Biocompatible Magnetic MWCNTs Based on Phytocomponents from *Eugenia carryophyllata*

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The aim of the present study was the phytocomponents extraction from the aromatic waters of Eugenia carryophyllata by magnetic MWCNT encapusaltion, in order to obtain biocompatible nanoparticles with clinical applications. Eugenia carryophyllata dried buds were hydrodistilled, in microwave conditions, using a Neo-Clevenger device. The aqueous residue was separated in two equal parts, one for the chloroform extraction and the other for the extraction with magnetic MWCNT. The chemical composition of the aqueous residue extracts was established by GC-MS analyses. The high content of eugenol and α-cariophyllen was proven in both magnetic MWCNT assisted and chloroform extraction. The MWCNT antimicrobial activity was tested against 20 Staphylococcus aureus strains. The MIC value for all tested strains was 50mg/mL, smaller concentrations being promoters for bacterial growth probably due to the large surface offered by MWCNT for microbial adherence and to the subinhibitory concentration of encapuslated phytocomponents with antimicrobial activity.

Keywords: magnetic nanoparticles, Eugenia carryophyllata, encapsulated phytocomponents, MWCNT

Nanoparticles are generally defined as having carbon nanotubes (CNTs) have a lot of applications since they were discovered. CNTs are crystalline graphitic sheets rolled up into a seamless cylindrical shape [1]. Since their discovery diverse and unique physical properties of CNTs have been revealed. Therefore, many novel applications such as nanotube-based magnets, solar cells, superconductors, displays, air pollution filter, hydrogen storage, medicine and clinical microbiology have been conceived. However, due to their macromolecular structure and their affinity to agglomerate, carbon nanotubes are insoluble [2]. This is a serious problem that obstructs their uniform incorporation into matrices for the fabrication of advanced materials. This deficiency can be attenuated through functionalization of carbon nanotube surfaces [3]. Therefore, in many cases, carbon nanotubes of either multi walled (MWCNT) or single-walled (SWCNT) structure are oxidized along their sidewalls in a non destructive way by a HCl/HNO_3 treatment which functionalizes the surfaces through hydroxyl, carboxyl, and carbonyl groups [4]. The magnetic properties of MWCNTs make them possess unique potential applications as magnetic data storage [5], magnetic force microscopes [6], microwave absorbing materials [7], or sau magnetic nanomaterials for drug delivery [8-10].

Essential oils are complex mixtures of phytocomponents with synergic or concerted action. The fitochemistry studies of different plant extracts are oriented to the component isolation and identification from complex mixtures in order to establish correlations between the structure and the biological and pharmacological activity, respectively. The determination of the essential oil pharmacological activity is relatively difficult due to their intra and interspecific variability, and to the working conditions (low solubility in aqueous media, high volatility). The simplification of the phytocomponents complex mixture from the essential oils considerably reduces the synergic/concerted action implications, thus allowing the determination of the role of the isolate component. For that purpose, aromatic waters represent a remarkable alternative due to the fact that they contain principal components of aqueous medium solubilized oils.

The antimicrobial activity of some volatile oils and their components was proven towards a large variety of microorganisms, fungi, Gram-positive and Gram-negative bacteria. It seems like Gram-negative bacteria are more resistant to the activity of biocides obtained from plants, due to lipopolisacharides that are found in the external membrane; but there are also exceptions [11,12]. Bacterial resistance at drug substances together with the decline registered in the formulation of new antibiotics represent a threat for human health [13].

In this context, our study focused on the development of a new, easy and rapid method of obtaining encapsulated magnetic MWCNT for biomedical applications, based on the antimicrobial activity of phytocomponents extracted from aromatic waters of *E. carryophyllata*.

Experimental part

Aromatic water preparation from Eugenia carryophyllata samples

E. carryophyllata dried buds were purchased from a local supplier and used for the essential oil extraction [14]. Three extractions were performed in microwave conditions using a Neo Clevenger type apparatus. The essential oils and the aromatic water were collected separately [15]. In scheme 1 the experimental steps are presented.

Synthesis and characterization of magnetic nanoparticles

Magnetic MWCNTs were synthesized by plasma processing and purified as follows: solvent extraction (with benzene,

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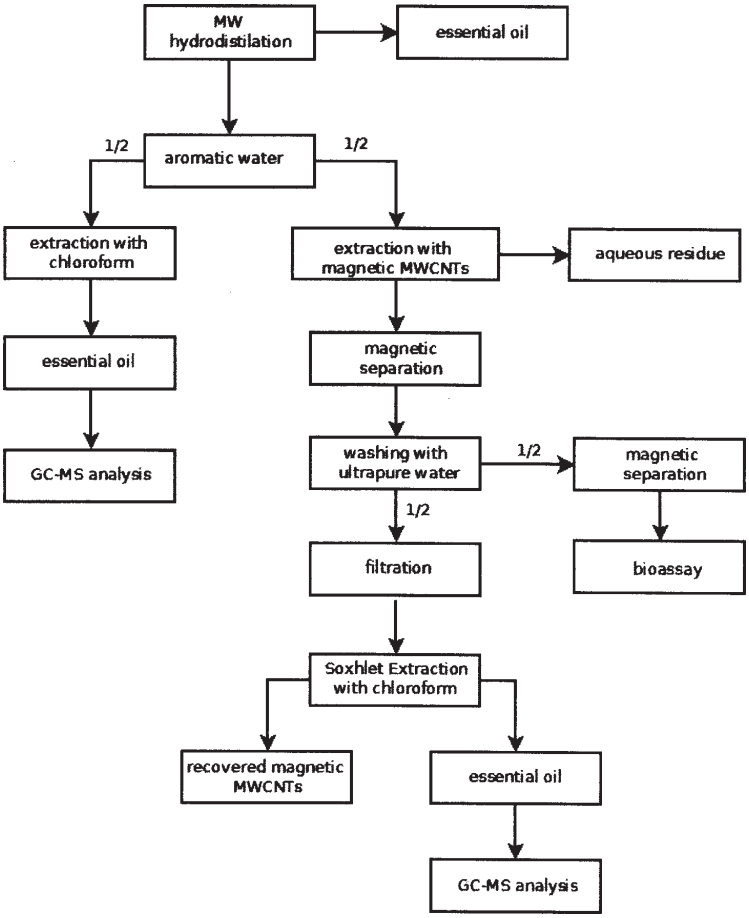


Scheme 1. Schematic diagram of the experimental part

dichloromethane and o-dichlorobenzene, successively), elimination of inorganic impurities by nitric acid/concentrated hydrochloric acid treatment, washing with ultra-pure water, thermal treatment at 300 °C. The morphology was determined by Transmission Electron Microscopy (TEM). A TEM image of the obtained magnetic nanoparticles is presented in figure 1.

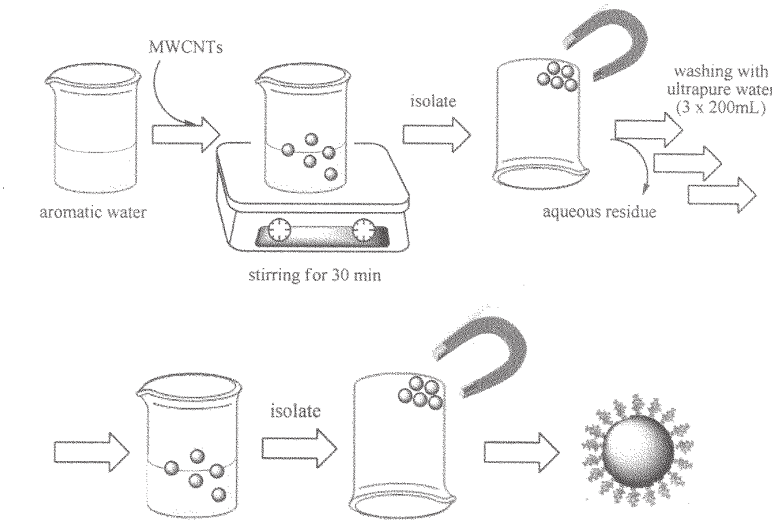
Phytocomponents extraction from aromatic waters

Aqueous residues from the hydrodistillation of *Eugenia carryophyllata* samples were combined and filtered, 500 mL of aromatic water being obtained. This was extracted by two procedures: simple solvent extraction (with chloroform) and magnetic MWCNT extraction (scheme 2) using 800 mg of nanoparticles. For the phytocomponents encapsulation, the nanoparticle-aromatic water mixture was stirred for 30 min in order to suspend the nanotubes and then felt in a magnetic field for 5 min. The resulted nanoparticles were isolated by placing a strong magnet Nd–Fe–B, (100 kgf) on the bottom of the suspension beaker, the aqueous residue being thus eliminated [16].

The isolated encapsulated MWCNTs were washed with ultra-pure water (3×200 mL) to remove the hydrosoluble compounds. Encapsulated MWCNTs were divided in two halfs, one being used in the GC-MS analysis and the other one for the biological activity testing.

GC-MS analysis

Gas chromatographic analysis was performed using an Agilent 6890 Series GC System. Detection was carried out with a 5973 mass-selective single quadrupole detector (Agilent technologies). Operation control and data process were carried out by Agilent Technologies ChemStation software (Santa Clara, CA, USA). The mass spectrometer was calibrated before use with perfluorotributylamine (PFTBA) as a calibration standard.



Scheme 2. Phytocomponents extraction procedure from aromatic water, using magnetic MWCNTs

Nr. crt.	Bacterial strain and internal codes	Clinical specimen
1	*S. aureus* ATCC 5538	reference strain
2	*S. aureus* 5re	bronchial secretion
3	*S. aureus* 6re	bronchial secretion
4	*S. aureus* 7re	bronchial secretion
5	*S. aureus* 8re	bronchial secretion
6	*S. aureus* 10re	bronchial secretion
7	*S. aureus* 11re	bronchial secretion
8	*S. aureus* 12re	bronchial secretion
9	*S. aureus* 13re	bronchial secretion
10	*S. aureus* 4 pl	wound secretion
11	*S. aureus* 11 pl	wound secretion
12	*S. aureus* 21 pl	wound secretion
13	*S. aureus* 22 pl	wound secretion
14	*S. aureus* 23 pl	wound secretion
15	*S. aureus* 33 pl	wound secretion
16	*S. aureus* 35 pl	wound secretion
17	*S. aureus* 36 pl	wound secretion
18	*S. aureus* 40pl	wound secretion
19	*S. aureus* 1F	pharyngeal exudate
20	*S. aureus* 3F	pharyngeal exudate

Table 1

The working conditions were: H_2-carrier gas, flow: 1,2 mL/min, temperature program 50/300°C with a ramp rate of 5°C/min; the temperature of the injector and of the detector was 250°C, and a DB5-MS (30m; 0.25 mm id; 0.25 μm) column.

Antimicrobial activity determination of magnetic MWCNTs

Microbial strains

The antimicrobial activity of MWCNTs was tested against 20 *Staphylococcus aureus* strains, recently isolated from clinical specimens, and a reference strain. The strains were identified with a Vitek automatic system II [17]. The bacterial suspensions used were obtained from bacterial cultures of 15-18 h grown in solid media (Chapman medium), that were adjusted at an optic density appropriate with the McFarland 0.5 standard (containing $1.5x10^8$ colony forming units (UFC)/mL) [18].

Quantitative determination of minimal inhibitory concentration (MIC) by serial microdilution technique

The MIC value [19] for MWCNT was determined by twofold microdilution technique, in 96 multi-well plates, starting from 50 to 0.39mg/mL, for each tested bacterial strain. Positive and negative controll (serial dillutions for MWCNT) was used for microbial growth. The plates were incubated for 24 h at 37°C, and MIC values were determined by macroscopic examination [20] (as the lowest concentration of compound which inhibited the microbial growth) and spectrofotometric (A_{620} for the obtained microbial cultures) [21].

Results and discussions

HR-TEM characterisation

Two representative HR-TEM images of MWCNTs are shown in figure 1. The multiple graphitic walls of the CNTs are clearly visible in this TEM image that also shows a 10-11 nm diameter tube.

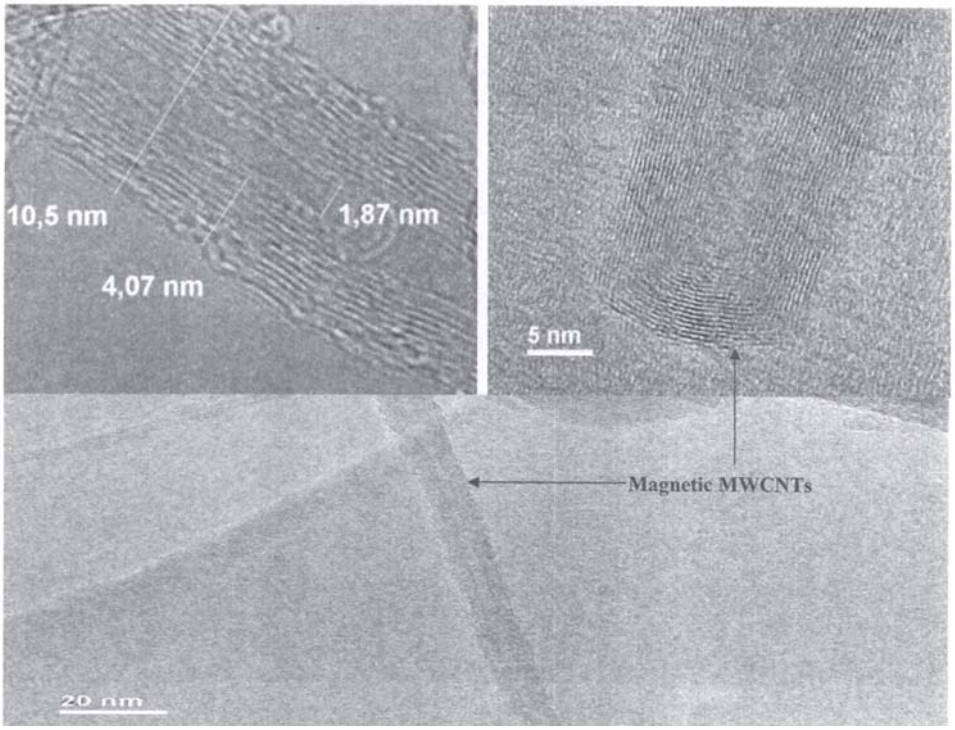


Fig. 1. Transmission Electron Microscopy images of the MWCNTs

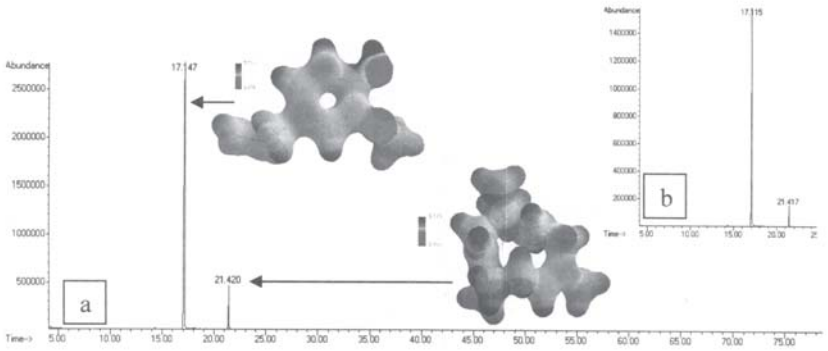
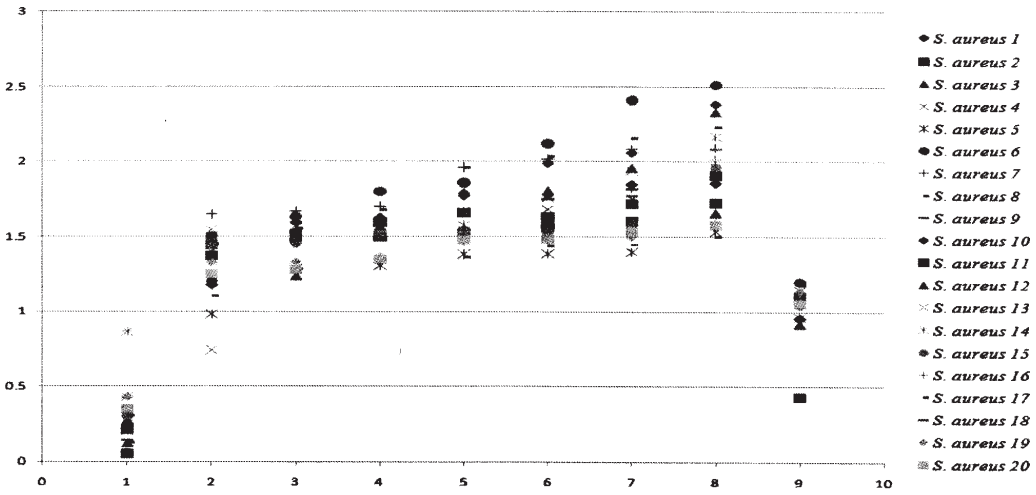


Fig. 2. Aromatic water chromatogram after extraction with magnetic MWCNTs (a) and chloroform extraction (b)



1	2	3	4	5	6	7	8	9
50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL	1.56 mg/mL	0.78 mg/mL	0.39 mg/mL	Pozitive control

Fig. 3. Graphical representation of microbial cultures optical densities at 24h of incubation (A_{490} nm), at different concentrations of the coated magnetic MWCNTs

GC-MS analysis

Total ion chromatograms (TIC) for the two extraction methods are presented in figure 2. The identified phytocomponents were the same in both methods: eugenol and α-cariophyllene. The principal components percentage was 92.42% and 5.44% from the total area, for the MWCNT extraction.

Minimal Inhibitory Concentration

The MIC value for all tested strains was the same (50mg/mL). The subinhibitory concentrations are bacterial growth promoters, fact that can be attributed to the large surface offered by the MWCNTs for microbial adherence (this favours the metabolic process acceleration) as well as to the low concentrations of antimicrobial compounds from charged MWCNT (fig. 3).

Conclusions

Encapsulation of carbon nanotubes was proven to be an efficient method of extraction of aromatic waters compounds, in comparison with the control extraction with organic solvent. The TIC analysis for the two extraction methods – chloroform and MWCNTs – has demonstrated the presence of principal components from the *E.* carriophyllata volatile oil, eugenol and α-cariophyllene, in the aromatic water. Also, it was proven that the phytocomponents extracted from the *E. carriophyllata* aromatic water exhibit antibacterial activity on all of the *S. aureus* tested strains, at 50 mg/mL, while lower concentrations stimulated the bacterial growth. This was probably due to the specific nature of MWCNTs that offer a large contact surface for microbial adherence.

In conclusion, magnetic MWCNTs can become efficient target systems for natural compounds with antimicrobial activity, having applications in the anti-infectious therapy field.

Acknowledgment: The present study was financed by the POSDRU 107/1.5/S/80765 European Program and Human Resources no. 135/2010 (Contract nr. 76/2010).

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Manuscript received: 14.11.2011

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