, purified EC and grafted EC gum at varied concentrations.

| Sample            | Concentration of Gum |
|-------------------|----------------------|
|                   | 2%       | 4%       | 6%       | 8%       | 10%      |
| Crude EC gum      | 3.23 ± 0.02 | 3.85 ± 0.03 | 3.97 ± 0.01 | 4.27 ± 0.03 | 4.5 ± 0.01 |
| Purified EC gum   | 2.11 ± 0.02 | 2.45 ± 0.01 | 2.93 ± 0.05 | 3.69 ± 0.02 | 3.88 ± 0.03 |
| EG-g-PAM gum      | 2.56 ± 0.01 | 2.97 ± 0.03 | 3.18 ± 0.02 | 3.72 ± 0.02 | 4.59 ± 0.01 |

3.7. In vitro toxicity by hemolytic activity

Erythrocytes membranes are affected by the consumption of natural bioactive compounds originating from ethnobotanical plants and their products (including gums). There exists negligible previous toxicological evidences with regards to E. camaldulensis gum within the established literature. The native, purified and synthesized E. camaldulensis gum samples underwent screening analyses utilizing a fast assay on human erythrocytes. The percentage lysis increased with increasing concentrations of the gum samples (table 1). Very low toxicity is observed by purified gum but somewhat comparable hemolytic capability is recorded for crude and grafted samples at elevated concentrations. The results indicate that there exists prohibitive correlation between the hemolytic activity and concentration of gum. At the same concentrations, crude extracts exhibit considerably higher hemolytic activity in comparison with other gum extracts and resulted in elevated total percentage lysis of 3.23%, 3.85%, 3.97%, 4.27% and 4.5% at 2%, 4%, 6%, 8% and 10% gum concentrations, respectively. In case of grafted gum extracts, the results demonstrate comparatively lower lysis percentage than crude extracts yet higher than that of purified gum extracts. Similarly, at gum concentrations of 2%, 4%, 6%, 8% and 10% the observed lytic percentages is 2.56%, 2.97%, 3.18%, 3.72% and 4.59%. However, at higher concentration i.e. 10%, no significant change in activity between the crude and grafted extracts is observed. Purified gum extracts on the whole exhibit lower toxicological activity i.e. 2.11%, 2.45%, 2.93%, 3.69% and 3.88% respectively at 2%, 4%, 6%, 8% and 10% gum concentrations.
Table 2. Cumulative release of EG-g-PAM gum at different time intervals. All values are represented as mean ± standard deviation. Standard error of mean < 0.635.

| EG-g-PAM Gum Concentration (w/w) | 5 min | 10 min | 15 min | 20 min | 25 min | 30 min |
|----------------------------------|-------|--------|--------|--------|--------|--------|
| 1%                               | 02.16 ± 0.76 | 04.75 ± 0.9 | 07.01 ± 1.1 | 09.19 ± 0.9 | 11.24 ± 0.38 | 13.27 ± 0.8 |
| 2%                               | 02.02 ± 0.9 | 04.66 ± 0.76 | 06.89 ± 0.76 | 09.11 ± 1.1 | 11.20 ± 1.1 | 13.25 ± 0.8 |
| 3%                               | 01.83 ± 0.76 | 04.54 ± 1.1 | 06.80 ± 0.9 | 09.09 ± 0.9 | 11.18 ± 0.9 | 13.11 ± 1.1 |
| 4%                               | 01.77 ± 1.1 | 04.41 ± 0.9 | 06.78 ± 1.1 | 09.05 ± 0.35 | 11.13 ± 0.55 | 13.08 ± 0.9 |
| 5%                               | 01.60 ± 0.76 | 04.39 ± 0.9 | 06.75 ± 0.8 | 09.02 ± 0.38 | 11.09 ± 0.8 | 13.05 ± 1.1 |

3.8. In vitro drug release analysis

A comparative study of drug release profiles for paracetamol is executed within gastro-enteric media by using USP dissolution apparatus. The revolutions of the paddle and temperature of the isothermal bath are maintained at 50 rpm and 37 ± 1 °C, respectively. The formulations are prepared by the assimilation of 1%, 2%, 3%, 4% and 5% concentrations of native, purified and synthesized EC-g-PAM gum respectively in place of binding material. The corresponding drug release profile i.e. cumulative drug release (%/age) versus time (minutes) is recorded in triplicates measurements. The crude EC gum formulations show fast release characteristics, however the release is slow at elevated gum levels. It may be due to the reason that with the increase in concentrations of the gum from 1% to 5%, the tablet formulations exhibit an effective control on the binding characteristics (table S11). Within the 5 min of dissolution time, the formulations with 1%, 2%, 3%, 4% and 5% gum concentrations demonstrated 03.93 ± 0.76%, 03.91 ± 1.1%, 03.87 ± 0.76%, 03.85 ± 1.1% and 03.81 ± 0.76% drug release, respectively. Similarly, at 10 min dissolution time, with similar gum binder concentrations (1% to 5%), demonstrate 05.96 ± 0.8%, 05.94 ± 0.76%, 05.92 ± 0.9%, 05.89 ± 0.9% and 05.90 ± 0.8% drug release. Furthermore, dissolution is evaluated as 09.00 ± 0.8% for 1% binder concentration, 08.98 ± 0.9% for 2% binder concentration, 08.95 ± 0.9% for 3% binder concentration, 08.92 ± 0.76% for 4% binder concentration and 08.89 ± 0.8% for 5% binder concentration for the subsequent 15 min time interval. After 20 min, 10.03 ± 0.8% drug is dissolved from 1% binder concentration, however, a slightly lower drug release (09.94 ± 0.9%) is observed from 5% binder concentration. As the concentration of gum binder increase from 1% to 5% for the subsequent 25 and 30 min time intervals, the dissolution rate decreased from 12.05 ± 0.9% to 11.95 ± 0.8% and from 4.08 ± 0.9% to 13.98 ± 0.8%, respectively. Effectively moderate drug release rates i.e. intermediate between those of crude and modified gum release profiles is observed in case of purified EC gum formulations. During the course of initial 5 min of assay in gastro-enteric media, the cumulative dissolution is 03.50 ± 1.1%, 03.48 ± 0.9%, 03.46 ± 0.76%, 03.43 ± 0.9% and 03.39 ± 1.1% for 1%, 2%, 3%, 4% and 5% of binder concentrations respectively (table S11). Conclusively, it is found that high gum concentration from 1 to 5% effectively develop the binding characteristics of the formulations. Gum concentrations of 1%, 2%, 3%, 4% and 5% display moderately gentle rates of dissolution subsequent to 10 min time-lapse i.e. 05.70 ± 0.9%, 05.94 ± 0.76%, 05.92 ± 0.9%, 05.89 ± 0.9% and 05.90 ± 0.8% correspondingly. Additionally, measured disintegration rates - 08.76 ± 0.8%, 08.74 ± 0.55%, 08.73 ± 0.55%, 08.70 ± 0.9% and 08.69 ± 0.38% are attained during succeeding time interval for the increase of the gum concentration from 1% to 5%, respectively. At 20 min, as the concentration of gum increase from 1% to 5%, the dissolution decrease from 09.78 ± 0.38% to 09.49 ± 0.76%. The dissolution decreased from 11.86 ± 0.9% to 11.75 ± 0.8% for the same increase of binder concentration. It can be inferred that on increasing the concentrations of the gum from 1% to 5%, the tablet formulations present an effective control on the binding characteristics for the subsequent time-lapse i.e. 14.00 ± 0.8%, 13.98 ± 1.1%, 13.95 ± 0.38%, 13.92 ± 0.76% and 13.90 ± 1.1% respectively. Binder concentrations of purified EC gum i.e. 1%, 2%, 3%, 4% and 5% show lower percentage release than the corresponding concentrations of crude EC gum, yet, greater than the corresponding concentrations of grafted EC gum at all disintegration time intervals of 5, 10, 15, 20, 25 and 30 min.

With reference to the evaluation of its binding characteristics in comparison with crude and purified EC gum, the enhanced concentrations of EG-g-PAM gum from 1% to 5% exhibit encouraging outcomes on the whole (table 3). From the drug release statistics, it can be inferred that appropriate grafting of EG-g-PAM resulted in sustained drug release rates. The grafted EC gum demonstrated itself as an effective binder at all concentrations as compared to the crude and purified gum binders. At 5 min dissolution time, the formulations incorporated with 1%, 2%, 3%, 4% and 5% binder gum concentrations demonstrated 02.16 ± 0.76%, 02.02 ± 0.9%, 01.83 ± 0.76%, 01.77 ± 1.1% and 01.60 ± 0.76% drug release, respectively. Similarly, binder gum concentrations of 1%, 2%, 3%, 4% and 5% displayed moderately gentle rates of dissolution (04.75 ± 0.9%,
0.466 ± 0.76%, 0.45 ± 1.1%, 0.41 ± 0.9% and 0.39 ± 0.9% at 10 min time-lapse. Similar gum concentrations (1% to 5%) demonstrated 0.71 ± 1.1%, 0.69 ± 0.76%, 0.68 ± 0.9%, 0.68 ± 1.1% and 0.67 ± 0.8% dissolution rates, respectively, during 10 min time-lapse. Moreover, as the concentration of gum binder increased from 1% to 5% for subsequent 25 and 30 min time intervals, the dissolution decreased from 0.919 ± 0.9% to 0.902 ± 0.38% and from 1.124 ± 0.38% to 1.109 ± 0.8%, correspondingly. The formulations with 5% binder proportions of EC-g-PAM gum demonstrated as the appropriate formulations on account of the fact that these exhibit least percentages of release (0.16 ± 0.76%, 0.39 ± 0.9%, 0.67 ± 0.8%, 0.91 ± 0.38%, 1.10 ± 0.8% and 1.30 ± 1.1%) subsequent to consistent time-lapses (5, 10, 15, 20, 25 and 30 min). The sustained release of formulations can be attributed to their chemical binding with EC-g-PAM. That tendency could be explicated by the fact that with correspondent increase in percentage grafting, the magnitude and number of grafted chains increase, hence, more effective linkage is formed among them which lowers the solubility of the polymeric matrix, resulting in lesser breakdown of the formulations and disintegration of the enclosed drug formulations [36, 37].

4. Conclusion

The physicochemical characterization of native, purified and modified *Eucalyptus camaldulensis* gum are evaluated in this study. Prior to modification, native gum exhibited rough and irregular external morphologies that transformed into fibrillar structure. Grafting of purified gum lead to even and non-porous textures. In case of EC-g-PAM, thermo-gravimetric analysis (TGA) curves justified the thermal stabilization where initial decomposition curve appeared at 90 °C rather than at 50 °C and one more distinctive zone in EC-g-PAM correspondence to the native EC gum. The characteristics of synthesized *E. camaldulensis* gum are analyzed at distinctive concentrations i.e. 1%, 2%, 3%, 4% and 5% and *Eucalyptus camaldulensis* gum demonstrated sustained-release drug dosage formulations. This show that *Eucalyptus camaldulensis* gum could be advantageous as a binding agent due to remarkable mechanical strength. So, it is incorporated into the paracetamol, *in vitro* formulations. Drug release profile of modified EC gum revealed excessive controlled drug-release profiles within distinctive media, comparable to that exhibited by various plants' gum binding agents. Grafted EC-g-PAM gum engrossed into the particulates, thus, minimized their enlargement within gastro-enteric systems. These modifications suggest a more advantageous oral administered and site-specified drug transference system. The current investigations on *Eucalyptus camaldulensis* gum reveal that the gum can be employed as an innocuous potential binder within time release dosage formulations, especially for drug carrier systems designed to achieve prolonged therapeutic effectiveness through continuously releasing medication over an extended period of time, subsequent to single dose administrations.

Acknowledgments

Authors acknowledge Higher Education Commission (HEC) of Pakistan for financial support. Furthermore, authors declare

ORCID iDs

Dilshad Hussain @ https://orcid.org/0000-0001-6187-5626

References

[1] Urtti A 2006 Challenges and obstacles of ocular pharmacokinetics and drug delivery Adv. Drug Delivery Rev. 58 1131–5
[2] Dengale S, Grohgzah H, Rades T and Lobmann K 2016 Recent advances in co-amorphous drug formulations Adv. Drug Delivery Rev. 100 116–25
[3] Sharma A K, Keservani R K and Kesharwani R K 2018 Nanobiomaterials: Applications in Drug Delivery (Boca Raton, FL: CRC Press)
[4] Ting J M, Porter W W III, Mecca J M, Bates F S and Reineke T M 2018 Advances in polymer design for enhancing oral drug solubility and delivery Bioconjugate Chem. 29 939–52
[5] Date A A, Desai N, Dixit R and Nagarsenker M 2010 Self-nanoemulsifying drug delivery systems: formulation insights, applications and advances Nanomedicine 5 1595–616
[6] Wieczorek S, Schaett T, Senge M O and Börner H G 2015 Specific drug formulation additives: revealing the impact of architecture and block length ratio Biomacromolecules 16 3308–12
[7] Rautio J, Meanwell N A, Di L and Hageman M J 2018 The expanding role of prodrugs in contemporary drug design and development Nat. Rev. Drug Discovery 17 559–87
[8] Al-Khattawi A and Mohammed A R 2013 Compressed orally disintegrating tablets: excipients evolution and formulation strategies Expert Opinion on Drug Delivery 10 651–63
[9] Pandey S P, Shukla T, Dhote V K, Mishra D K, Maheshwari R and Tekade R K 2019 Use of Polymers in Controlled Release of Active Agents, Basic Fundamentals of Drug Delivery (Amsterdam: Elsevier) pp 113–72

[10] Bharate S S, Bharate S B and Bajaj A N 2016 Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: a comprehensive review Journal of Excipients and Food Chemicals 1 3–26 https://scinapse.io/papers/2341071777

[11] Pauk V, Pluháček T, Havlíček V and Lemr K 2017 Ultra-high performance supercritical fluid chromatography-mass spectrometry procedure for analysis of monosaccharides from plant gum binders Anal. Chim. Acta 989 112–20

[12] Prajapati V D, Jami G K, Moradiya N G and Randeria N P 2013 Pharmaceutical applications of various natural gums, mucilages and their modified forms Carbohydrate Polym. 92 1685–99

[13] Scholtz J, Van der Colff J, Steenkamp J, Stieger N and Hamman J 2014 More good news about polymeric plant- and algae-derived biomaterials in drug delivery systems Current Drug Targets 15 486–501

[14] Nayak A K and Saquib H 2019 Plant Polysaccharides-Based Multiple-Unit Systems for Oral Drug Delivery (Berlin: Springer)

[15] Choudhary P D and Pawar H A 2014 Recently investigated natural gums and mucilages as pharmaceutical excipients: an overview Journal of pharmaceuticals 2014 204849

[16] Benecke G, Vílaseca A and Hamman J 2009 Polymeric plant-derived excipients in drug delivery Molecules 14 2602–20

[17] Yadav P, Pandey P and Parashar S 2017 Pectin as natural polymer: an overview Research Journal of Pharmacy and Technology 10 1225

[18] Bhata S 2016 Plant derived polymers, properties, modification & applications Natural Polymer Drug Delivery Systems (Berlin: Springer) pp 119–84 https://ojs.abs.fit/ojs/index.php/jfc/article/view/26/43%20English

[19] Jani G K, Shah D P, Prajapati V D and Jain V C 2009 Gums and mucilages: versatile excipients for pharmaceutical formulations Asian J. Pharm. Sci. 4 309–23 http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.472.4557

[20] Dharmendra S 2012 Natural excipients a review International Journal of Pharmaceutical & Biological Archive 3 33889043 https://www.jipba.info/jipba/index.php/article/view/815

[21] Saha A, Tyagi S, Gupta R K and Tyagi Y K 2017 Natural gums of plant origin as edible coatings for food industry applications Crit. Rev. Biotechnol. 37 959–73

[22] Prajapati V D, Jami G K and Khanda S M 2013 Pullulan: an exopolysaccharide and its various applications Carbohydrate Polym. 95 540–9

[23] Goswami S and Naik S 2014 Natural gums and its pharmaceutical application Journal of Scientific and Innovative Research 3 112–21 www.jsirjournal.com

[24] Nayak A K and Pal D 2018 Functionalization of tamarind gum for drug delivery Functional Biopolymers (Berlin: Springer) pp 25–56

[25] Setia A 2018 Applications of graft copolymerization: a revolutionary approach Biopolymer Grafting (Amsterdam: Elsevier) pp 1–44

[26] Nayak A K, Bera H, Hasnain M S and Pal D 2018 Synthesis and characterization of graft copolymers of plant polysaccharides Biopolymer Grafting (Amsterdam: Elsevier) pp 1–62

[27] Sen G, Sharon A and Pal S 2011 Grafted polysaccharides: smart materials of the future, their synthesis and applications Biopolymers: Biomedical and Environmental Applications 99–127 Chapter 5

[28] Shahid M, Bukhari S A, Gul Y, Munir H, Anjum F, Zuber M, Jamil T and Zia K M 2013 Graft polymerization of guar gum with acrylamide using an ultrasound-assisted method J. Drug Delivery Sci. Technol. 56 101572

[29] Bal T, Yadav S K, Rai N, Swain S, Shambhavi, Garg S and Sen G 2020 In vitro evaluations of free radical assisted microwave irradiated polyacrylamide grafted cashew gum (CG) biocompatible graft copolymer (CG-g-PAM) as effective polymeric scaffold J. Drug Delivery Sci. Technol. 1101572

[30] Nandi G, Chandra G and Ghosh I K 2019 Graft–copolymer of polyacrylamide-tamarind seed gum: synthesis, characterization and evaluation of flocculating potential in peroral paracetamol suspension Carbohydrate Polym. 215 213–25

[31] Sand A and Kwaak Y J 2017 Modification of guar gum through grafting of acrylamide with potassium bromate/thiourea redox initiating system Fibers Polym. 18 675–81

[32] Klein J M, Silva de Lima V, Couto da Feira J M, Nichele Brandalise R and de Camargo Forte M M 2016 Chemical modification of cashew gum with acrylamide using an ultrasound-assisted method J. Appl. Polym. Sci. 133 43634

[33] Sand A and Vyas A 2020 Superabsorbent polymer based on guar gum-graft-acrylamide: synthesis and characterization J. Polym. Res. 27 43

[34] McDonald M, Brooker M and Butcher P 2009 A taxonomic revision of Eucalyptus camaldulensis (Myrtaceae) Australian Systematic Botany 22 257–85

[35] Sen G, Mishra S, Jha U and Pal S 2010 Microwave initiated synthesis of polyacrylamide grafted guar gum (CG-g-PAM)—characteristics and application as matrix for controlled release of 5-amino salicylic acid Int. J. Biol. Macromol. 47 164–70

[36] Tripathi R and Mishra B 2012 Development and evaluation of sodium alginate–polyacrylamide graft co–polymer–based stomach targeted hydrogels of famotidine AAPS PharmSciTech 13 1091–102

[37] Kumar D, Pandey J, Raj V and Kumar P 2017 A review on the modification of polysaccharide through graft copolymerization for various potential applications Open Med. Chem. J. 11 109–26