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(54) **Title**: A NANOBIOCOMPOSITE FORMULATION FOR WOUND HEALING AND A PROCESS FOR THE PREPARATION THEREOF

(57) **Abstract**: The present invention involves the isolation of plant based CNCs from the leaves of S. cumini. For the formation of NCs, a novel greener approach using S. cumini LE as reducing agent for in situ impregnation of AgNPs as fillers into CNCs as matrix is reported. The silver nitrate solution in three different concentrations of 1 mM, 5 mM and 10 mM was used to form NCs where AgNPs have been incorporated into CNCs matrix. The CNCs and NCs were characterized using SEM, TEM, XRD, Zeta potential, FT-IR, and UV-Vis spectroscopy. NCs developed in the form of film and ointment showed strong antimicrobial activity against both gram negative and gram positive bacteria. NCs wound dressing is capable of regulating wound exudates and providing moisture to wound responsible for faster healing of acute wounds. The observations from histopathological and biochemical assays confirmed that NCs enhance healing because of lesser inflammation, rapid angiogenesis, early collagen formation and enhanced rate of reepithelization.

AMENDED CLAIMS
received by the International Bureau on 28 August 2017 (28.08.2017)

We claim:

1. A nanobiocomposite formulation (NCs) comprising silver nanoparticles (AgNPs) and cellulose nanocrystals (CNCs) wherein the ratio of AgNPs and CNCs is in the range of 0.067% -0.4% w/w (AgNPs) : 7-8% w/w (CNCs), and wherein the cellulose is derived from *Syzygium cumini* leaves.

2. The nanobiocomposite formulation (NCs) as claimed in claim 1, wherein the formulation is in ointment or film form.

3. A process for the preparation of the nanobiocomposite formulation as claimed in claim 1 comprising the steps:

a) treating washed and dried *Syzygium cumini* leaves with bleaching agent at pH in the range of 3.0-4.0 for a time period ranging between 2-3 h at a temperature ranging between 70-100°C to obtain a bleached fibrous material;

b) filtering and washing the bleached fibrous material as obtained from step (a) followed by treating with acidified bleaching agent solution at a temperature ranging between 22-30°C for a time period ranging 16-18 h to obtain acidified fibrous material;

c) washing the acidified fibrous material as obtained from step (b) followed by keeping it into each of 2-18% base for 2 to 5 h each at 70 to 80°C under continuous stirring to obtain fibrous material;

d) treating the fibrous material from step (c) with 0.6 to 3% sodium chlorite solution for a time period ranging from 0.5 to 3 h at a temperature ranging from 80-100°C followed by filtrating and washing to obtain a chemically treated fiber;

e) treating the chemically treated fiber as obtained from step (d) with 64- 67% (v/v) acid in the ratio ranging from 1:5 -1:9 for a time period ranging from 30-

120 min at a temperature ranging from 40 to 80°C under continuous stirring to obtain acidified chemically treated fiber;

f) centrifuging and dialyzing the acidified chemically treated fiber as obtained from step (e) against water to remove the acid content until neutral to obtain cellulose nanocrystals, CNCs;

g) treating the cellulose nanocrystals, CNCs as obtained from step (f) mechanically by known methods for a time period ranging from 4-30 mM to individualize the fibers and freeze drying to obtain white solid CNCs;

h) dipping *Syzygium cumini* leaves in water for a period of time in the range of 20-28 h at a temperature in the range 20-30°C followed by filtration and storing at a temperature 4-10°C to obtain leaf extract as a biological reducing agent;

i) freeze drying of CNCs as obtained from step (g) and adding each of 1 mM, 5 mM and 10 mM (1-10 mM) silver nitrate solution and sonicating for 2 to 5 min to obtain a mixture;

j) adding 10-20% v/v of *Syzgium cumini* leaf extract as obtained from step(h) to each of the mixture as obtained from step (i) under continuous stirring for a time period ranging from 4 to 8 h at a temperature in the range of 20-30°C;

k) centrifuging the mixture as obtained in step (j) to obtain nanobiocomposite, NC formulation;

4. The process as claimed in claim 3, wherein the bleaching agent used is selected from a group consisting of sodium chlorite , ammonium persulphate and hydroxide peroxide.

5. The process as claimed in claim 3, wherein the acid used is selected from a group of sulphuric acid , nitric acid, hydrochloric acid or a mixture thereof.

6. The process as claimed in claim 3, wherein the base used is selected from a group of potassium hydroxide, sodium hydroxide.

7. The process as claimed in claim 3, wherein the NC formulation is used for wound healing in the form of strip or ointment.

8. The process for the preparation of the nanobiocomposite formulation as claimed in claim 3, wherein the NCs pellet is directly mixed with Vaseline base in the ratio of 1:1 to obtain ointment

9. The process as claimed in claim 3, wherein the NC formulation is dissolved in water and vortexed properly to obtain aqueous suspension which is casted into a mold and allowed to oven dry in the form of NCs strip.

STATEMENT UNDER ARTICLE 19(1)

With reference to the search report and written opinion of the International Searching Authority [ISA/EP], we are suitably amending the claims to improve their clarity and to establish the novelty and inventive step in the present application.

1. Claims 1, 2 and 3-9 (original claims 2-8) have been suitably amended.
2. New dependent claim 2 is incorporated.
3. Claim 1 is amended to incorporate the that the cellulose is derived from *Syzygium cumini* leaves.
4. Claim 3 is amended to rectify the improper dependency.
5. Any addition in the claims has been indicated by underline and any deletion in the claims has been indicated by strike through.

The Applicant undertakes that no new subject matter has been added in claims and the amended claims do not go beyond the disclosure of the international application as-filed and have no impact on the description and drawings as filed.