Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2015, 6 (6):69-74
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

A comparitive study of drug release profile of propolis and diclofenac for efficient wound healing

Preethi Ramadoss* **and V. Subha**

Crystal Growth Centre, Anna University, Chennai

ABSTRACT

Diclofenac and Propolis based scaffolds were compared for their in vitro drug delivery efficacy. The fabricated Gelatin scaffolds were then soaked in Diclofenac and Propolis followed by thorough drying. The drug loaded scaffolds were tested for their anti-microbial and anti-fungal efficiency. The scaffolds loaded with the two different drugs were also subjected to drug release analysis in vitro. Propolis was found to possess a controlled release pattern when compared to that of Diclofenac.

Keywords: Propolis, anti-inflammatory, anti-bacterial and anti-fungal, drug release

---

INTRODUCTION

Skin injuries that require a skin replacement can demand an autograft wherein the skin from another part of the same individual is used to replace the damaged part. But this technique requires a lot of immune suppressive medications to avoid inflammations that arise because of infections, which will lead to severe immne deficiency. This is why autografts are losing out on interest. Therefore bioengineered skin substitutes are being used to replace any damaged part of the human skin. Wound healing generally undergoes an inflammatory phase, epithelial phase, proliferation phase, and maturation phase [1]. If the inflammation phase continues for a long time, the next stage is delayed. For this reason, inflammatory phase is the most significant of the 4 stages. Major problems related to artificial skin are its vulnerability in bacterial and fungal infection. It can take a week or two for the blood vessels (which holds the immune system's infection-fighting cells) to connect to the newly forming dermis. In the meantime, before the formation of the blood vessels bacteria may cause infection, which will destroy the graft and result in a fresh wound [2]. A potential skin graft should be capable of overcoming these two issues, namely inflammation and infection. Tissue repair is normally accompanied by fibrotic reactions that result in scarring. Nevertheless, certain mammalian tissues have the capability to regenerate completely without scarring; examples are embryonic or fetal skin and the ear of the MRL/MpJ mouse. Research about these revealed that, to achieve complete regeneration, the inflammatory response is modified so that the fibrosis and scarring can be averted [3]. Propolis is a natural product derived from plant resins collected by honeybees. Honeybees use propolis as a glue to seal the beehives. Bee propolis has been used for centuries in folk medicines. Propolis is known to possess anti- microbial, antioxidative, anti-ulcer and anti-tumor activities. Hence, it has attracted a lot of attention in recent years as a potential material used in medicine and cosmetics products. The chemical composition of propolis is quite complicated. The contents in it depend on the bee's collecting location, time and plant source. Therefore, propolis gathered from different geographical areas and time periods vary greatly [3]. It is used to seal the hives and, more importantly, to prevent the decomposition of creatures that have been killed by bees after invading the hive [4, 5]. The propolis is used as an antiseptic and

healing in the treatment of wounds and as a mouthwash, and its use in the Middle Ages perpetuated among the Arab doctors [6]. Also, it was widely used in the form of ointment and cream in the treatment of wounds in the battlefield, because of their healing effect. This healing property of propolis is known as "Balm of Gilead," which is also mentioned in the Holy Bible [7]. There was also the investigation of antiseptic and healing properties of propolis in subjects admitted to various hospitals and the results were extremely positive [8]. Azulene (an isomer of naphthalene present in chamomile flower), which is inferred from the Propolis of honey bee hives with the help of GCMS analysis, has revealed anti-inflammatory attributes. Traditionally, chamomile has been used as an anti-inflammatory, antioxidant and as a mild astringent for healing purposes. Chamomile is widely used to treat inflammations of the skin and mucous membranes, bacterial infections of the skin, oral cavity and gums, and respiratory tract because one of the major activities of azulene involves the suppression of LPS-stimulated prostaglandin E (2) release and attenuation of cyclooxygenase (COX-2) enzyme activity without affecting the constituent form of COX-1, thereby greatly reducing the inflammation [9]. Hence Propolis was compared with Diclofenac in this study for its analgesic effect which in turn exhibits immunomodulatory behavior as well, thus eliminating the use of any immune suppressive medications to the patient.

MATERIALS AND METHODS

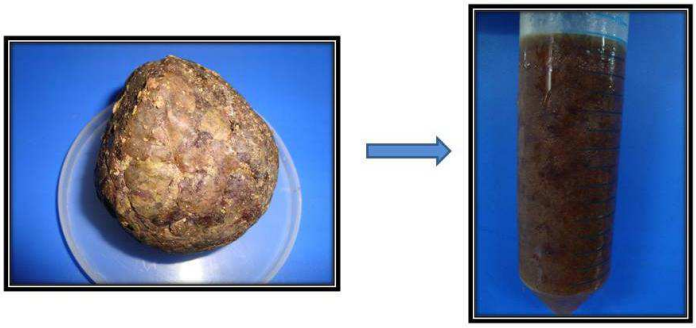
2.1 Scaffold Preparation:

12% (w/v) Gelatin (Sigma Aldrich) was used to fabricate Gelatin scaffold. 3.6g of Gelatin was mixed in 30 ml of deionised water and allowed to stir at 60°C for 6 hours. The obtained mixture was allowed to cool at 25°C followed by pre-freezing at -25°C for 24hrs before lyophilising the composites at -110°C. The freeze dried scaffolds were then crosslinked with 0.4% Gluteraldehyde at room temperature for 2 hours and then washed before being lyophilized once again.

2.2 Extraction of Propolis:

Extraction of Propolis was carried out by adding 100 g of Propolis in 500 ml of acetone and stirred for 48 hours at room temperature until it gets completely dissolved. The solution was then strained through a filter paper and the obtained Propolis extract was stored at 4°C thereafter (Figure 1).

Figure 1: Acetone extraction of Propolis



2.3 Drug loading for scaffolds:

The cross-linked scaffolds were loaded with 2 different drugs for comparing their efficacy as a safe anti-microbial, anti-fungal and anti-inflammatory action. The scaffolds were weighed to 50mg each and one of these scaffolds were soaked in 3ml (75 mg/ml) of Diclofenac sodium drug for 4 hours and dried at 37°C and the other scaffold was soaked in 1ml (200 mg/ml) of propolis and dried at 37°C for 48 hrs. The quantity of drug loaded into the scaffolds was estimated by variation in concentration of the drug, before and after loading measured with the help of UV spectrophotometer (UV-3900, Hitachi High-Tech).

2.4 Drug Release:

The in vitro release of diclofenac and propolis was carried out by immersing the scaffolds in 4ml of saline solution and incubating at 37±0.1 °C placed in an orbital shaker set at 100 RPM. The medium was withdrawn at various time intervals and replenished with fresh medium. All experiments were carried out in triplicates. The drug release was measured for the two drugs by the optical density using a UV spectrophotometer (U-3900 Spectrophotometer,

Hitachi High-Tech) at their corresponding wavelengths (280 nm for Diclofenac and 326 nm for Propolis). The Diclofenac and Bee Propolis drug loaded scaffolds were represented as a GD, GBP respectively.

2.5 Antibacterial/Antifungal Activity:

A comparative study was performed between the Diclofenac drug loaded and Bee Propolis drug loaded set of samples to check their antibacterial and anti-fungal activity. Two species of gram positive bacteria were chosen Staphylococcus aureus and Staphylococcus epidermidis. Apart from these, a fungal species, Candida albicans was also used for the study. Mueller Hinton Agar (MHA, Himedia) was applied as a base medium for bacterial samples and potato dextrose was used as a base medium for Candida albicans and nutrient broth were used for the preparation of inoculum of the bacterial species. 50 µl of inoculum was taken and spread onto the MHA plate thoroughly. The zone of inhibition for the scaffolds was determined for the two drugs.

2.6 GC-MS

The mass spectrometry analysis was carried out for the extracted propolis sample with the help of JEOL GCMATE II GC-MS in order to identify the individual components present in the sample (drug). The following important compounds were identified from the acetone extracted propolis (Table 1). In this the azulene (derived from chamomile flower) is the component that possesses anti-inflammatory properties.

Table 1: List of compounds identified in acetone extracted Propolis

Name of the compound	Percentage of the compound present in 200 mg/ml of propolis
Azulene	7.89 %
Naphthalene	7.89 %
Santalol	8.54 %
Pentadecanoic acid	4.14 %
Pentacosane	5.7 %
Heptacosane	5.57 %

RESULTS AND DISCUSSION

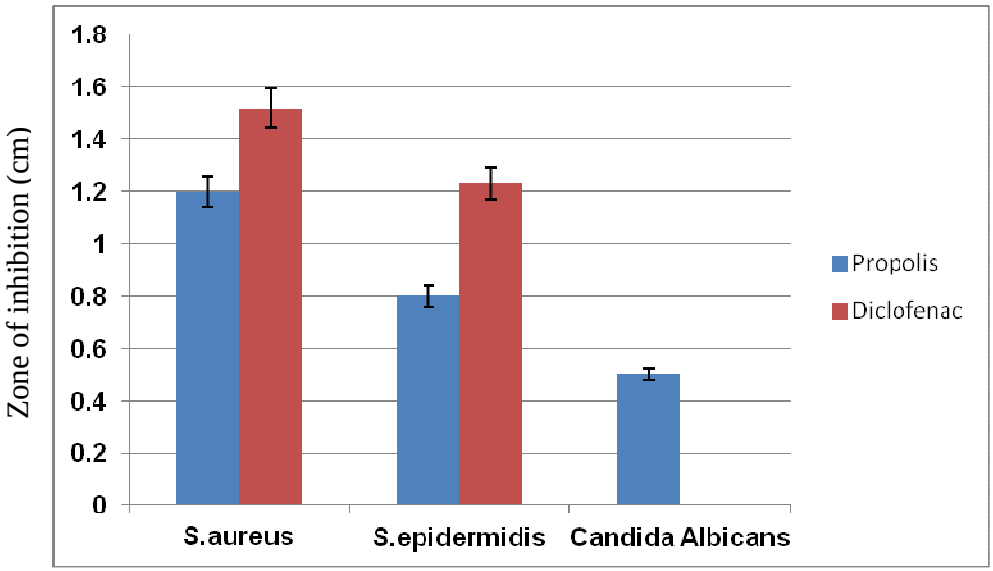
3.1 Antimicrobial

Antimicrobial and antifungal effect of two different drugs was analyzed (Figure 2). Major problems associated with artificial skin are its vulnerability to bacterial and fungal infection. When the skin is healing, blood vessels takes a week or two to form, thereby making the wound so vulnerable to bacterial, viral and fungal attacks which in turn delays the healing process [2]. Major skin-graft failures occur because of the following bacteria

- Staphylococcus aureus (gram-positive)
- Staphylococcus epidermidis (gram-positive)
- Candida Albicans (fungi)

Bee propolis showed strong inhibitory activity (Figure 2) for gram-positive bacteria, this could be because of the presence of the phenolic compounds in it. Takasi et al (1994) [10] stated that the propolis inhibits bacterial growth by preventing cell division. In addition, propolis disorganized the cytoplasm, the cytoplasmic membrane and the cell wall, caused a partial bacteriolysis and inhibited protein synthesis. The most common pathogens affecting the skin implants are pseudomonas aeruginosa, staphylococcus aureus, staphylococcus epidermidis and candida albicans. Propolis is effective against gram positive bacteria and not against gram negative (pseudomonas sp.); Gram-negative bacteria have a cell membrane chemically more complex and a higher fat content, which may explain the higher resistance [11]. Decanoic acid alias capric acid and azulene contribute to the bactericidal action of propolis. Earlier studies have shown that Capric acid, a 10-carbon saturated fatty acid, causes the most effective killing of all three strains of *C. albicans* that was tested, leaving their cytoplasm disorganized and shrunken because of a disrupted or disintegrated plasma membrane [12].

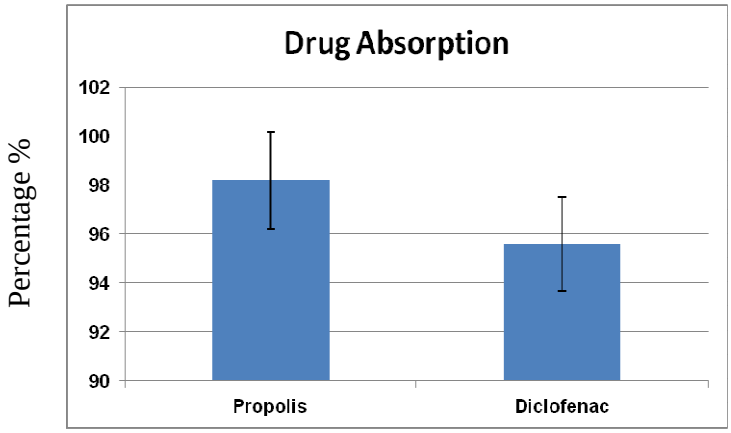
Figure 2: Zone of inhibition for the three pathogens



3.2 Drug loading:

The percentage of drug loading was calculated using the formula, "Percentage of drug that was loaded= [(Y−X) /X] ×100", where X represents the initial concentration of the drug and Y is the final concentration of drug after removing the scaffold from the drug [13]. X and Y was determined using UV spectrophotometer at 280 nm for diclofenac sodium and 326 nm wavelength for propolis drug respectively. All experiments were performed in triplicate. The percentage of the drug loaded into the scaffolds is shown below (Figure 3).

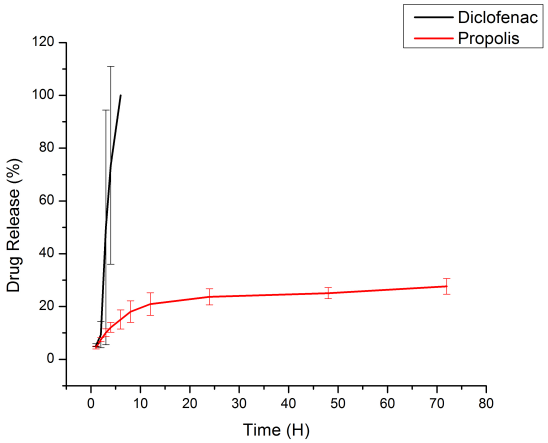
Figure 3: Percentage of drug absorbed by scaffold



3.3 Drug Delivery:

The release percentage of the two drugs was taken (Figure 11) for the intervals of 1h, 2h , 3h, 4h, 6h, 8h, 12h, 24h, 42h and 72 h and it was found that Propolis showed a sustained release of ~30% of the total drug concentration for upto 72 hours (Figure 4). This can be attributed to the presence of many hydrogen bonds and hydrophobic interactions between the Gelatin and the various compounds present in Propolis such as capric acid, azulene, naphthalene, etc, and also because of the strong intermolecular bonds formed between the compounds of Propolis. On the other hand, Diclofenac showed a 100% release (Figure 4) of the total drug concentration within a period of 3 hours. Besides, Diclofenac is a water soluble drug hence it gets easily released into the body fluid, whereas propolis contains a number of fatty acids such as decanoic acid and hence the release is sustained. Hydrogen bonding also plays a major role in stabilizing protein-ligand complexes.

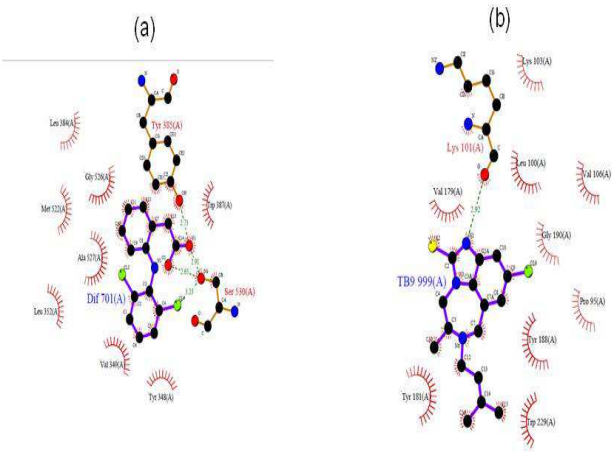
Figure 4: Percentage of drug release of Diclofenac and Propolis



3.4 Protein-ligand docking:

The drug propolis has UV absorption at 326 nm. At this wavelength the compound that gets absorbed is azulene which plays a major role in inhibiting the inflammation phase. Hence the docking was carried out between the protein (Gelatin) and the ligand (azulene) with the help of the software PatchDock. The obtained file was uploaded in the software PDBSum for protein-ligand interaction analysis. The number of non-bonded interactions between azulene and gelatin (Figure 5b) is higher when compared to that of gelatin and diclofenac (Figure 5a). Non-bonded interactions are nothing but the electrostatic and hydrophobic interaction between the two proteins. There are three types of non-bonded interactions, which are electrostatic interaction, the van der Waals forces and hydrophobic interactions, of which the ion-ion (electrostatic) interaction is stronger than the dipole-dipole (vaan der Waals) interaction. The binding energy of azulene and gelatin (-20.7605 kcal/mol) is higher than that of gelatin and diclofenac (-55.8037 kcal/mol), moreover azulene and decanoic acid are lipophilic compounds that will not dissolve in water (saline); hence the release is delayed when compared to diclofenac which is a hydrophilic compound. Another reason for the controlled release of azulene in a saline solution is that azulene forms strong covalent bonds with other compounds such as naphthalene, pentacosane and hexacosane, etc also present in propolis that makes it difficult to break free and release.

Figure 5: Protein-Ligand docking of (a) Gelatin and Diclofenac, (b) Gelatin and Azulene



CONCLUSION

Propolis contains number of flavonoids and phenolic compounds which makes strong hydrogen bonds with Gelatin. The diclofenac sodium drug is water soluble; hence it gets released rapidly within 3 hours in vitro. The lipophilicity of the phenolic acids in propolis has made it less soluble in water, thereby sustaining the drug release pattern. Molecular docking of Gelatin and the two drugs has shown that the binding energy of Azulene and Gelatin is higher

than that of diclofenac and gelatin thereby facilitating a sustained release. Besides, the bonding between the compounds present in Propolis itself is so strong that it makes it difficult for azulene to break free from the complex and release itself into the system. The inhibition zones were observed against staphylococcus aureus, staphylococcus epidermidis and candida albicans, which could be attributed to the presence of azulene, decanoic acid, naphthalene, prentacosane, etc present in propolis. Candida albicans is a common fungal infection that occurs on skin wounds which can delay the healing process [14], propolis showed a clear inhibitory zone against it. Thus, Propolis exhibited an excellent anti-microbial, anti-fungal and a site specific sustained release pattern making it an outstanding alternative to immune suppression medications.

Acknowledgement
Preethi Ramadoss would like to thank Dr. V.R. Giridev and Mr. Shiva Shankar of the Department of Textile Technology for having helped with UV spectrophotometer characterization of the scaffolds. I duly acknowledge Dr. S. Narayana Kalkura, Professor, Crystal Growth Centre, Anna University for providing me with all the facilities to carry out my research work.

REFERENCES

[1] Kang JS & Kang jin-sung. Plastic surgery, 3rd Gun-ja Publication: Seoul, **2004**.
[2] IV Yannas, Tissue and organ regeneration in adults, Springer, New York, **2001**.
[3] L Mahmoud, *Asia Pacific Journal of Cancer Prevention*, **2006**, 7(1), 22-31.
[4] Ghisalbert EL, *Bee World*, **1979**, 60, 59–84.
[5] C Garcia-Viguera, W Greenaway, FR Watley, Z Naturforsch & Tubigen, **1992**, 47c, 634.
[6] S Castaldo & F Capasso, *Fitoterapia*. **2002**, 73, S1- S6.
[7] YK Park, SM Alencar, FF Moura & FFM Ikegaki, **1999**, 27, 46-53.
[8] AMT Grégio, AAS Lima, MO Ribas, APM Barbosa, ACP Pereira, F Koike & CEP Repeke, *Estud Biolog*, **2005**, 27, 58.
[9] JK Srivastava, M Pandey & S Gupta, *Life Sci*, **2009**, 85, 663–669.
[10] K Takasi, NB Kikuni & H Schilr, *Povenance Planta Med*, **1994**, 60, 222-227.
[11] S Stepanović, N Antić, I Dakić & M Svabić-Vlahović, *Microbiological Research*, **2003**, 158(4), 353-357.
[12] Gudmundur Bergsson, Jóhann Arnfinnsson, Ólafur Steingrímsson & Halldor Thormar, *Chemother*, **2001**, 45(11), 3209–3212.
[13] V Siva Kumar & KP Rao, *Biomaterials*, **2002**, 23, 3175–318.
[14] DF Williams, *Biomaterials*, **2009**, 30, 5897–5909.