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(71) Applicant *(for all designated States except US)*: **THE ROYAL INSTITUTION FOR THE ADVANCEMENT OF LEARNING/MCGILL UNIVERSITY** [CA/CA]; 845 Sherbrooke Street West, Montréal, Québec H3A 2T5 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **TUFENKJI, Nathalie** [CA/CA]; 1009, rue des Pervenches, Laval, Québec H7Y 2H8 (CA). **EYDELNANT, Irwin Adam** [CA/CA]; 885 Queen Street West, Toronto, Ontario M6J 1G5 (CA).

(74) Agent: **OGILVY RENAULT LLP / S.E.N.C.R.L., S.R.L.**; Suite 2500, 1 Place Ville Marie, Montréal, Québec H3B 1R1 (CA).

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(54) Title: USE OF PROANTHOCYANIDINS AS AN ANTI-APOPTOTIC AGENT AND ANTI-ADHESIVE BACTERIAL AGENT

(57) Abstract: The present disclosure relates to the use of proanthocyanidins, particularly those isolated from cranberry, as an anti-apoptotic and anti-bacterial agent, the anti-bacterial property also being due to the anti-adhesive properties of PACs when comprised in a material.

USE OF PROANTHOCYANIDINS AS AN ANTI-APOPTOTIC AGENT AND ANTI-ADHESIVE BACTERIAL AGENT

TECHNICAL FIELD

[0001] The present disclosure relates to a composition comprising proanthocyanidins, a biomaterial comprising proanthocyanidins and the use of proanthocyanidins as an anti-apoptotic, anti-invasion and anti-adhesive bacterial agent.

BACKGROUND ART

[0002] It is estimated that one of every ten patients admitted to hospital will develop a hospital acquired infection (HAI). In Canada alone, more than 200,000 cases of HAI are reported each year, leading to the death of approximately 8,000 Canadians and millions of dollars in associated healthcare costs. The vast majority of HAIs occur as a result of contamination of implanted devices such as heart valves, stents, and catheters.

[0003] For example, urinary catheters are used within patient care facilities in order to aid in bladder drainage for patients who are either incontinent or lack mobility. The catheter is passed through the urethra and into the bladder where a balloon is expanded in order to collect fluids. This commonly performed procedure often results in Catheter Associated Urinary Tract Infections (CAUTI). CAUTI accounts for 40% of the 200,000 hospital acquired infections that occur in Canada each year, significantly increasing patient mortality.

[0004] Approximately 25% of all patients that are admitted to hospital are catheterized with a urinary catheter, increasing the risk for the development of hospital acquired infections and thereby leading to increased patient morbidity and healthcare costs. Currently, direct costs associated with each case of CAUTI range from $500 to $1000 for complete treatment, accumulating upwards of $40 million to $80 million in Canadian healthcare dollars. Clinicians have sought varying methods to decrease the rates of catheter associated urinary tract infection including bladder irrigation, the addition of antibacterial

agents to collection bags, and metal cleaning that have all failed as promising solutions.

[0005] With an increasing prevalence of multi-drug resistant pathogens (e.g., antibiotic resistant bacteria) in hospitals, the emergence of novel non-antibiotic based preventative strategies has risen. A non-antibiotic target of interest is the prevention of initial bacterial adhesion to surfaces. After bacteria attach to a surface they often begin to grow and develop into complex microbial communities known as biofilms. It is the development of biofilm that generally results in HAI and patient complications.

[0006] It would thus be highly desirable to be provided with an anti-infective compound for medical applications in order to reduce contamination of implanted devices. It would also be desirable to be provided with a strategy to block the initial adhesion process of bacteria limiting the biofilm formation and potentially reducing infection.

SUMMARY

[0007] In accordance with the present disclosure, there is now provided an anti-infectious composition comprising proanthocyanidins extracted from cranberry and a carrier, the composition preventing adhesion and propagation of bacteria.

[0008] It is also provided an anti-infectious composition comprising proanthocyanidins extracted from cranberry and a carrier, the composition preventing invasion of bacteria in a cell, infection and apoptosis of the cell.

[0009] In an embodiment, cranberry is of the species *Vaccinium macrocarpon.*

[0010] In a further embodiment, bacteria are Gram-positive or Gram-negative, such as for example but not limited to *Escherichia coli*, *Pseudomonas* or *Enterococcus faecalis.*

[0011] In another embodiment, the proanthocyanidins prevent biofilm formation.

[0012] In a further embodiment, the composition described herein further comprises a therapeutic agent, such as for example, but not limited to, an anti-bacterial agent or an antibiotic.

[0013] In another embodiment, the composition described herein also has anti-inflammatory property.

[0014] It is also provided herein a material comprising a composition as described herein.

[0015] In an embodiment, the material is a heart valve, a stent, an artificial limb, an angioplasty balloon, a shunt, a scalpel or a catheter.

[0016] In another embodiment, the catheter is a urinary catheter or a central line catheter, is made of a polymer, and more precisely it can also be made of latex, silicone or Teflon.

[0017] In a further embodiment, the material is a packaging material, such as for example but not limited to a food packaging material or a cosmetic packaging material.

[0018] It is also provided the use of proanthocyanidins extracted from cranberry in the manufacture of a medicament to prevent apoptosis of cells.

[0019] In an embodiment, the medicament is for improving the condition of a patient suffering from cardiovascular disease, liver disorder, kidney disorder or complication of heart transplant.

[0020] It is also provided the use of proanthocyanidins extracted from cranberry in the manufacture of a material free of bacteria.

[0021] It is further provided the use of proanthocyanidins extracted from cranberry for improving the condition of a patient suffering from cardiovascular disease, liver disorder, kidney disorder or complication of heart transplant.

[0022] It is also provided the use of proanthocyanidins extracted from cranberry for preventing apoptosis of cells.

[0023] It is also provided a method of preventing bacterial colonization and biofilm formation on the surface of a material comprising the step of contacting the material with proanthocyanidins extracted from cranberry.

[0024] It is further provided a method of preventing apoptosis of cells in a patient comprising administering to the patient proanthocyanidins extracted from cranberry.

[0025] In an embodiment, proanthocyanidins further improve the condition of a patient suffering from cardiovascular disease, liver disorder, kidney disorder or complication of heart transplant.

[0026] It is also provided a method for improving the condition of a patient comprising administering to the patient proanthocyanidins extracted from cranberry, the condition being cardiovascular disease, liver disorder, kidney disorder or complication of heart transplant.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Reference will now be made to the accompanying drawings.

[0028] Fig. 1A illustrates the results of adhesion experiments conducted with latex microspheres on different biomaterials wherein in the image on the left, latex microspheres (white dots) have adhered onto a polycarbonate (PC) surface (2.5 % w/v in chloroform); in the image on the right, microspheres (white dots) have adhered onto a polycarbonate surface (2.5 % w/v in chloroform) that was first modified with proanthocyanidins (PACs), whereby the PACs were embedded into the polycarbonate; both surfaces (PC on the left and PAC-PC on the right) were exposed to latex microspheres at a concentration of 10^8 particles/mL, and microscope images were taken of the materials to allow enumeration of attached microspheres.

[0029] Fig. 1B illustrates an histogram of polycarbonate (PC) control material versus PACs incorporated polycarbonate (PAC-PC) showing samples challenged with latex microspheres (lanes 1 and 2) and uropathogen *E. faecalis* 29212 (lane 3) with a significant reduction of adhesion observed.

[0030] Fig. 2 illustrates PACs' cytoprotective effects by SEM microscopy images of Madin-Darby canine kidney cells (MDCK) exposed to *Pseudomonas aeruginosa* for two hours in phosphate buffered saline (PBS) (left panel) and MDCK cells exposed to *Pseudomonas aeruginosa* for two hours in PACs supplemented PBS (75 ug/ml; right panel).

[0031] Fig. 3 illustrates the proliferative effects of cranberry extracted PACs on MDCK cells incubated in PBS for 3 hours in the presence of various concentrations of cranberry extracted PACs (panel 1: 0 μg/ml of PACs; panel 2: 25 μg/ml of PACs; panel 3: 50 μg/ml of PACs; panel 4: 75 μg/ml of PACs , panel 5: 100 μg/ml of PACs, panel 6: 150 μg/ml of PACs), imaged by epifluorescence microscopy using Live/Dead staining.

[0032] Fig. 4 illustrates representative fluorescence microscope images of bacteria adhered to (A) a polycaprolactone (PCL) coated glass disk (control) and (B) PCL-2.5wt%PAC coated glass disk, following an adhesion experiment conducted in a parallel-plate flow cell (PPFC), the images showing considerably greater adhesion on the control (clean) material than the polymer containing cranberry PAC.

[0033] Fig. 5 illustrates a comparison of the number of adhered bacteria to polycaprolactone (PCL) coated glass disks (lanes 2) versus PCL-2.5wt% PAC glass disks (lanes 1), on three different parallel-plate flow cell (PPFC) experiments.

[0034] Fig. 6 illustrates the effect of cranberry derived PACs on adhesion and invasion of four bacterial pathogens to kidney epithelial cells: *E. faecalis* (EF), *P. aeruginosa* (PA), *E. coli* O157:H7 (EC1), and *E. coli* CFT073 (EC2) pre-incubated in DMEM with or without PAC (50 μg/ml) for 1 hour (data presented

as fold-change from untreated conditions; p <0.01, Student's two-tailed t-test; error bars indicate 95% confidence intervals).

[0035] Fig. 7 illustrates the effect of cranberry derived PACs on invasion of MDCK cells by *E. coli* O157:H7, CLSM images of (A) MDCK cells infected with *E. coli* O157:H7, and (B) MDCK cells infected with *E. coli* O157:H7 cells pre-incubated in PAC supplemented media (50 µg/ml), cells stained for actin (red) and invaded bacteria (green).

[0036] Fig. 8 illustrates a FTIR spectra for PAC, PCL, and PCL-2.5wt%PAC composite, the peaks specific to PAC which can be observed in the composite spectrum have been identified at 1610, 1524, and 825 cm^{-1}.

[0037] Fig. 9 illustrates a release profile of proanthocyanidins from polycaprolactone films in water plotted as a function of time.

[0038] Fig. 10 illustrates a release profile of proanthocyanidins from polycaprolactone films in PBS plotted as a function of time.

[0039] Fig. 11 illustrates a percentage release profile of proanthocyanidins from polycaprolactone films in water plotted as a function of time.

[0040] Fig. 12 illustrates a percentage release profile of proanthocyanidins from polycaprolactone films in PBS plotted as a function of time.

[0041] Fig. 13 illustrates Differential Scanning Calorimetry (DSC) results for assessing the thermal properties of clean PCL and PAC-embedded PCL films. DSC heating (A) and cooling (B) second cycle curves of PCL, PCL-2.5wt%PAC, and PCL-5wt%PAC thin films obtained at a heating rate of 20°C/minute, the Tm (melting temperature) is presented on graph (A).

DETAILED DESCRIPTION

[0042] It is provided the anti-apoptotic, anti-invasion and anti-adhesive bacterial properties of proanthocyanidins.

[0043] It is disclosed herein the anti-adhesive activity of North American cranberry (*Vaccinium macrocarpon)* extracted proanthocyanidins (PACs) embedded into materials. It is disclosed that pretreatment of surfaces or biomaterials with these compounds have a protective effect, anti-apoptotic and anti-invasion effect on subsequent bacterial infection of cells. Preferably, PACs are isolated as described in Howell et al. (2005, Phytochemistry, 66: 2281-2291).

[0044] It is known that cranberry has been administered in the treatment of bacterial infections, particularly for urinary tract infection (UTI). However, it is only over the last 20 years that basic research has been performed to elucidate the active components responsible for the beneficial effects of cranberries. PACs are one group of bioactive molecules that have been identified. These are large molecules present in several different foods including chocolate, red wine, and blueberries.

[0045] PACs (also known as procyanidin oligomeric proanthocyanidins (OPC), pycnogenol, leukocyanidin and leucoanthocyanin) are a class of flavanols. Proanthocyanidins are essentially polymer chains of flavonoids such as catechins.

[0046] Proanthocyanidins are a class of polyphenolic compounds found in several plant species composed of flavan-3-ol most commonly linked through either 4 → 6 or 4 → 8. Procyanidins are the most common classes that have chains of catechin, epicatechin, and their gallic acid esters. Structural isomers exist including the formation of second interflavanoid bonds by C-O oxidative coupling forming A-type oligomers. B-type oligomers are singly linked and are more abundant in nature.

[0047] Proanthocyanidin trimers derived from *Vaccinium macrocarpon* exhibit bioactivity against pathogenic P-fimbriated *E. coli* to uroepithelial cells. Specifically these trimers exhibit A-type interflavanoid linkages that are absent in proanthocyanidins isolated from other sources. The trimers epicatechin-(4βf6)-epicatechin-(4βf8, 2βf(f7)-epicatechin, epicatechin-(4βf8, 2βf(f7)-

epicatechin-(4βf8)-epicatechin, and epicatechin-(4βf8)-epicatechin-(4βf8, 2βf(f7)-epicatechin are known to exhibit significantly greater activity than weakly active epicatechin-(4βf8, 2βf(f7)-epicatechin (procyanidin A2) and inactive epicatechin monomers and their dimers epicatechin-(4βf8)-epicatechin (procyanidin B2).

[0048] In a screening of nearly 100 common foods, only cranberry, plum, avocado, peanut, curry and cinnamon exhibited A-type proanthocyanidins (Gu et al., 2003, Journal of Agricultural and Food Chemistry, 51: 7513-7521). Cranberry and peanuts exhibit A-type structures as both terminal and extension structures whereas plum presents only terminal units; avocado, curry, and cinnamon have extension structures. Others have confirmed the presence of A-type linkages in other Vaccinium species including lingonberry, bilberry and bog whortleberry (Maatta-Riihinen et al., 2005, Journal of Agricultural and Food Chemistry, 53: 8485-8491).

[0049] PACs produced by cranberries have a particular structure that has been demonstrated to prevent the adhesion of bacteria to the cells found in the internal lining of the urinary tract, thereby preventing the onset of UTI (Howell et al., 2005, Phytochemistry, 66: 2281-2291).

[0050] Whole cranberry proanthocyanidin fraction demonstrates complex series of oligomers with both A-type (containing at least one double linkage) and B-type (exclusively single linked) oligomers. The A-type oligomers found in cranberry have only a single double linkage per oligomer as opposed to those found in other species with multiple double linkages. Blueberry in contrast only exhibits B-type proanthocyanidins. Peanuts and cinnamon have A-type linkages.

[0051] High molecular weight PACs have a significant higher binding efficiency to lipopolysaccharides (LPS) on various strains of pathogenic bacteria than PACs derived from tea and grapes (Delehanty et al., 2007, Journal of Natural Products, 70: 1718-1724). This LPS binding potential has been exploited for the development of new materials in the removal of LPS from

solution. Derivatives from Vaccinium species were found to bind the pili of N. meningitidis with greater efficiency than a wide range of extracts derived from other berry sources, with implication in the prevention of infection (Toivanen et al., 2009, Journal of Agricultural and Food Chemistry, 57: 3120-3127).

[0052] It is demonstrated herein that cranberry derived proanthocyanidins, when embedded in a material, effectively prevent the adhesion of both Gram-positive and Gram-negative pathogenic bacteria to commonly used biomaterial surfaces, independent of anti-bacterial mechanisms. This is an important finding as by preventing adhesion instead of killing bacteria, these compounds reduce the risk of propagating new resistant bacterial strains. The observed reduction ranged from 50% to 90% (Fig. 1B), indicating a strong potential for prolonging the non-infected lifespan of the tested materials when PACs are embedded in the carrier material.

[0053] Solutions of neat polycaprolactone (PCL) and PCL-2.5wt% PAC composite were prepared and glass disks mounted on a spin coater and spin coated with the solutions. Fig. 4 shows representative fluorescence microscope images of a PCL-2.5wt%PAC coated glass disk, and PCL coated glass disk, following the full PPFC experiment described hereinabove. The adhesion data recited in Table 1 hereinbelow is presented as the average number of bacteria adhering to the biomaterials surfaces. The ratio of adhered bacteria between the control and the treatment represents the fold reduction in bacterial adhesion (see Fig. 5). Bacterial adhesion was shown to be 5.5 times greater on the PCL coated glass disks than on the PCL-2.5wt% PAC glass disks.

[0054] It is disclosed herein that PACs activity when embedded and/or coated into a material such as a biomaterial result in a reduction in adhesion of bacteria due to steric interference by PACs adsorbed to the surface. Mammalian (kidney) cells maintained normal stretched morphology when challenged with bacteria (*P. aeruginosa*) in the presence of PACs as seen in Fig. 2, supporting PACs cytoprotective effects. In an embodiment, cranberry-derived PACs can be use to prevent infections related to a broad range of

indwelling devices (e.g., heart valves, stents, artificial limbs, etc) and medical conditions.

[0055] For example, it is disclosed a new catheter system embedded with cranberry extracted PACs, yielding biomaterial products with a greater resistance to bacterial colonization and biofilm formation. This novel system provides healthcare facilities with an innovative means to reduce costs and improve patient care.

[0056] Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface, tissue or cells for example. These first colonists adhere to the surface initially through weak, reversible van der Waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili. The first colonists facilitate the arrival of other cells by providing more diverse adhesion sites and beginning to build the matrix that holds the biofilm together. Some species are not able to attach to a surface on their own but are often able to anchor themselves to the matrix or directly to earlier colonists. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. The final stage of biofilm formation is known as development, and is the stage in which the biofilm is established and may only change in shape and size. The development of a biofilm may allow for the aggregate cell colony(ies) to be increasedly antibiotic resistant.

[0057] The catheter system disclosed herein or other material can be made of numerous polymers. Alternatively, it can be made of flexible latex, silicone, or Teflon tubing embedded with cranberry derived proanthocyanidins (PACs). It is the PACs embedded into the material that prevents initial bacterial adhesion and increases the infection-free lifespan of the catheter and thereby protects the patient. Catheter diameter ranges from 4 mm to 10 mm with a standard size of 4.6 mm. Terminal balloons vary in size from 5 cm^3 to 30 cm^3 as required for different applications. A dual channel design allows for balloon inflation with sterile water or saline solution. These treated urinary catheter systems are readily broadened to include infusion, cardiovascular, haemodynamic

monitoring, neurological, and renal catheters. Value added characteristics of these catheters lie in the reported inhibitory effects of PACs against inflammation and oxidative stress. Not only do these catheters prevent the onset of bacterial infection but they also reduce patient discomfort and reduce urethral or arterial irritation and cell death typically associated with catheterization.

[0058] The antiseptic catheter market is presently dominated by hydrogel-coated, antibiotic-coated, and silver-imbedded catheters. The most effective technology to date is the Bard® Hydrogel system combined with the BactiGuard® silver alloy coating. Though costs associated with these catheters are in excess of $5.00 more than for untreated catheters, clinical and economical evaluations have estimated a 47% decrease in the incidence of CAUTI resulting in a 20% decrease in associated overall health care costs.

[0059] The catheter system disclosed herein represents a more economical alternative to antiseptic catheter present in the market today, with a cost reduction of $2.00 to $3.00 or more per unit.

[0060] In addition, the effectivity of silver lies in its ability to catalyze oxidation reactions thereby denaturing protein disulfide bonds. Denaturing these proteins has a bactericidal function, which has resulted in the emergence of silver resistant strains, thereby further complicating infection treatment options. PACs treated catheters offer a novel approach. It is disclosed herein that PACs inhibit bacterial adhesion to surfaces independent of anti-bacterial mechanisms, a significant advantage since the potential for more resistant bacteria is reduced.

[0061] Thus, it is disclosed the use of PACs through the incorporation, coating and/or embedding of the compound in biomaterial or drug delivery vehicles in a range of applications that require sterility, including but not limited to medical devices, materials used in the food industry, materials used in pediatric applications, angioplasty balloons, urinary catheters, central line catheters, shunts, scalpels, and implanted materials.

[0062] It is intended herein as "embedding" rendering PACs integrally part of the material by infiltrating, enclosing or incorporating PACS during manufacture.

[0063] The anti-adhesive properties of PACs disclosed herein can also be used for a wide range of specialized industries to protect surfaces from bacterial colonization and biofilm formation. The anti-adhesive properties of PACs disclosed herein can be embedded into any material where sterility is a concern. For example, within the agricultural industry there are applications for PACs such as a natural food preservative if embedded into packaging materials. Other applications include cleaning products such as cleaning brushes, sponges or utensils.

[0064] Further applications relate to protecting foods from potential agroterrorism related microbial contamination. As a naturally derived compound, the addition of PACs compounds as disclosed herein to cosmetics may alleviate bacterial contamination of these products and the resulting irritations.

[0065] A material referred herein is intended to mean any material, natural or man-made, such as a biomaterial, metals, food preparation surfaces, wherein PACs can be coated on, incorporated in and/or embedded therein without limitation to. Biomaterials are intended to mean material that can contain whole or part of a living structure or biomedical device which performs, augments, or replaces a natural function. It is also intended herein to include all materials used as packaging materials for food preservation or cosmetic products. For example, and without limitation to, such biomaterial is a heart valve, a stent, an artificial limb, an angioplasty balloon, a shunt, a scalpel, and a catheter. The catheter can be for example biodegradable, as known in the art (US application publication nos. 2009/311337; 2009/299465; 2009/299292).

[0066] It is also disclosed herein the use of cranberry derived proanthocyanidins (PACs) to prevent mammalian cell death. It is demonstrated that the exposure of mammalian cells to cranberry proanthocyanidins at concentrations up to 150 μg/ml promotes proliferative, anti-inflammatory, and anti-apoptotic functions (Fig. 2 and Fig. 3). With increasing concentrations of

PACs, as an example up to 150 μg/ml or up to 300 μg/ml, but not limited to such concentration, cell density was increased whereas cell death was notably decreased (Fig. 3).

[0067] An anti-apoptotic effect is intended to mean the prevention of apoptosis of bacteria and/or cell, apoptosis being a type of cell death in which a series of molecular steps in a cell leads to its death.

[0068] The *in-vitro* effectivity of cranberry derived proanthocyanidins (PACs) for the mitigation of kidney cell infection by selected uro- and entero-pathogens is described herein with an adhesion/invasion assay and confocal microscopy. This study demonstrated that PACs effectively reduce invasion of canine kidney cells by pathogenic bacteria: *E. coli* CFT073 and O157:H7, *E. faecalis* 29212, and *P. aeruginosa* 10145. These effects demonstrate the potential for cranberry derived PACs as a useful tool in the prevention of kidney infection.

[0069] Although previous studies demonstrate that cranberry derived products can prevent adhesion of *E. coli* to selected eukaryotic cells (Ahuja et al., 1998, The Journal of Urology, 159: 559-562; Gupta et al., 2007, The Journal of Urology, 177: 2357-2360; Howell, et al., 1998, New England Journal of Medicine, 339: 1085-1086) and biomaterials (Eydelnant & Tufenkji, 2008, Langmuir, 24: 10273-10281), the potential effectivity of PACs in preventing cellular invasion has not been previously examined and demonstrated. It is described herein for the first time the effects of PAC on bacterial adhesion to and invasion of kidney epithelial cells. Moreover, this is the first disclosure presenting PAC effectivity on enteropathogenic *E. coli*, *Pseudomonas*, and Gram-positive *E. faecalis*. As invasion of kidney cells is a key step in the development of Gram positive and Gram-negative initiated pyelonephritis, the results described allow conceiving clinical implementation of PACs.

[0070] An invasion by bacteria of a cell is intended to mean the penetration of the bacteria into the cell by phagocytosis or fusion with the cell, allowing the infection by the bacteria, the bacteria growing and dividing in the cell cytoplasm

and gaining entry to neighbouring cells by bursting through and digesting membranes.

[0071] This new area of application of the PACs is fundamental as these compounds will prevent cellular inflammatory response and improve resistance to apoptosis. Their anti-inflammatory and anti-apoptotic effects will reduce the incidence of chronic illnesses such as cardiovascular diseases, liver/kidney disorders and others complications following heart transplant. The increased totipotency/proliferation effect of PACs on healthy mammalian cells will impact positively human well-being.

[0072] PACs according to the disclosure herein can also be used in the medical field as part of a composition to protect patients from the harmful effects of inflammation and cell death. In addition, the present disclosure provides the use of a natural product which is safe, non-toxic, and available in large quantities for a range of applications mentioned herein. In particular this is an exciting advance over existing technology as cranberry derived proanthocyanidins also reduce adhesion of bacteria to abiotic surfaces thereby serving as a 'two-for-one' strategy in clinical applications. The relatively low cost of PACs per application creates an economically viable solution to alleviate clinical expenditures. In an embodiment, it is disclosed an anti-apoptotic and/or anti-bacterial composition comprising PACs formulated in different forms such as powder form, pills, softchews and food beverages. The composition comprises also a pharmaceutically acceptable carrier, adjuvant or vehicle.

[0073] The term "carrier", also known as adjuvant or vehicle, refers to a carrier, adjuvant or vehicle that may be administered to a subject, incorporated into a composition of the present invention, and which does not destroy the pharmacological activity thereof. Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions disclosed herein include, but are not limited to, the following: ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems ("SEDDS"), surfactants used in pharmaceutical dosage forms such as Tweens™ or other similar polymeric delivery matrices, serum proteins such as

human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α-, β- and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-β-cyclodextrins, or other solubilized derivatives may also be used to enhance delivery of the compositions of the present invention.

[0074] The compositions disclosed herein may contain other therapeutic agents, and may be formulated, for example, by employing conventional solid or liquid vehicles or diluents, as well as pharmaceutical additives of a type appropriate to the mode of desired administration (for example, excipients, binders, preservatives, stabilizers, flavors, etc.) according to techniques such as those well known in the art of pharmaceutical formulation.

[0075] The compositions disclosed herein may be administered by any suitable means, for example, orally, such as in the form of tablets, capsules, granules or powders; sublingually; buccally; parenterally, such as by subcutaneous, intravenous, intramuscular, or intrastemal injection or infusion techniques (e.g., as sterile injectable aqueous or non-aqueous solutions or suspensions); nasally such as by inhalation spray; topically, such as in the form of a cream or ointment; or rectally such as in the form of suppositories; in dosage unit formulations containing non-toxic, pharmaceutically acceptable vehicles or diluents. The present compositions may, for example, be administered in a form suitable for immediate release or extended release. Immediate release or extended release may be achieved by the use of suitable pharmaceutical compositions, or, particularly in the case of extended release, by the use of devices such as subcutaneous implants or osmotic pumps.

[0076] Exemplary compositions for oral administration include suspensions which may contain, for example, microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners or flavoring agents such as those known in the art; and immediate release tablets which may contain, for example, microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and/or lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants such as those known in the art. The present compounds may also be delivered through the oral cavity by sublingual and/or buccal administration. Molded tablets, compressed tablets or freeze-dried tablets are exemplary forms which may be used. Exemplary compositions include those formulating the present compositions with fast dissolving diluents such as mannitol, lactose, sucrose and/or cyclodextrins. Also included in such formulations may be high molecular weight excipients such as celluloses (avicel^{TM}) or polyethylene glycols (PEG). Such formulations may also include an excipient to aid mucosal adhesion such as hydroxy propyl cellulose (HPC), hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose (SCMC), maleic anhydride copolymer (e.g., Gantrez^{TM}), and agents to control release such as polyacrylic copolymer (e.g., Carbopol^{TM} 934). Lubricants, glidants, flavors, coloring agents and stabilizers may also be added for ease of fabrication and use.

[0077] The effective amount of composition as disclosed herein may be determined by one of ordinary skill in the art, and includes exemplary dosage amounts for an adult human from about 0.1 to 500 mg/kg of body weight of active compound per day, which may be administered in a single dose or in the form of individual divided doses, such as from 1 to 5 times per day. It will be understood that the specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion and clearance, drug combination, and severity of the particular

condition. Preferred subjects for treatment include animals, most preferably mammalian species such as humans, and domestic animals such as dogs, cats and the like, subject to anti-apoptotic and/or anti-bacterial dependence or apoptotic and/or bacterial associated disorders.

[0078] The term "therapeutically effective amount" refers to an amount that is sufficient to effect a therapeutically or prophylactically significant reduction of a disease or condition when administered to a typical subject of the intended type. In aspects involving administration of PACs or composition comprising PACs to a subject, typically the PACs, formulation, or composition should be administered in a therapeutically effective amount.

[0079] The compositions disclosed herein may be employed alone or in combination with other suitable therapeutic agents useful in promoting anti-apoptotic, anti-invasive and anti-bacterial effects.

[0080] The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE 1

Bacterial adhesion assessments using PAC embedded biomaterial

[0081] Solutions of neat polycaprolactone (PCL) and PCL-2.5wt% PAC composite are prepared. Four glass disks, 14 mm in diameter (Biosurface Technologies), are washed with soap and rinsed thoroughly with distilled deionised water, then dried under ultrapure nitrogen. The disks are stored in sterile Petri dishes until use. The disks are then mounted on a spin coater (WS-400B-86NPP-LITE, Laurell Technologies Corporation), and spin coated for 5 minutes, at 3000 rpm, with an average of 175 µl of solution using a glass syringe. The analysis of PCL thin film preparation presented by Simon *et al.* (2007, Journal of Applied Polymer Science, 103: 1287-1294) is used as guidelines to decide upon the ideal spin coating parameters for this part of the experiment. The solutions are emptied from the syringe at a rate of 17.5 µl/s to

obtain uniform films. Two of the glass disks are coated with neat PCL, the other two with PCL-2.5wt% PAC composite.

[0082] The gram negative bacteria *E. coli* D21 are used. The bacterial cell suspensions are prepared from pure cultures, previously maintained at -80°C in Luria-Bertani Lennox broth (20 g/l) supplemented with 30% glycerol. Subsequently, cultures are streaked onto agar plates and incubated overnight (12h) at 37°C. The plates are stored for up to 7 days in the fridge at 4°C. For each experiment, a single colony of bacteria is inoculated into 150 ml of regular LB broth, and incubated at 200 rpm and 37°C for 4h. Afterwards, 30 ml of the cell suspension is centrifuged at 7000 rpm for 10 minutes (SS-34 rotor, Kendro) at 4°C. Then, the LB broth is decanted and the pellet is resuspended in 30 ml of phosphate buffered saline (PBS). The centrifugation and resuspension are repeated twice more to remove all LB broth and metabolites. The concentration of the cell suspension is determined using a volume of 0.02 µl of the suspension in a Helber bacteria counting chamber (SV400, ProSciTech), after which the suspension is diluted to the desired final concentration of $1.85x10^8$ CFU/ml in PBS.

[0083] First, the PCL and PCL-PAC coated glass disks are carefully placed in a dual channel parallel plate flow cell (PPFC), each channel containing 2 disks coated with the same material. The disks are then covered with thin microscope slides, previously washed with soap and DI water, and dried under ultrapure nitrogen. The PPFC is then closed, and two 20 ml syringes containing 85% ethanol are connected to it. All of these procedures are conducted under a biological safety cabinet (BSC) to reduce the risks of bacterial contamination. Using a syringe pump (Model 230, KD Scientific), the flow chambers are first rinsed with 85% ethanol contained in the previously connected syringes, at a flow rate of 2 ml/min. This procedure is repeated with DI water and then PBS, to allow the PPFC to be cleaned and then equilibrated with the same solution as the one in which the bacteria are suspended (PBS). Next, 5 ml syringes are filled with the bacterial suspensions, and the suspensions are injected in the flow cell at a rate of 0.5 ml/min. Once again the PPFC is rinsed with PBS contained in two 20 ml syringes, in order to remove any unattached cells.

Finally, the PPFC is covered in aluminium foil to reduce light incidence, and two 5 ml syringes with solutions of LIVE/DEAD BacLight™ bacterial viability kit (Invitrogen) prepared from 100 µl of green-fluorescent SYTO® 9 staining reagent, and 100 µl of red-fluorescent propidium iodide staining reagent, completed to 5 ml with DI water, are attached to the PPFC. The staining reagents are injected at a rate of 0.5 ml into the PPFC, and the system is incubated for 15 minutes in the dark at room temperature.

[0084] Following the incubation of the PPFC system with the LIVE/DEAD BacLight™, images are acquired using fluorescence microscopy (IX-71, Olympus). An average of 12 images per type of material (PCL and PCL-2.5wt%PAC) is acquired, and then processed using the public domain software ImageJ™. The attached bacteria are enumerated manually.

[0085] Fig. 4 shows representative fluorescence microscope images of a PCL-2.5wt%PAC coated glass disk, and PCL coated glass disk, following the full PPFC experiment described hereinabove.

[0086] The adhesion data recited in Table 1 is presented as the average number of bacteria adhering to the biomaterials surfaces, with the standard deviation from the sample presented in between parentheses. The ratio of adhered bacteria between the control and the treatment represents the fold reduction in bacterial adhesion (see Fig. 5). The average fold reduction was found to be 5.5; namely, bacterial adhesion is 5.5 times greater on the PCL coated glass disks than on the PCL-2.5wt% PAC glass disks. In all three experiments, the number of bacteria was shown to be significantly higher on the PCL coated coupons than on the PCL-2.5wt%PAC coated coupons. A Student one-tailed t-test was used to compare the means of the two treatments and a value of $p<0.01$ was chosen to show a statistically significant difference.

Table 1

Summary of Bacterial Adhesion Experiment Results in the Presence and Absence of PACs

Mean Adhered Bacteria (cells/cm^2) to PCL-2.5wt%PAC coupons	Mean Adhered Bacteria (cells/cm^2) to PCL coupons	Ratio of Adhered Bacteria (PCL/PCL-2.5wt%PAC)	Student t-test
1.666E+04 (1.202E+04)	1.507E+05 (6.892E+04)	9.1	p<0.01
2.782E+04 (1.223E+04)	9.980E+04 (2.444E+04)	3.6	p<0.01
1.004E+04 (5.941E+03)	3.778E+04 (1.911E+04)	3.8	p<0.01

EXAMPLE 2

Preventing pathogen invasion of kidney epithelial cells

[0087] Dry PAC extract purified by HPLC (Marucci Center for Blueberry and Cranberry Research, Rutgers University) is ground using a mortal and pestle then solubilized in deionized (DI) (Milli-Q) water to obtain a PAC stock solution (1.5 mg/mL). PAC stock solution is filtered through a 0.45 m syringe filter prior to experimentation. The average molecular weight of PAC used is ~15 kDa.

[0088] Axenic cultures of uropathogenic *E. coli* CFT073 ATCC 700928, uropathogenic *E. faecalis* ATCC 29212, environmental isolate *P. aeruginosa* 10145, and enteropathogenic *E. coli* O157:H7 ATCC 700927 are used. *E. coli* CFT073 is a Gram negative clinical isolate from the blood and urine of a woman with acute pyelonephritis and its complete genome has been sequenced. *E. faecalis* 29212 is a well-characterized Gram-positive uropathogenic bacterium isolated from urine. *P. aeruginosa* is a common Gram-negative opportunistic pathogen that typically infects the pulmonary tract and urinary tract. *E. coli* O157:H7 is a Gram-negative enterohemorrhagic strain of *E. coli* that causes acute gastroenteritis. Pure cultures are maintained at -80°C in Luria-Bertani Lennox broth (20 g/l) supplemented with 30% glycerol. Cultures are streaked onto LB agar plates, then incubated 24 h at 37°C. For each experiment, a single colony from a fresh plate was used to inoculate 15 ml of LB broth (in a 50 ml Erlenmeyer flask). Cultures are incubated at 37°C for 18 h at 200 rpm, then

harvested by centrifugation at 5860 *g* for 15 min (SS-34 rotor, Kendro) at 4°C. The growth media is decanted and the pellet is resuspended in Dulbecco's Modified Eagle Medium (DMEM). Centrifugation and resuspension are repeated one additional time to remove any traces of growth media and metabolites. The concentration of cells is determined with a Helber (SV400, Proscitech) bacteria counting chamber and the suspensions are diluted accordingly to achieve a multiplicity of infection of 50:1 in DMEM with or without PAC supplementation (50 g/ml).

[0089] Madin-Darby Canine Kidney (MDCK) cells are cultured in DMEM containing 4.5 g/l glucose, 10% fetal bovine serum (FBS, Invitrogen) supplemented with 10 mM Hepes, 100 U/ml of penicillin, 100 g/ml streptomycin and non-essential amino acids. The cells are incubated under conventional cell culture conditions at 37°C in a humidified incubator containing 5% CO_2. One day prior to experimentation, MDCK cells are seeded on glass coverslips in 24-well culture plates, then incubated 24 hr to 80% confluency.

[0090] MDCK cells are washed in phosphate buffered saline (PBS, Sigma-Aldrich) then infected with the respective bacteria at a multiplicity of infection of 50:1 for 3 hrs at 37°C. In the determination of total associated bacteria (TAB), MDCK cells are washed with PBS and lysed in 0.1% Triton™ X-100 (Sigma). Supernatant is diluted in maximum recovery diluent (Fisher) and plated in triplicate on LB agar plates for bacteria enumeration. Invasion assays followed the same infection protocol, with the exception that after infection, MDCK cells are incubated for 1 hr in gentamiacin (100 µg/ml, Sigma-Aldrich) to eliminate extracellular bacteria prior to lysis and plating. Control experiments determined these conditions as sufficient for complete elimination of all bacteria examined.

[0091] For immunofluorescence imaging, MDCK cells infected with *E. coli* O157:H7, prior to Triton™-X lysis, are washed with PBS, fixed in 2.5% paraformaldehyde (Fluka) for 15 minutes, washed again using PBS, then blocked and permeabilized with 4% bovine serum albumin/0.1% Triton™-X/PBS (BPBS) for 30 min. Infected MDCK cells are incubated for 1 hr with fluorescein-labeled antibody directed to *E. coli* O157:H7 (Kirkegaard and Perry

Laboratories), washed with PBS, incubated with Texas-Red phalloidin (Invitrogen), and washed again with PBS. Internalized bacteria are imaged by confocal laser scanning microscopy and enumerated manually (30 cells per condition are imaged).

[0092] The presence of PAC significantly inhibited bacterial invasion of MDCK cells as determined by the adhesion/invasion assay (AIA) (Fig. 6; open bars). The noted reduction in invasion (i.e., internalization) was further confirmed by analysis of CLSM images of stained MDCK cells infected by *E. coli* O157:H7 (Fig. 6; solid bar and Fig. 7).

EXAMPLE 3

Release study of PACs in water and PBS

Composite cranberry PAC-PCL film preparation

[0093] A solvent casting method is used to produce neat polymer and composite films. In all cases, 1g of PCL is dissolved in 10 ml of chloroform. The PAC powder is added to the polymer solution in the following concentrations for the composite films: PCL-2.5wt% PAC and PCL-5wt% PAC. Once the PAC is incorporated into the PCL solution, the glass vials are covered in aluminium foil to reduce light incidence. The solutions are then sonicated (Ultrasonic Cleaner FS20, Fisher Scientific) for 1h to obtain a homogeneous dispersion of the PAC in the polymer solution. On glass Petri dishes, the solutions are cast and the solvent is allowed to evaporate in a dark room for 48h. Six circular coupons 15 mm in diameter of each type (neat PCL, PCL-2.5wt% PAC, and PCL-5wt% PAC) are then cut out of the films and stored with the rest of the films in the dark in desiccators until further analysis.

Fourier Transform Infrared (FTIR) Analysis

[0094] Some Fourier Transform Infrared Analysis are conducted for pure PACs, a PCL film, and a PCL-2.5wt%PAC composite film. The FTIR spectroscopy is done with a PE IR SPECTRUM ASCII PEDS 1.60(spectrometer (Perkin-Elmer Instruments).

Differential Scanning Calorimetry

[0095] A Differential Scanning Calorimetry method is used to assess the thermal properties of the PCL film, as well as of the composite film of PCL-2.5wt% PAC and PCL-5wt% PAC. The tests are performed using a Perkin-Elmer Pyris Diamond Differential Scanning Calorimeter (Perkin-Elmer Instruments). Samples for each material weighing on average 5.5 mg ± 0.1 are used. All samples are first brought to a temperature of -50°C and held at that temperature for 1 minute, after which they are heated up to 100°C at a rate of 20°C/minute, and again held at that temperature for 1 minute. The samples are subsequently cooled back to -50°C at the same rate. This heat/cool cycle is repeated three times. All tests are carried out under ultrapure nitrogen.

Release study of PAC in water and phosphate buffered saline (PBS)

[0096] A release study is conducted to quantify the behaviour of PAC when soaked in either water or PBS. A 137 mM NaCl, 2.7 nmM KCl, and 10mM PBS solution (Sigma Aldrich, pH 7.40±0.02 at 25°C) is prepared using ultrapure water (18.2 MΩ·cm). 9 sterile plastic vials are filled with 20 ml of this solution; another 9 sterile plastic vials are filled with 20 ml of ultrapure water. All vials are then covered in aluminium foil and incubated for 12h at 37°C±0.5. One polymer or composite coupon is added to each vial, to obtain the following design:

Table 2

Experimental design for the release study of PAC in water and PBS

Sample Name	Type of media	Polymer type
A1, A2, A3	Ultrapure water	Neat PCL
B1, B2, B3	PBS	Neat PCL
C1, C2, C3	Ultrapure water	PCL -2.5wt% PAC
D1, D2, D3	PBS	PCL -2.5wt% PAC
E1, E2, E3	Ultrapure water	PCL -5wt% PAC
F1, F2, F3	PBS	PCL -5wt% PAC

[0097] Prior to their incorporation into the vials, all coupons are weighed, and their average thickness is established. Samples are then returned to the

incubator, before being withdrawn again for further analysis at set time points: 1h, 2h, 4h, 8h, 1d, 2d, 3d, 7d, 14d, and 21d. At each time point, each sample is removed from their respective solution, gently dabbed with absorbing paper, and weighed. Afterwards, aliquots of 0.5 ml are taken from each solution and analysed using a UV-Visible spectrophotometer (Agilent Technologies 8453 with ChemStation) at 280 nm to determine the concentration of PAC. The set wavelength of 280 nm is known to be specific for the absorbance of PAC.

[0098] The incorporation of cranberry derived proanthocyanidins into a polymer matrix of polycaprolactone, and subsequent release of the compound in an aqueous environment is thus proven successful herein.

[0099] The ATR spectra for PAC, neat PCL, and the PCL-PAC composite can be seen in Fig. 8. The presence of PACs could be observed in the composite material spectrum, more specifically at 1610, 1524, and 825 cm^{-1}. Following the incorporation of the PACs in the PCL polymer matrix, the peaks at 1695–1735 cm^{-1} associated with carbonyl stretching, as well as the peaks at 2800–3000 cm^{-1}, typical for CH_2 symmetrical and asymmetrical stretching of the PCL backbone are still strongly present, suggesting that the PACs and PCL are not covalently bonding.

[00100] Data relative to the thermal properties of PCL, PCL-2.5wt%PAC, and PCL-5wt%PAC is seen in Fig. 13 and Table 3, showing the comparison of the three different materials, both in the heating and the cooling cycles. In addition, specific properties of the materials are extracted, more specifically the melting temperatures (T_m), and the heat of fusion (ΔH_f), both taken from the second heating cycles. The first cycles could not be used, as the materials could have undergone different treatments prior to testing, and these first heating and cooling processes allowed for the materials to regain uniform properties. It is found that the different materials, with or without cranberry PACs, have almost identical thermal properties, when it comes to melting temperature and heat of fusion.

Table 3

Thermal properties of PCL, PCL-2.5wt%PAC and PCL-5wt%PAC thin films

Properties *	PCL	PCL-2.5wt% PAC	PCL-5wt%PAC
Tm (°C)	56.2	56.3	56.2
ΔHf (J/g)	65.0	62.3	63.1

[00101] The release study is conclusive in demonstrating that PAC could be released from a PCL polymer matrix. The results presented in Fig. 9 and Fig. 10 show the average cumulative release of PAC in water and PBS, at concentrations of 2.5 wt% and 5 wt% of PAC in the composites, with the error bars representing the standard deviations from the mean values. It is confirmed through the UV-Vis analysis that only the cranberry proanthocyanidins compounds could be observed at 280 nm. Therefore, as expected, the concentrations for the neat PCL coupons remained at zero throughout the experiment and are not presented on these figures. It can be seen that the release profiles for PCL-5wt%PAC are almost identical for both water and PBS, as well as for PCL-2.5wt%PAC. The final maximal concentrations of PACs in ultrapure water (after 21 days), for the PCL-2.5wt%PAC coupons is of 0.036mg/ml±0.003, and of 0.082 mg/ml±0.005 for the PCL-5wt%PAC coupons. In contrast, in PBS, the concentrations after 21 days are of 0.043mg/ml±0.004 for the PCL-2.5wt%PAC coupons, and of 0.082mg/ml±0.010 for the PCL-5wt%PAC coupons. These variations can be explained by the poor control on the homogeneity of the composite films, especially for lower concentrations of PACs. This led to the difficulty of precisely assessing the percentage release of PAC in water and PBS. However, it can be estimated from Fig. 11 and Fig. 12 that for all films most of the PACs are released. The fact that some PACs might have been lost in the casting process combined with the poor control on the homogeneity of the films most probably lead to an error in the estimation of the initial amount of PACs in the films, which is the basis for the estimation of the percentage of PACs released, and therefore explaining the large standard deviation error bars present in Figs. 11 and 12.

[00102] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1- An anti-infectious composition comprising proanthocyanidins extracted from cranberry and a carrier, said composition preventing adhesion and propagation of bacteria.

2- The composition of claim 1, wherein said cranberry is of the species *Vaccinium macrocarpon.*

3- The composition of claim 2, wherein said bacteria are Gram-positive or Gram-negative.

4- The composition of any one of claims 1-3, wherein said proanthocyanidins prevent biofilm formation.

5- The composition of any one of claim 1-4, further comprising a therapeutic agent.

6- The composition of claim 5, wherein said therapeutic agent is an anti-bacterial agent or an antibiotic.

7- The composition of any one of claims 1-6, wherein said composition also has anti-inflammatory property.

8- An anti-infectious composition comprising proanthocyanidins extracted from cranberry and a carrier, said composition preventing invasion of bacteria in a cell, infection and apoptosis of the cell.

9- The composition of claim 8, wherein said cranberry is of the species *Vaccinium macrocarpon.*

10- The composition of claim 8, wherein said bacteria are Gram-positive or Gram-negative.

11- The composition of any one of claims 8-10, wherein said proanthocyanidins prevent biofilm formation.

12- The composition of any one of claims 8-11, wherein said composition further prevents adhesion and propagation of bacteria.

13- The composition of any one of claim 8-12, further comprising a therapeutic agent.

14- The composition of claim 13, wherein said therapeutic agent is an anti-bacterial agent or an antibiotic.

15- The composition of any one of claims 8-14, wherein said composition also has anti-inflammatory property.

16- A material comprising a composition as defined in any one of claims 1-15.

17- The material of claim 16, wherein said material is a heart valve, a stent, an artificial limb, an angioplasty balloon, a shunt, a scalpel or a catheter.

18- The material of claim 17, wherein said catheter is a urinary catheter or a central line catheter.

19- The material of claim 18, wherein said catheter is made of a polymer.

20- The material of any one of claims 16-19, wherein said material is a packaging material.

21- The material of claim 20, wherein said packaging material is a food packaging material or a cosmetic packaging material.

22- Use of proanthocyanidins extracted from cranberry in the manufacture of a medicament to prevent apoptosis of cells.

23- The use of claim 22, wherein said medicament is for improving the condition of a patient suffering from cardiovascular disease, liver disorder, kidney disorder or complication of heart transplant.

24- The use of claim 22, wherein said cranberry is of the species *Vaccinium macrocarpon*.

25- Use of proanthocyanidins extracted from cranberry in the manufacture of a material free of bacteria.

26- The use of claim 25, wherein said cranberry is of the species *Vaccinium macrocarpon*.

27- Use of proanthocyanidins extracted from cranberry for improving the condition of a patient suffering from cardiovascular disease, liver disorder, kidney disorder or complication of heart transplant.

28- Use of proanthocyanidins extracted from cranberry for preventing apoptosis of cells.

29- The use of claim 27 or 28, wherein said cranberry is of the species *Vaccinium macrocarpon*.

30- Method of preventing bacterial colonization and biofilm formation on the surface of a material comprising the step of contacting said material with proanthocyanidins extracted from cranberry.

31- The method of claim 30, wherein said cranberry is of the species *Vaccinium macrocarpon*.

32- The method of claim 30, wherein said material is a heart valve, stent, artificial limb, angioplasty balloon, shunt, scalpel or catheter.

33- The method of claim 32, wherein said catheter is a urinary catheter or a central line catheter.

34- The method of claim 33, wherein said catheter is made of a polymer.

35- The method of claim 30, wherein said material is a packaging material.

36- The method of claim 35, wherein said packaging material is a food packaging material or a cosmetic packaging material.

37- A method of preventing apoptosis of cells in a patient comprising administering to said patient proanthocyanidins extracted from cranberry.

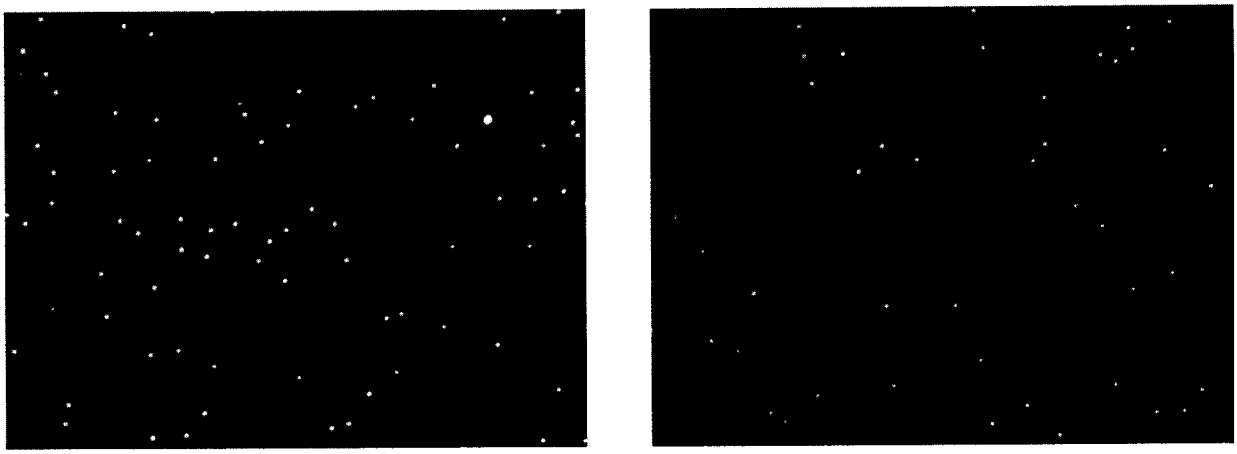
38- The method of claim 37, wherein said proanthocyanidins further improve the condition of a patient suffering from cardiovascular disease, liver disorder, kidney disorder or complication of heart transplant.

39- The method of claim 37, wherein said cranberry is of the species *Vaccinium macrocarpon*.

40- A method for improving the condition of a patient comprising administering to said patient proanthocyanidins extracted from cranberry, the condition being cardiovascular disease, liver disorder, kidney disorder or complication of heart transplant.

41- The method of claim 40, wherein said cranberry is of the species *Vaccinium macrocarpon*.

A



Control = 4x PAC = 4x

B

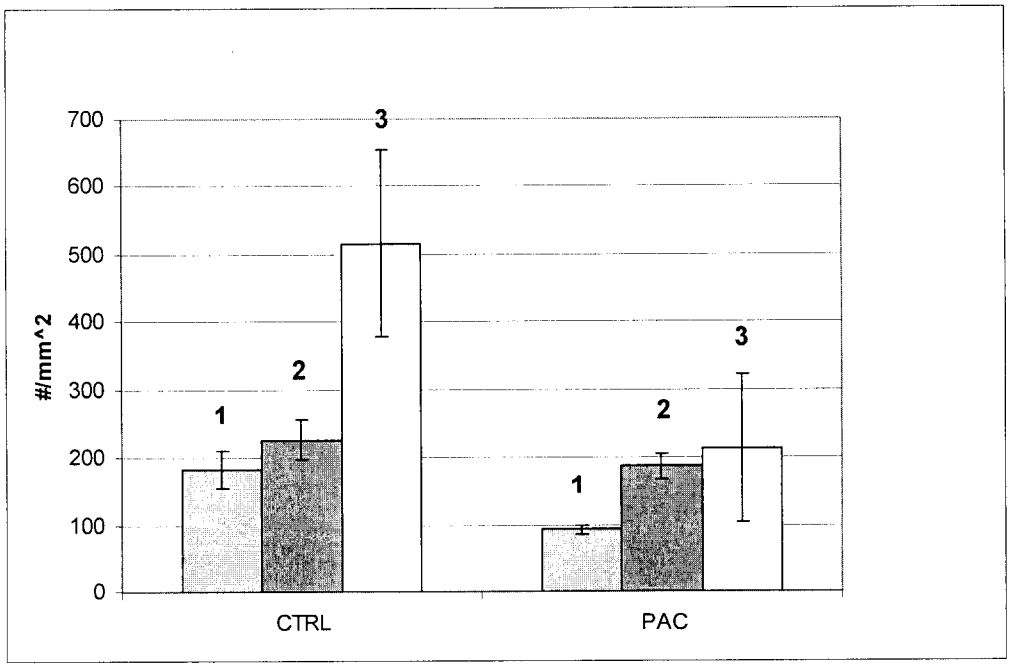


Fig. 1

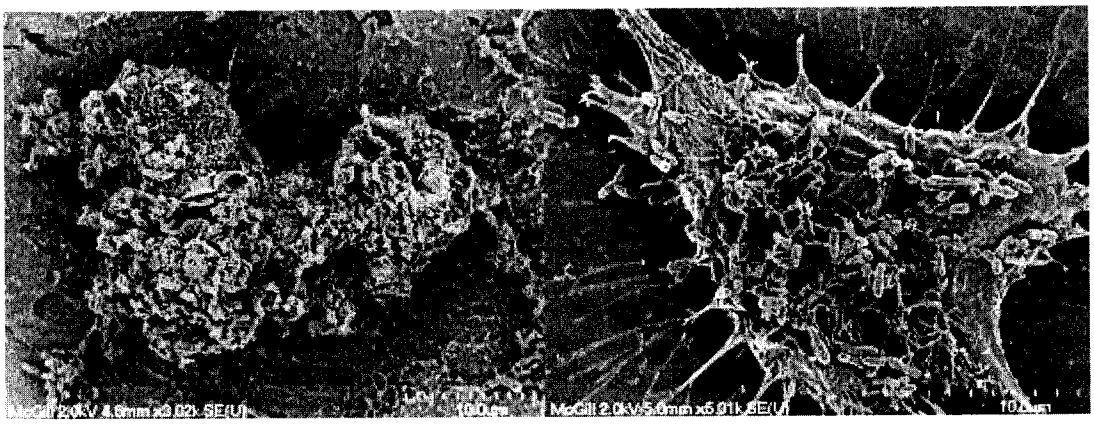


Fig. 2

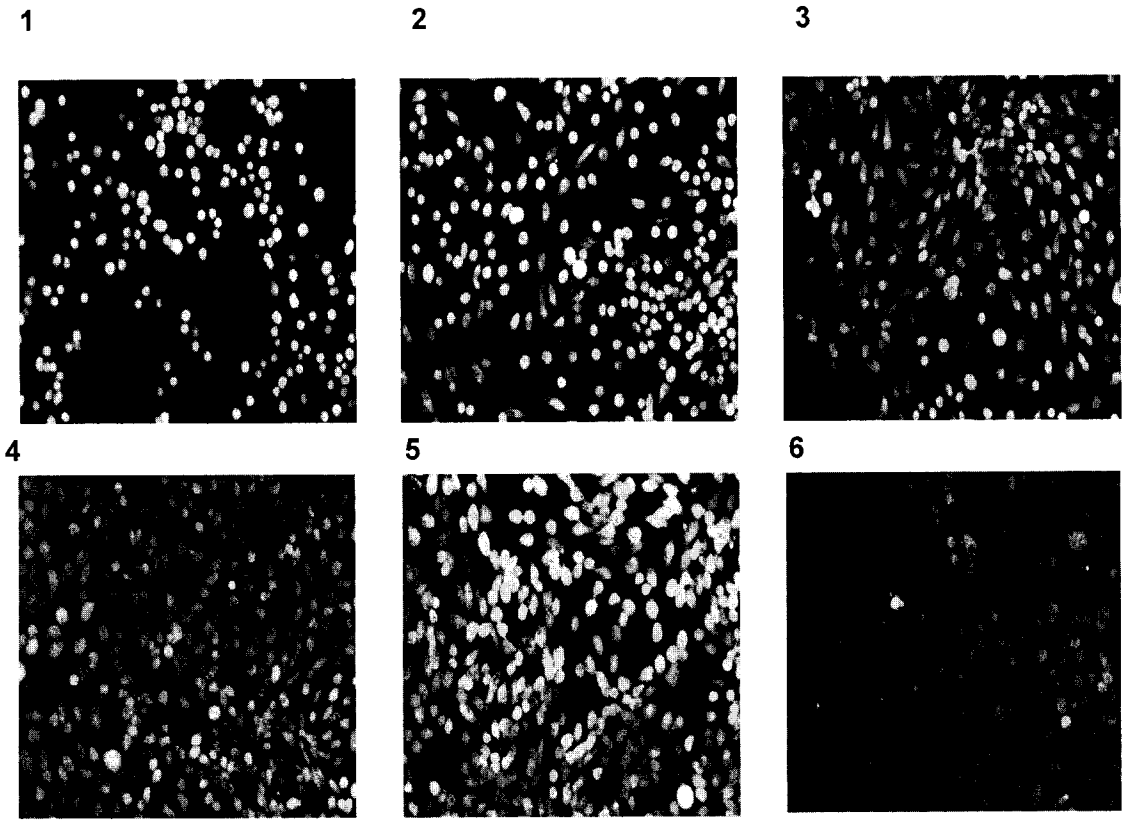


Fig. 3

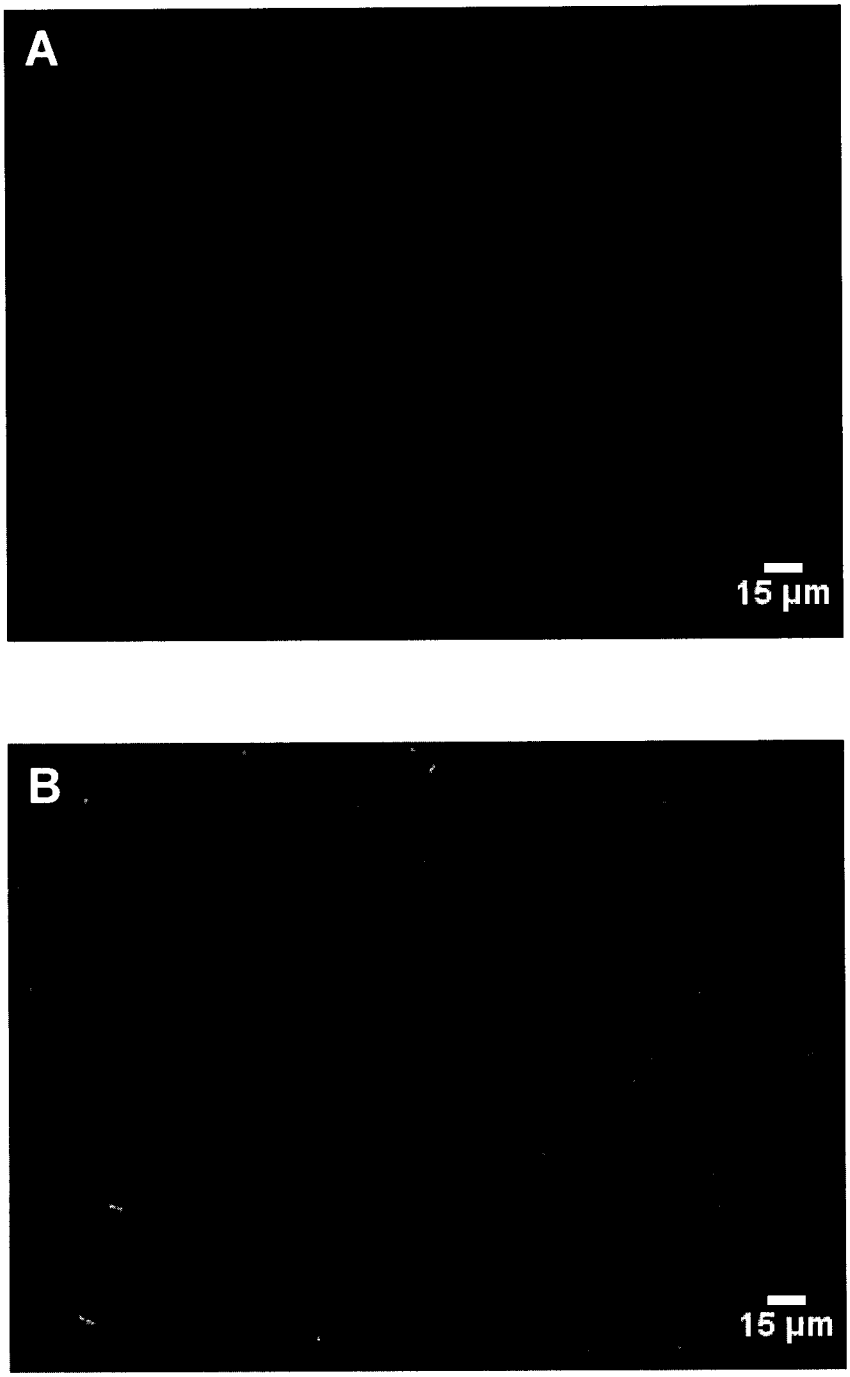


Fig. 4

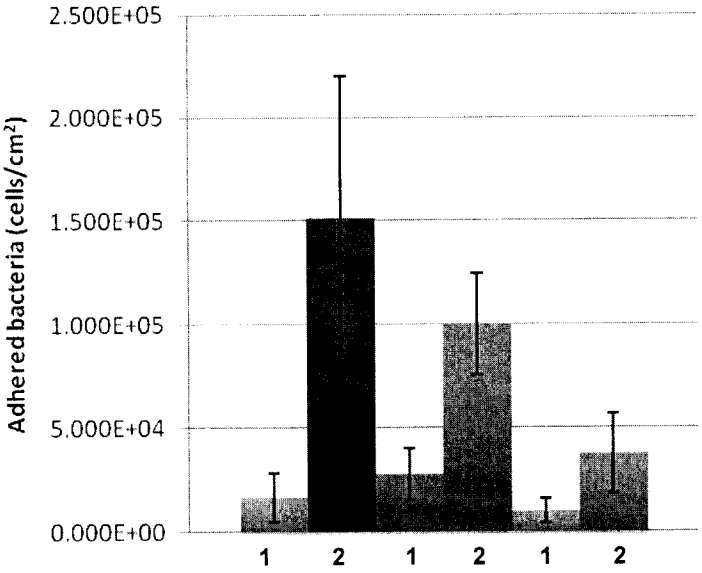

2.500E+05
2.000E+05
1.500E+05
1.000E+05
5.000E+04
0.000E+00
Adhered bacteria (cells/cm²)
1
2
1
2
1
2

Fig. 5

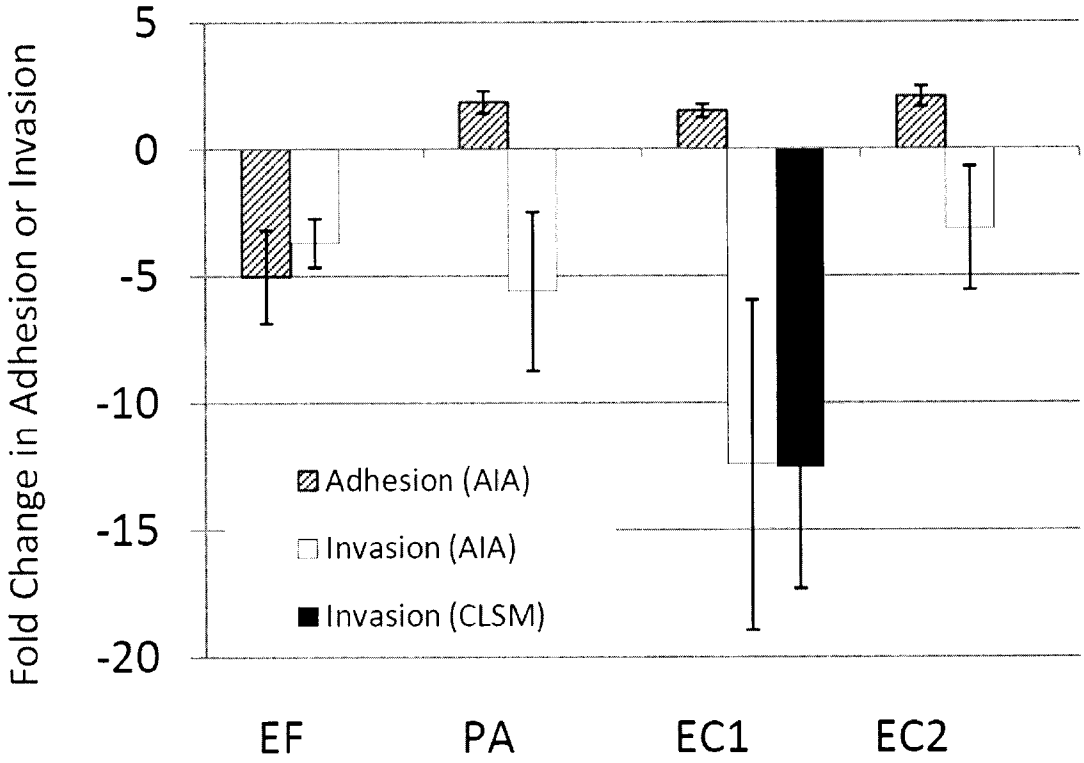

Fold Change in Adhesion or Invasion
5
0
-5
-10
-15
-20
Adhesion (AIA)
Invasion (AIA)
Invasion (CLSM)
EF
PA
EC1
EC2

Fig. 6

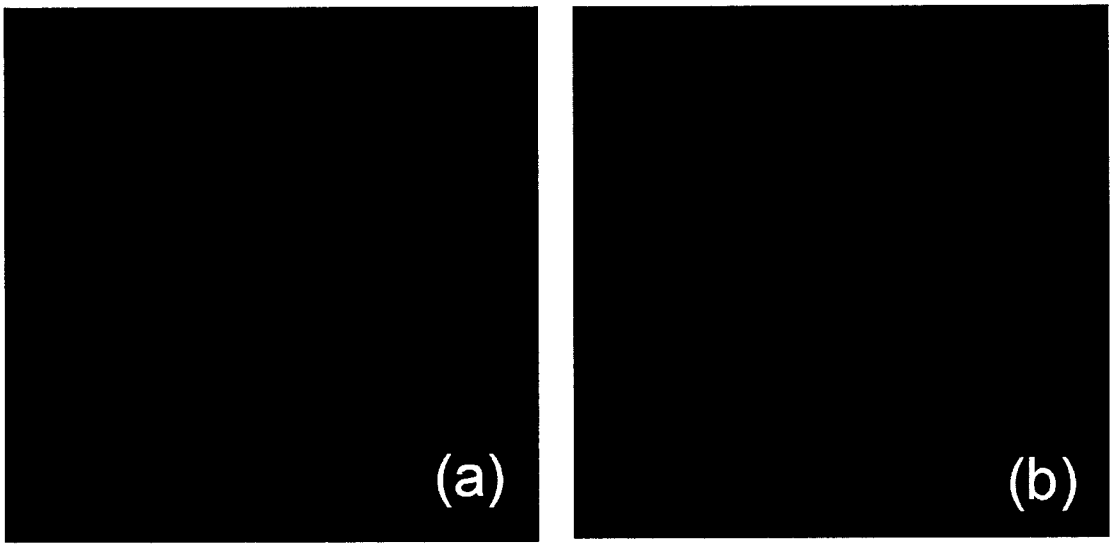

(a)
(b)

Fig. 7

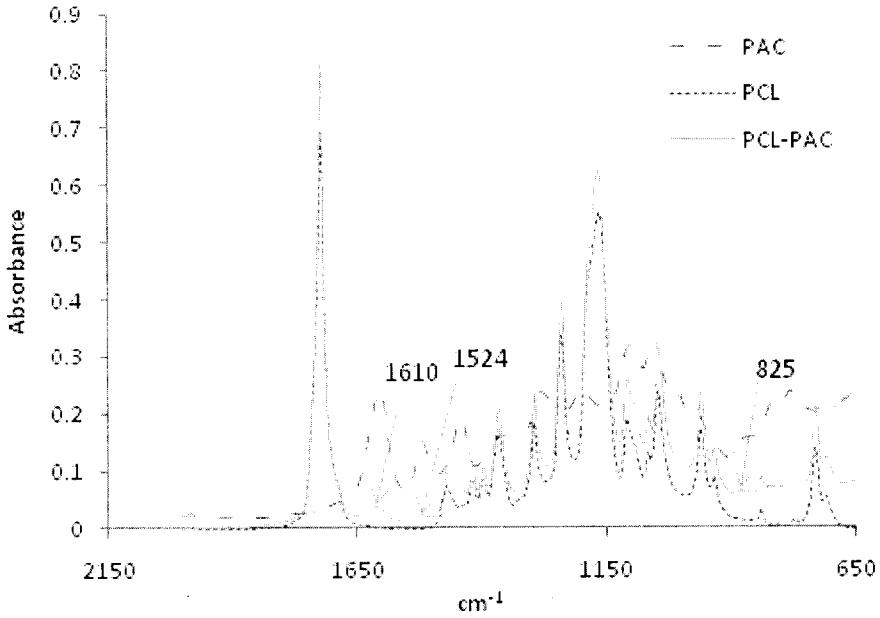

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance
PAC
PCL
PCL-PAC
1610
1524
825
2150
1650
1150
650
cm-1

Fig. 8

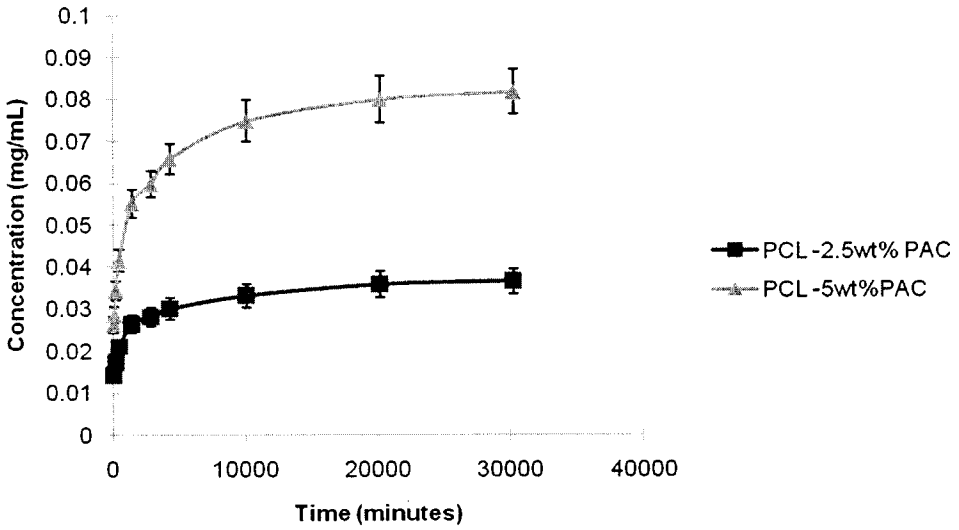

0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Concentration (mg/mL)
0
10000
20000
30000
40000
Time (minutes)
PCL-2.5wt% PAC
PCL-5wt%PAC

Fig. 9

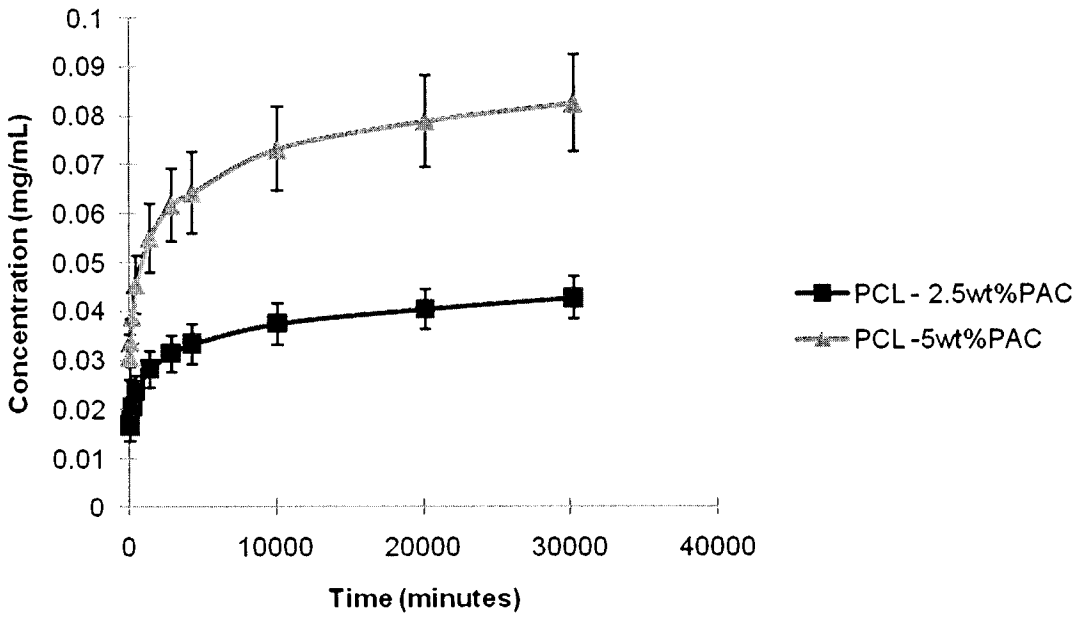

0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Concentration (mg/mL)
0
10000
20000
30000
40000
Time (minutes)
PCL - 2.5wt%PAC
PCL -5wt%PAC

Fig. 10

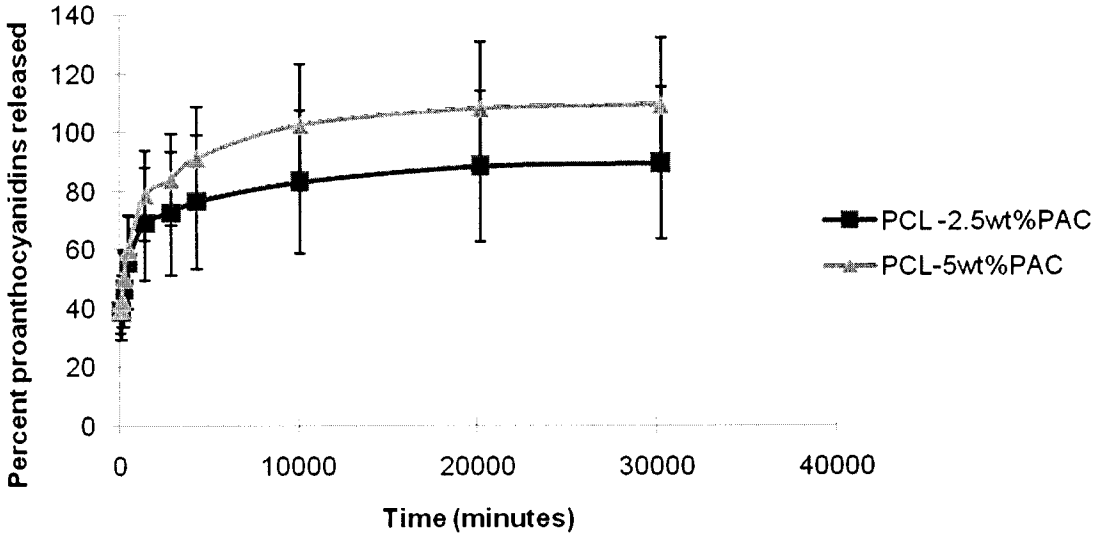

Percent proanthocyanidins released
140
120
100
80
60
40
20
0
0
10000
20000
30000
40000
Time (minutes)
PCL -2.5wt%PAC
PCL-5wt%PAC

Fig. 11

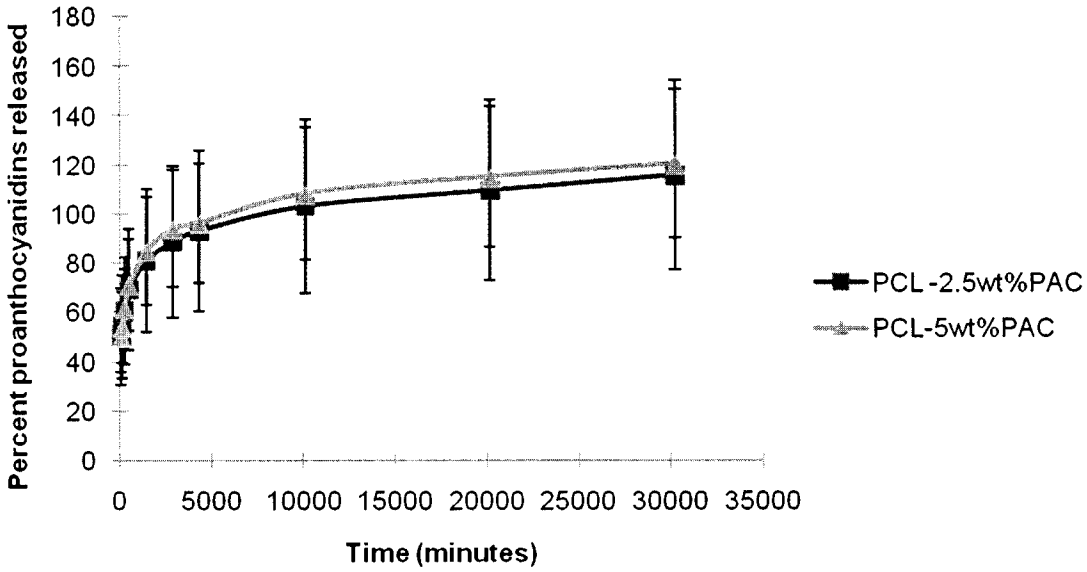
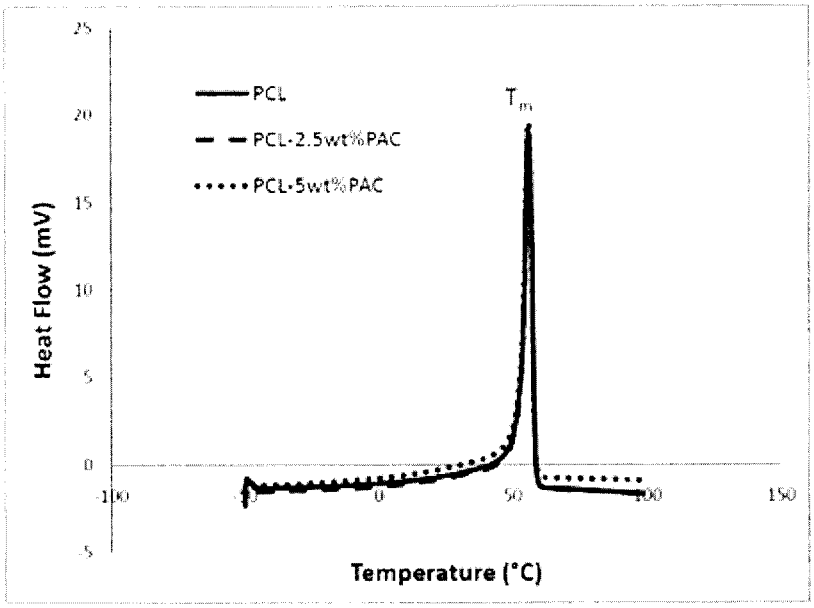

Percent proanthocyanidins released
180
160
140
120
100
80
60
40
20
0
0
5000
10000
15000
20000
25000
30000
35000
Time (minutes)
PCL -2.5wt%PAC
PCL-5wt%PAC

Fig. 12

A


PCL
PCL-2.5wt%PAC
PCL-5wt%PAC
T_m
Heat Flow (mV)
Temperature (°C)

B

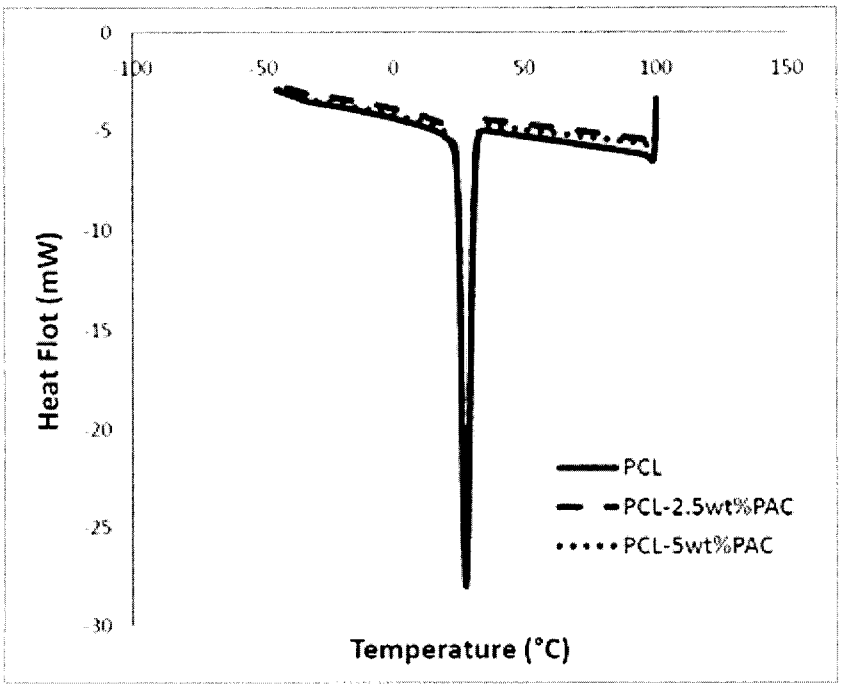

Heat Flot (mW)
Temperature (°C)
PCL
PCL-2.5wt%PAC
PCL-5wt%PAC

Fig. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2010/000044

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 36/45*** (2006.01) , ***A61K 31/352*** (2006.01) , ***A61L 2/16*** (2006.01) , ***A61L 27/54*** (2006.01) , ***A61L 29/16*** (2006.01) , ***A61L 31/16*** (2006.01), ***A61P 31/04*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: ***A61K 36/45*** (2006.01) , ***A61K 31/352*** (2006.01) , ***A61L 2/16*** (2006.01) , ***A61L 27/54*** (2006.01) , ***A61L 29/16*** (2006.01) , ***A61L 31/16*** (2006.01), ***A61P 31/04*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

CANADIAN PATENT DATABASE, EPOQUE, USPTO/WEST, ESPACENET, STN/CAPLUS, PUBMED, GOOGLE; Keywords: proanthocyanidins, cranberry, *Vaccinium macrocarpon*, infection, bacteria, apoptosis, antibiotic, anti- inflammatory, anti-bacterial, anti-adhesive, cardiovascular disease, liver disorder, kidney disorder, complication of heart transplant.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EYDELNANT, T.A. and TUFENKJI, N. Cranberry derived proanthocyanidins reduce bacterial adhesion to selected biomaterials. Langmuir. 16 September 2008, Vol. 24, No.18, pages 10273-10281, ISSN 0743-7463. See entire document.	1-21, 25, 26, and 30-36
X	WO 2008/018111 A2 (FALCONE, G. and BOLDRINI, E.) 14 February 2008. See page 4, lines 14-20; and page 4, line 31 to page 5, line 3.	1-3, 8-10, and 12

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 April 2010 (10-04-2010)	3 May 2010 (03-05-2010)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Qianfa Chen (819) 994-1374

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2010/000044

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 30-41

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 30-41 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search under Rule 39.1 (iv) of the PCT. Regardless, this Authority has carried out a search based on the alleged effect(s) or purpose(s)/use(s) of the product defined in claims 30-41.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/0054924 A1 (LEAHY, M.M. et al.) 9 May 2002. See paragraphs 0007, 0011, 0079, 0082 and 0105.	22-24, 27-29, and 37-41
X	RASMUSSEN, S.E. et al. Dietary proanthocyanidins: occurrence, dietary intake, bioavailability, and protection against cardiovascular disease. Mol. Nutr. Food Res. February 2005, Vol.49, No.2, pages 159-174, ISSN 1613-4125.	22-24, 27-29, and 37-41
A	GU, L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. J. Agric Food Chem. 3 December 2003, Vol.51, No.25, pages 7513-7521, ISSN 0021-8561. See entire document.	1-41
A	LAURA, A.K. Cranberry proanthocyanidins induce apoptosis and inhibit acid-induced proliferation of human esophageal adenocarcinoma cells. J. Agric. Food Chem.. 23 January 2008, Vol.56, No.3, pages 676–680, ISSN 0021-8561. See entire document.	1-41

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2010/000044

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2008018111 A2	14-02-2008	ITRM20060435A1	09-11-2006
		WO2008018111A3	20-03-2008
US2002054924 A1	09-05-2002	AU5161001A	07-11-2001
		CA2405348A1	01-11-2001
		WO0180870A2	01-11-2001
		WO0180870A3	21-08-2003