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# AABC

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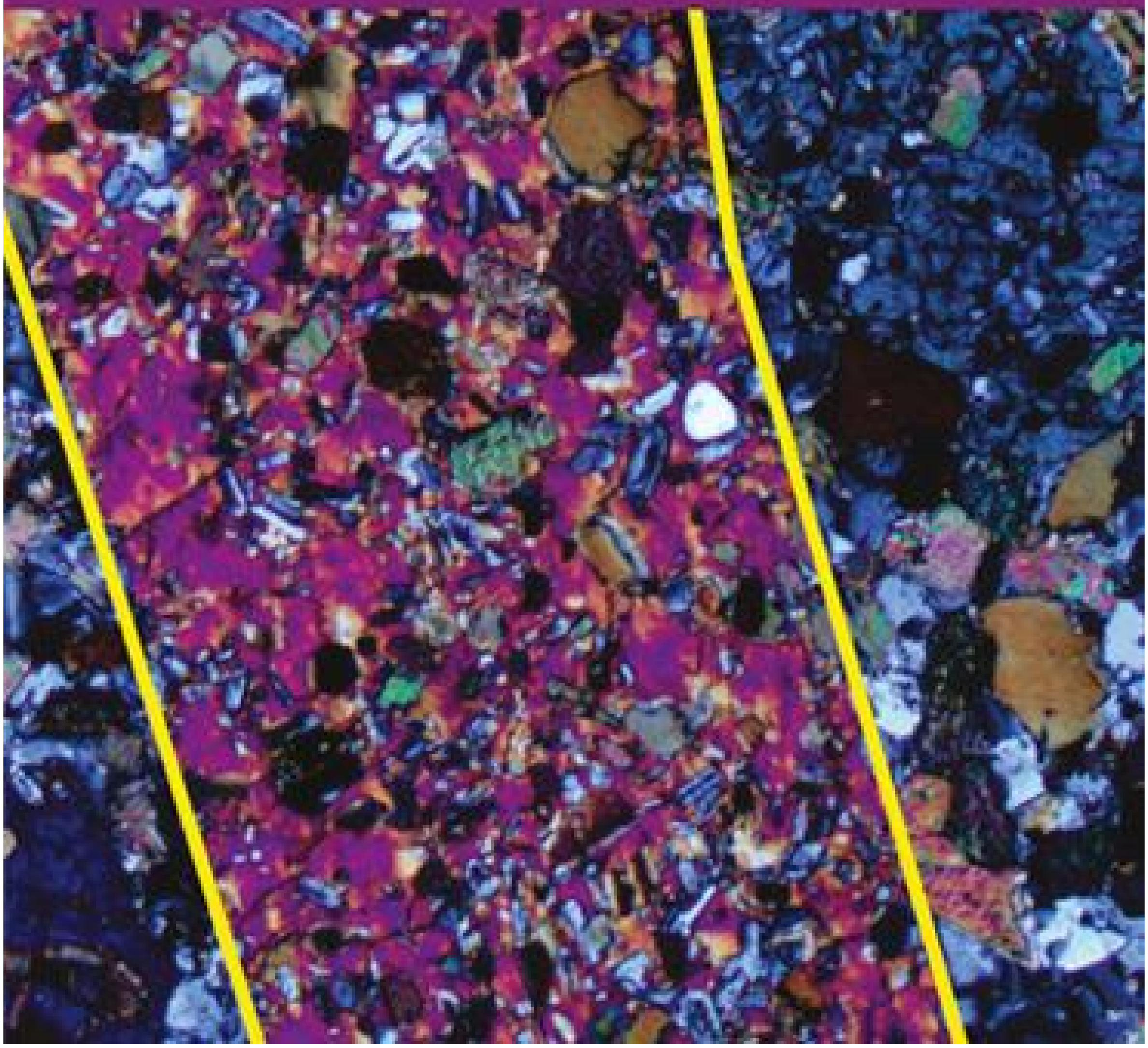

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# Anais da Academia Brasileira de Ciências

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**CELLULAR AND MOLECULAR BIOLOGY**

# The effect of chitosan addition on cellular uptake and cytotoxicity of ursolic acid niosomes

ANDANG MIATMOKO, BERLIAN S. HARIAWAN, DEVY M. CAHYANI, RETNO SARI, ARISTIKA DINARYANTI & ERYK HENDRIANTO

**Abstract:** This study evaluated the cellular uptake and cytotoxicity of low permeable Ursolic acid (UA) on cancer cells using niosomes composed of span 60 and cholesterol. The results showed that the addition of chitosan increased particle sizes and ζ-potentials. The UA niosomes with chitosan layers had higher cytotoxicity in HeLa cells than without chitosan, however, there was no improvement observed for Huh7it cells. Moreover, chitosan layers improved the cellular uptake, which clathrin-mediated endocytosis may determine the cellular transport of UA niosomes. In conclusion, the addition of chitosan improved cellular uptake and cytotoxicity of UA niosomes in the HeLa cells.

**Key words:** Chitosan, niosomes, ursolic acid, cytotoxicity, cell uptake, cancer.

## INTRODUCTION

Ursolic acid (UA) is a pentacyclic triterpene which promotes anti-cancer activity in humans (Ali et al. 2019). They revealed the role of UA in accelerating liver proliferation, restoring biochemical and histological functions in liver cells damaged by hepatocarcinoma, and protecting the integrity of hepatocytes against liver damage.

The use of UA in anti-cancer therapy has drawbacks related to its poor permeability and water solubility. Lawsone, an anti-cancer drug that demonstrates poor solubility and permeability, is reported to induce high cytotoxicity when in the form of niosomes (Barani et al. 2018). The increased cytotoxic effect was due to the internalization effect of the niosomes in MCF-7 breast cancer cells which was superior to that of the free form and the resulting sustained-release effect.

Research conducted by Song et al. (2014) shows that the mechanism transporting niosomes into cells is active and linked to the endocytosis pathway. Chitosan, a natural polysaccharide (Szymańska & Winnicka 2015) and a cationic polymer, has been shown to increase cell uptake (Li et al. 2013, Zhang et al. 2013). The cationic polymer charge significantly affects cellular absorption *in vitro* (Li & Ju 2017) due to the electrostatic interaction between cells and positively charged cationic polymers.

Against this background, research was carried out on cell uptake and *in vitro* cytotoxicity of UA niosomes with chitosan coating on HeLa and Huh7it cells (Guo et al. 2019, Purnamasari et al. 2019).

## MATERIALS AND METHODS

In this study, UA niosomes (Nio-UA) was composed of Span 60, Cholesterol, UA at a molar ratio of 6:4:1, respectively. A solution of UA, Span 60, and Cholesterol was mixed in a round bottom flask, then the organic solvents was evaporated until a thin lipid film was formed. This film was hydrated using Phosphate buffered saline solution pH 7.4. Furthermore, a solution of 0.005% w/v chitosan was added for producing Nio-UA-CS.

The particle size and ζ-potential were measured by means of dynamic and electrophoresis light scattering method, respectively, by the use of a Delsa™ Nano C Particle Analyzer at 25°C.

A cytotoxicity test was performed using an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on HeLa and Huh7it cells after incubating Nio-UA and Nio-UA-CS for 48 hours. The absorbance of MTT was measured at λ: 560 and 750 nm using a GloMax-Multi Microplate Multimode Reader (Promega).

Furthermore, the cell uptake test was carried out on HeLa cells after incubating the UA niosomes labeled Coumarin-6 (Cou-6= 10 μg/mL). In order to determine the mechanism of cell uptake, pre-incubation was carried out with endocytosis specific inhibitors, namely; 200 μM of genistein and 450 mM of sucrose. Pre-incubation was performed on sucrose for one hour and on genistein for 30 minutes at 37$^{o}$C. After pre-incubation, the media was replaced with 10 μg/mL of coumarin 6-labeled UA niosomes in the medium and incubated for two hours at 37°C. The the fluorescence intensity of Coumarin-6 in the cells was measured at $\lambda_{ex}$: 475 nm and $\lambda_{em}$: 500-550 nm using the Glomax Microplate Reader. The cell uptake was also evaluated by using a fluorescence microscope.

For statistical analysis, significance was indicated by $P<0.05$ by using one-way analysis of variance followed by a least significant difference test.

## RESULTS

The data shown in Table I indicates that the particle size of Nio-UA-CS was larger than that of Nio-UA. Similarly, for niosomes labeled with coumarin, the size of Nio-UA-CS-Cou6 was greater than that of Nio-UA-Cou6. The cytotoxicity results show that the $IC_{50}$ Nio-UA was higher than that of $IC_{50}$ Nio-UA-CS in HeLa cells (Figure 1a). Thus, chitosan coating can increase the cytotoxicity of UA niosomes with regard to HeLa cells; however, the Huh7it cells were less sensitive to UA than HeLa cells as shown in Figure 1b.

Observations were further conducted using a fluorosense microscope after HeLa cells had been treated with UA niosomes for two hours. It can be seen in Figure 2 that the Coumarin-6 intensity of the Nio-UA-CS was higher than that of Nio-UA. Moreover, the appearance of cells in the treatment of Nio-UA indicates that Nio-UA slightly entered the cell and still mainly remained in the membrane, while Nio-UA-CS appear evenly across the cell membrane and were accumulated highly inside the cells. The Figure 2 also show that after genistein

**Table I. Physical characteristics of Nio-UA, Nio-UA-CS, Nio-UA-Cou6, and Nio-UA-CS-Cou6.**

| Parameter | Nio-UA | Nio-UA-CS | Nio-UA-Cou6 | Nio-UA-CS-Cou6 |
|---|---|---|---|---|
| Particle Size (nm) | 198.7±13.8 | 237.7±6.2 | 163.0±5.3 | 203.6±6.7 |
| ζ-Potential (mV) | -57.5±11.9 | 3.88±1.5 | -64.8±0.2 | -45.7±7.6 |
| Polydispersity index | 0.29±0.02 | 0.33±0.03 | 0.16±0.03 | 0.22±0.09 |

pretreatment as transport inhibitor of Nio-UA, the cells had less intense fluorescence than that of sucrose pretreatment. On the contrary, the cells treated Nio-UA-CS had a higher intensity than that without the addition of the inhibitor, and with the addition of sucrose, these cells had relatively lower intensities than that of genistein addition.

To determine the cellular uptake mechanism of the niosome, a test was carried out involving the addition of specific endocytosis inhibitors i.e. sucrose and genistein. As in Figure 3, with the addition of genistein or sucrose as an endocytosis inhibitor of the caveolae or clathrin pathway, respectively, the Nio-UA demonstrated relatively similar levels than the Nio-UA without inhibitor. The contrast results were produced by the Nio-UA-CS, which without inhibitor, it had significant lower levels than Nio-UA-CS with the genistein.

## DISCUSSION

Following the research conducted into the increase in particle size and changes in the ζ-potential value, it can be argued that chitosan layers on niosomes induce this change (Aquila 2018). The chitosan layer may be formed by electrostatic interactions increasing in particle size (Guo et al. 2003). In the niosome, ionic attraction occurs between the ammonium group of chitosan and the phosphate group of phospholipids or other negatively charged groups in lipids (Frank et al. 2020). This interaction also causes changes in the ζ-potential of the niosome as shown in Table I where the ζ-potential value becomes positive after niosome coating.

In this study, HeLa and Huh7it cells had different sensitivity for cytotoxic study of UA and niosomes since each cell type has specific biological characteristics devoted for certain functions (von Gersdorff et al. 2006). The decrease in the $IC_{50}$ value of UA niosomes with

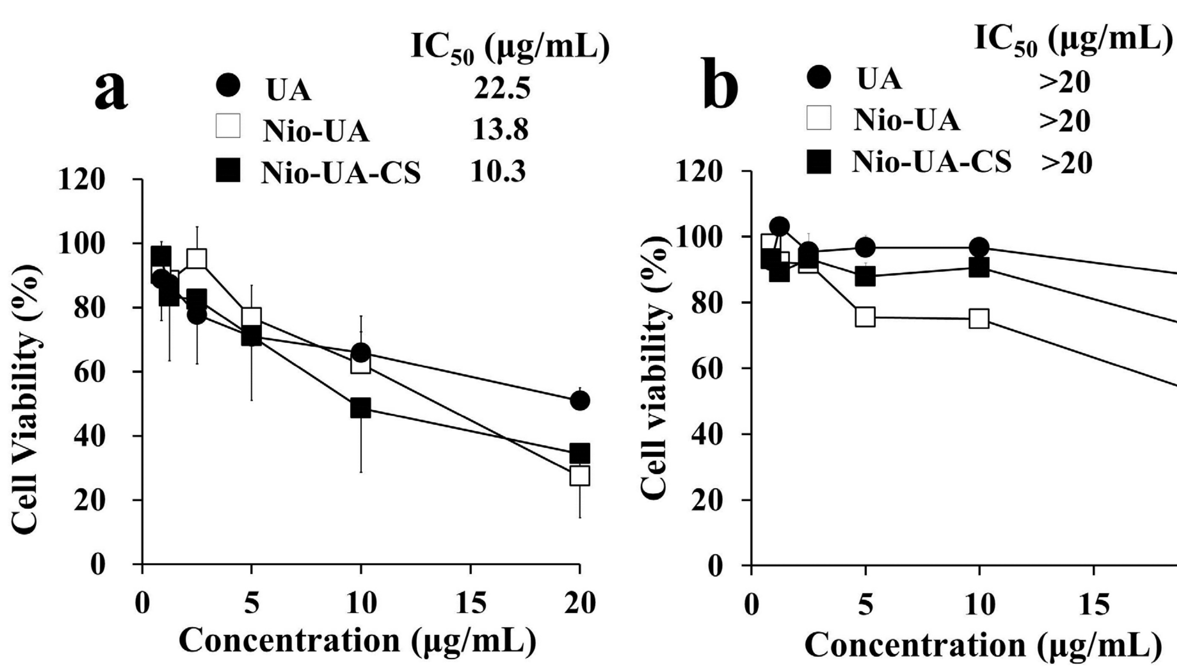


**Figure 1. Viability of HeLa (a) and Huh7it (b) cells in the presence of UA, Nio-UA and Nio UA-CS treatment after incubation for 48 hours at various concentrations.**

chitosan coating compared to UA niosomes is in accordance with the research findings of Ahmed et al. (2017) regarding the higher absorption of poly L-lysine silver nanoparticles than silver nanoparticles due to the interaction between positively charged poly L-lysine on the surface of the nanoparticles and the negatively charged HepG2 hepatoblastoma cell surface. In chitosan, the positive surface charge can increase the bio-nano interaction with negative surface charge of the cytoplasmic membrane resulting in an increase in cell uptake and cytotoxicity (Frank et al. 2020).

The photomicrographs of cellular uptake confirms that the niosome as a cell retrieval carrier accumulates on the surface during the cell uptake process (Kaksonen & Roux 2018). Since the addition of genistein produces higher yields, it clearly affects cell uptake from Nio-UA-CS (Quagliariello et al. 2019). Hence, the entry of Nio-UA-CS was improved with the presence of genistein as the inhibitor of the caveolae-mediated endocystosis, but no significant difference was observed after addition of sucrose. From these results, it can be concluded that Nio-UA-CS undergoes cell uptake through the clathrin-mediated endocytosis pathway, while the mechanism of Nio-UA uptake may involve both mechanisms. This finding is in accordance with that of Quagliariello's research from 2019 that cell uptake from Butyric acid in liposomes occurs through clathrin-mediated endocytosis whose mechanism is initiated when the endocytic protein layer of the cytosol clusters on the plasma membrane. After the coating is formed, the uptake molecules are concentrated in the layer area and a bond occurs which results in the flat plasma membrane becoming a 'clathrin-layered hole' containing the uptake molecules. Furthermore, the process of constriction and cutting of the neck membrane occurs with the result that the vesicles enter the cell (Kaksonen & Roux 2018).

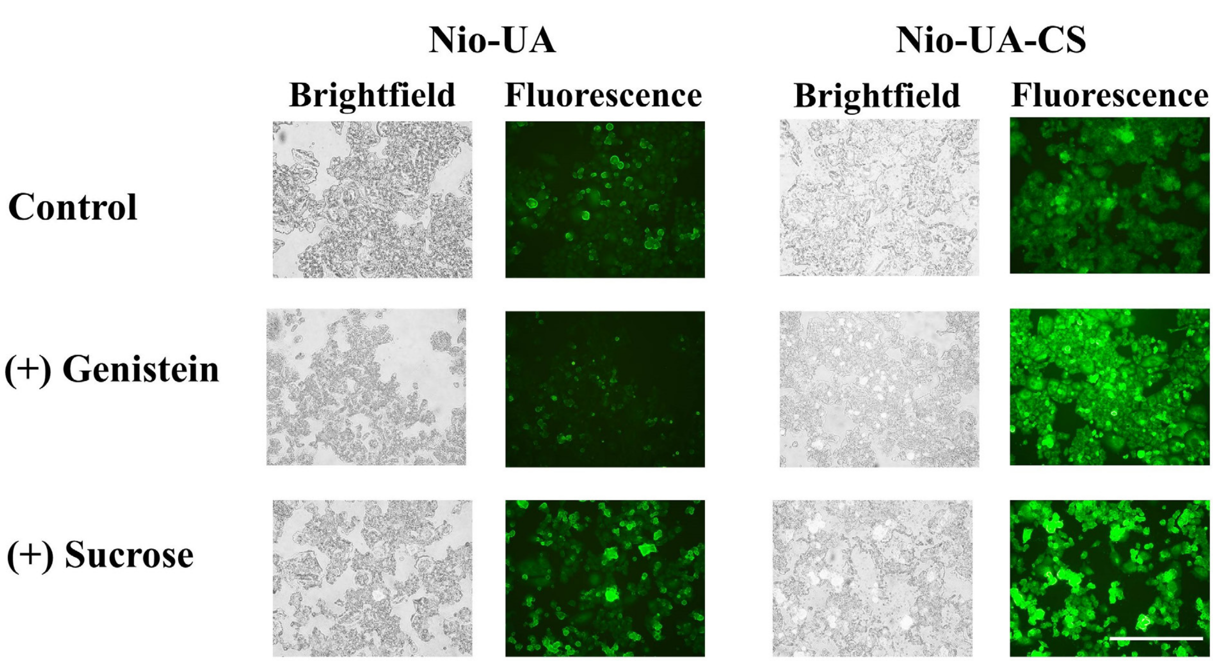


**Figure 2. Photomicrographs of cellular uptake of HeLa cells after incubated with Nio-UA and Nio-UA-CS containing Coumarin-6 for two hours without and with addition of genistein and sucrose pre-treatment. Scale bar= 100 μm.**

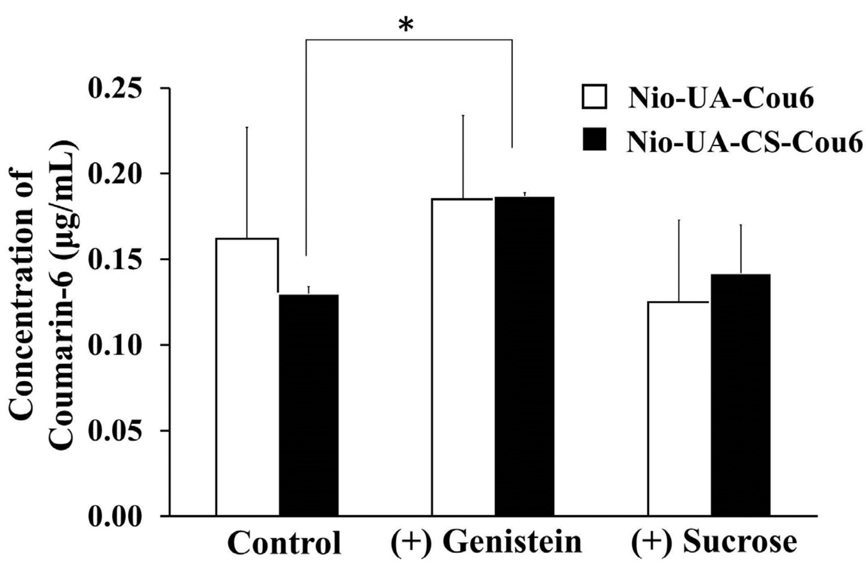


**Figure 3.** Coumarin-6 levels in HeLa cells after incubation with Nio-UA and Nio-UA-CS for two hours without and with addition of genistein and sucrose pre-treatment (*$P$<0.05).

## CONCLUSION

It can be seen from this study that the UA niosome with chitosan layers increased cytotoxicity in HeLa cells but it was less sensitive for Huh7it cells. The difference in cell uptake between Nio-UA and Nio-UA-CS should also be investigated at greater length in order to identify the effect of chitosan addition on cellular uptake pathways.

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Andang Miatmoko: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing-original draft, writing-review & editing. Berlian Sarasitha Hariawan: data curation, formal analysis, investigation, methodology, project administration, resources, writing original draft. Devy Maulidya Cahyani: data curation, formal analysis, investigation, project administration, resources. Retno Sari: conceptualization, data curation, formal analysis, supervision, validation, writing-review & editing. Aristika Dinaryanti: investigation, methodology, resources, validation, visualization. Eryk Hendrianto: investigation, methodology, validation, visualization.

# Anais da Academia Brasileira de Ciencias

**COUNTRY**

Brazil

Universities and research institutions in Brazil

**SUBJECT AREA AND CATEGORY**

Multidisciplinary
Multidisciplinary

**PUBLISHER**

Academia Brasileira de Ciencias

**H-INDEX**

62

**PUBLICATION TYPE**

Journals

**ISSN**

00013765, 16782690

**COVERAGE**

1945-1946, 1949, 1970-1992, 1994-2021

**INFORMATION**

Homepage

How to publish in this journal

aabc@abc.org.br

**SCOPE**

The Brazilian Academy of Sciences (BAS) publishes its journal, Annals of the Brazilian Academy of Sciences (AABC, in its Brazilianportuguese acronym ), every 3 months, being the oldest journal in Brazil with conkinuous distribukion, daking back to 1929. This scienkihic journal aims to publish the advances in scienkihic research from both Brazilian and foreigner scienkists, who work in the main research centers in the whole world, always looking for excellence. Essenkially a mulkidisciplinary journal, the AABC cover, with both reviews and original researches, the diverse areas represented in the Academy, such as Biology, Physics, Biomedical Sciences, Chemistry, Agrarian Sciences, Engineering, Mathemakics, Social, Health and Earth Sciences.

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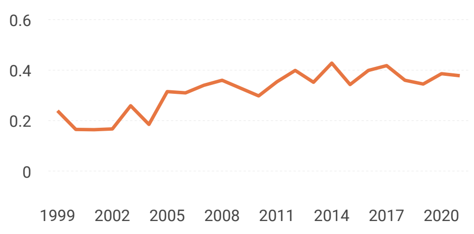


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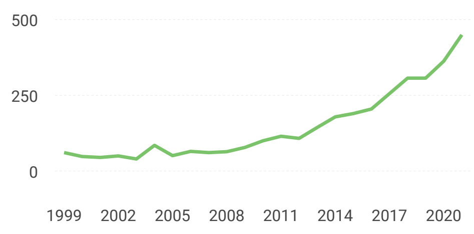


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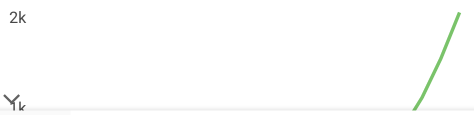


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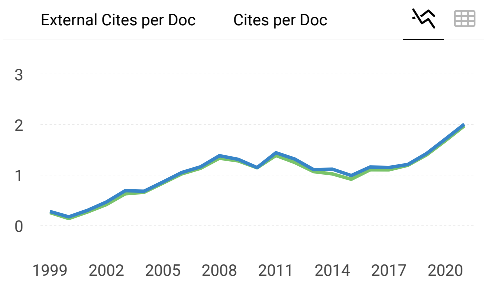


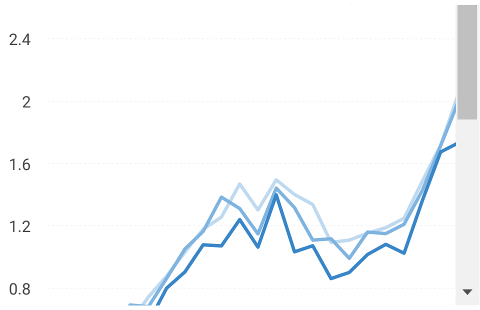



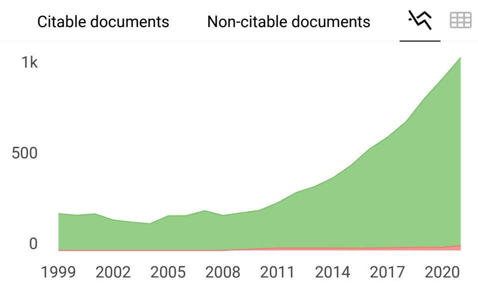


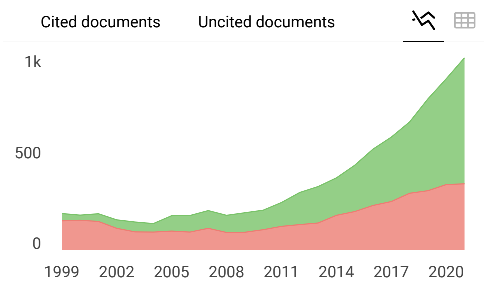



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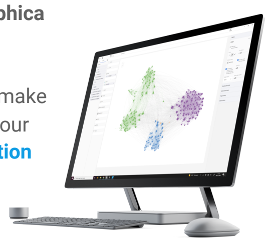



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**Melanie Ortiz** 3 years ago

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Olá,

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SJR 2021
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SNIP 2021
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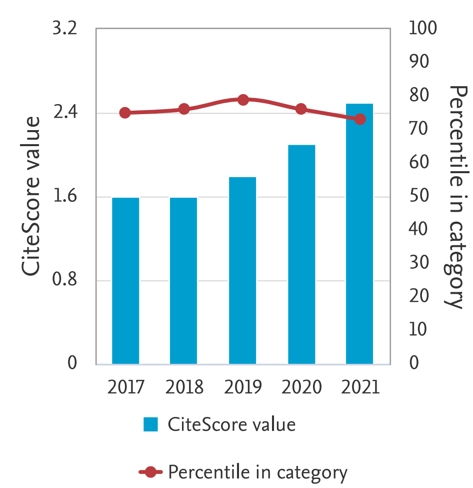
## CiteScore rank 2021

In category: Multidisciplinary

| Rank | Source title | CiteScore 2021 | Percentile |
|---|---|---|---|
| #32/120 | Anais da Academia Brasileira de Ciencias | 2.5 | 73rd percentile |

| Rank | Source title | CiteScore 2021 | Percentile |
|---|---|---|---|
| #1 | Nature | 70.2 | 99th percentile |
| #2 | Science | 57.8 | 98th percentile |
| #3 | Nature Communications | 23.2 | 97th percentile |
| #4 | National Science Review | 19.1 | 97th percentile |
| #5 | Science advances | 18.5 | 96th percentile |
| #6 | Proceedings of the National Academy of Sciences of the United States of America | 18.1 | 95th percentile |
| #7 | Science Bulletin | 17.2 | 94th percentile |
| #8 | Journal of Advanced Research | 17.1 | 93rd percentile |
| #9 | Research | 10.5 | 92nd percentile |
| #10 | The Innovation | 10.0 | 92nd percentile |
| #11 | Scientific Reports | 6.9 | 91st percentile |

## CiteScore trend



| Rank | Source title | CiteScore 2021 | Percentile |
|---|---|---|---|
| #12 | Tsinghua Science and Technology | 5.8 | 90th percentile |
| #13 | Royal Society Open Science | 5.7 | 89th percentile |
| #14 | iScience | 5.7 | 88th percentile |
| #15 | PLoS ONE | 5.6 | 87th percentile |
| #16 | Advanced Theory and Simulations | 5.5 | 87th percentile |
| #17 | Journal of King Saud University - Science | 5.3 | 86th percentile |
| #18 | Emerging Science Journal | 5.2 | 85th percentile |
| #19 | Karbala International Journal of Modern Science | 4.7 | 84th percentile |
| #20 | Journal of the Royal Society of New Zealand | 4.5 | 83rd percentile |
| #21 | Arabian Journal for Science and Engineering | 4.4 | 82nd percentile |
| #22 | Heliyon | 4.0 | 82nd percentile |
| #23 | International Journal of Bifurcation and Chaos in Applied Sciences and Engineering | 4.0 | 81st percentile |
| #24 | Transactions of Tianjin University | 3.7 | 80th percentile |
| #25 | Complexity | 3.5 | 79th percentile |
| #26 | Applied Network Science | 3.5 | 78th percentile |
| #27 | Facets | 3.4 | 77th percentile |
| #28 | International Journal of Agile Systems and Management | 3.3 | 77th percentile |
| #29 | International Journal of Service Science, Management, Engineering, and Technology | 3.2 | 76th percentile |
| #30 | Journal of the Indian Institute of Science | 2.9 | 75th percentile |
| #31 | Science, Technology and Society | 2.6 | 74th percentile |
| #32 | Anais da Academia Brasileira de Ciencias | 2.5 | 73rd percentile |
| #33 | Scientific African | 2.4 | 72nd percentile |
| #34 | Data in Brief | 2.4 | 72nd percentile |
| #35 | Sadhana - Academy Proceedings in Engineering Sciences | 2.3 | 71st percentile |
| #36 | Citizen Science: Theory and Practice | 2.2 | 70th percentile |

| ☆ | Rank | Source title | CiteScore 2021 | Percentile |
|---|---|---|---|---|
| ☆ | #37 | Foundations of Science | 2.1 | 69th percentile |
| ☆ | #38 | Kexue Tongbao/Chinese Science Bulletin | 1.9 | 68th percentile |
| ☆ | #39 | Pacific Science | 1.8 | 67th percentile |
| ☆ | #40 | Sains Malaysiana | 1.8 | 67th percentile |
| ☆ | #41 | Journal of Mathematical and Fundamental Sciences | 1.7 | 66th percentile |
| ☆ | #42 | Current Science | 1.6 | 65th percentile |
| ☆ | #43 | Kuwait Journal of Science | 1.6 | 64th percentile |
| ☆ | #44 | Universitas Scientiarum | 1.6 | 63rd percentile |
| ☆ | #45 | Springer Handbooks | 1.6 | 62nd percentile |
| ☆ | #46 | Perspectives on Science | 1.6 | 62nd percentile |
| ☆ | #47 | Latin American Research Review | 1.5 | 61st percentile |
| ☆ | #48 | Journal of Scientific and Industrial Research | 1.4 | 60th percentile |
| ☆ | #49 | Journal of Shanghai Jiaotong University (Science) | 1.4 | 59th percentile |
| ☆ | #50 | Fundamental Research | 1.4 | 58th percentile |
| ☆ | #51 | World Review of Science, Technology and Sustainable Development | 1.3 | 57th percentile |
| ☆ | #52 | Journal of the National Science Foundation of Sri Lanka | 1.3 | 57th percentile |
| ☆ | #53 | Tapuya: Latin American Science, Technology and Society | 1.3 | 56th percentile |
| ☆ | #54 | Journal of the Royal Society of Western Australia | 1.3 | 55th percentile |
| ☆ | #55 | Brazilian Archives of Biology and Technology | 1.3 | 54th percentile |
| ☆ | #56 | Science Progress | 1.2 | 53rd percentile |
| ☆ | #57 | Xinan Jiaotong Daxue Xuebao/Journal of Southwest Jiaotong University | 1.1 | 52nd percentile |
| ☆ | #58 | Gazi University Journal of Science | 1.1 | 52nd percentile |
| ☆ | #59 | Maejo International Journal of Science and Technology | 1.1 | 51st percentile |
| ☆ | #60 | Historia Agraria | 1.1 | 50th percentile |
| ☆ | #61 | ScienceAsia | 1.0 | 49th percentile |

| ☆ | Rank | Source title | CiteScore 2021 | Percentile |
|---|---|---|---|---|
| ☆ | #62 | Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition) | 1.0 | 48th percentile |
| ☆ | #63 | Tongji Daxue Xuebao/Journal of Tongji University | 0.9 | 47th percentile |
| ☆ | #64 | Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/Journal of Tianjin University Science and Technology | 0.9 | 47th percentile |
| ☆ | #65 | Beijing Daxue Xuebao (Ziran Kexue Ban)/Acta Scientiarum Naturalium Universitatis Pekinensis | 0.9 | 46th percentile |
| ☆ | #66 | Songklanakarin Journal of Science and Technology | 0.9 | 45th percentile |
| ☆ | #67 | Movement and Sports Sciences - Science et Motricite | 0.9 | 44th percentile |
| ☆ | #68 | Hunan Daxue Xuebao/Journal of Hunan University Natural Sciences | 0.9 | 43rd percentile |
| ☆ | #69 | Journal of Applied Science and Engineering (Taiwan) | 0.9 | 42nd percentile |
| ☆ | #70 | Interciencia | 0.9 | 42nd percentile |
| ☆ | #71 | Wuhan University Journal of Natural Sciences | 0.9 | 41st percentile |
| ☆ | #72 | AAS Open Research | 0.9 | 40th percentile |
| ☆ | #73 | Philippine Journal of Science | 0.8 | 39th percentile |
| ☆ | #74 | Malaysian Journal of Science | 0.8 | 38th percentile |
| ☆ | #75 | Walailak Journal of Science and Technology | 0.8 | 37th percentile |
| ☆ | #76 | International Journal of Innovative Research and Scientific Studies | 0.8 | 37th percentile |
| ☆ | #77 | Vibroengineering Procedia | 0.7 | 36th percentile |
| ☆ | #78 | Archives des Sciences | 0.7 | 35th percentile |
| ☆ | #79 | Journal of Sciences, Islamic Republic of Iran | 0.7 | 34th percentile |
| ☆ | #80 | Comptes Rendus de L'Academie Bulgare des Sciences | 0.7 | 33rd percentile |
| ☆ | #81 | Shanghai Jiaotong Daxue Xuebao/Journal of Shanghai Jiaotong University | 0.7 | 32nd percentile |
| ☆ | #82 | Bulletin of the Georgian National Academy of Sciences | 0.7 | 32nd percentile |
| ☆ | #83 | International Journal of Language and Culture | 0.7 | 31st percentile |
| ☆ | #84 | Constructivist Foundations | 0.6 | 30th percentile |

| ☆ | Rank | Source title | CiteScore 2021 | Percentile |
|---|---|---|---|---|
| ☆ | #85 | Proceedings of the Latvian Academy of Sciences, Section B: Natural, Exact, and Applied Sciences | 0.6 | 29th percentile |
| ☆ | #86 | Psychotherapy and Politics International | 0.6 | 28th percentile |
| ☆ | #87 | Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/Journal of Xi'an Shiyou University, Natural Sciences Edition | 0.6 | 27th percentile |
| ☆ | #88 | Papers and Proceedings - Royal Society of Tasmania | 0.6 | 27th percentile |
| ☆ | #89 | Shenyang Jianzhu Daxue Xuebao (Ziran Kexue Ban)/Journal of Shenyang Jianzhu University (Natural Science) | 0.6 | 26th percentile |
| ☆ | #90 | ASM Science Journal | 0.5 | 25th percentile |
| ☆ | #91 | Bulletin de la Societe Royale des Sciences de Liege | 0.5 | 24th percentile |
| ☆ | #92 | Ohio Journal of Sciences | 0.5 | 23rd percentile |
| ☆ | #93 | Scientific American | 0.5 | 22nd percentile |
| ☆ | #94 | Science and Technology Asia | 0.5 | 22nd percentile |
| ☆ | #95 | Zhongshan Daxue Xuebao/Acta Scientiarum Natralium Universitatis Sunyatseni | 0.5 | 21st percentile |
| ☆ | #96 | Journal of Zhejiang University, Science Edition | 0.4 | 20th percentile |
| ☆ | #97 | Science, Engineering and Health Studies | 0.4 | 19th percentile |
| ☆ | #98 | Acta Scientiae | 0.4 | 18th percentile |
| ☆ | #99 | Transdisciplinary Journal of Engineering and Science | 0.4 | 17th percentile |
| ☆ | #100 | Mindanao Journal of Science and Technology | 0.4 | 17th percentile |
| ☆ | #101 | Metode | 0.4 | 16th percentile |
| ☆ | #102 | Liaoning Gongcheng Jishu Daxue Xuebao (Ziran Kexue Ban)/Journal of Liaoning Technical University (Natural Science Edition) | 0.4 | 15th percentile |
| ☆ | #103 | Journal and Proceedings - Royal Society of New South Wales | 0.3 | 14th percentile |
| ☆ | #104 | Journal of Current Science and Technology | 0.3 | 13th percentile |
| ☆ | #105 | International Journal of Systematic Innovation | 0.3 | 12th percentile |
| ☆ | #106 | Know | 0.3 | 12th percentile |
| ☆ | #107 | Revista Lasallista de Investigacion | 0.3 | 11th percentile |

| ☆ | Rank | Source title | CiteScore 2021 | Percentile |
|---|---|---|---|---|
| ☆ | #108 | Chinese Annals of History of Science and Technology | 0.2 | 10th percentile |
| ☆ | #109 | Scientific Journal of King Faisal University Basic and Applied Sciences | 0.2 | 9th percentile |
| ☆ | #110 | Proceedings of Science | 0.2 | 8th percentile |
| ☆ | #111 | New Scientist | 0.2 | 7th percentile |
| ☆ | #112 | European Journal of Interdisciplinary Studies | 0.2 | 7th percentile |
| ☆ | #113 | Revista Conhecimento Online | 0.1 | 6th percentile |
| ☆ | #114 | International Journal of Advanced and Applied Sciences | 0.1 | 5th percentile |
| ☆ | #115 | Radovi Zavoda za Znanstvenoistrazivacki i Umjetnicki Rad u Bjelovaru | 0.1 | 4th percentile |
| ☆ | #116 | Edelweiss Applied Science and Technology | 0.1 | 3rd percentile |
| ☆ | #117 | Advanced Topics in Science and Technology in China | 0.1 | 2nd percentile |
| ☆ | #118 | Trends in Sciences | 0.0 | 2nd percentile |
| ☆ | #119 | Israa University Journal of Applied Science | 0.0 | 0th percentile |
| ☆ | #119 | Taiwan Review | 0.0 | 0th percentile |