**Evaluation Form for Manuscript Reporting the Anticancer Activity of Natural Compounds**

Instruction: Please fill out the form for your manuscript. The form covers all components of a manuscript reporting on the anticancer activity of natural compounds. Please indicate if each Item applies to your research (Yes and No) column. If **Yes** provide the Page **(Pg)** number and paragraph number **(Ph)** where the item is located in **your final revised draft of the manuscript**. If **No** or the item does not apply to your research provide an explanation why your study does not have that item. You can read more details of the tool at:  [10.31557/APJCP.2021.22.12.3735](https://dx.doi.org/10.31557/APJCP.2021.22.12.3735).

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| **Manuscript Section** | | **Item No** | | **Item Description** | **Is the item addressed in the manuscript** | **If Yes, the page Number (Pg)**  **The paragraph number (Ph)** | **If No, leave a comment. Why not?** | | **Other comments** |
| **Title and abstract and keywords** | | | | | |  |  | |  |
| Title | | A1 | | Be concise, clear, and comprehensive. Indicate the main variables, including the name of the natural product (generic or scientific), the histopathologic type of cancer, *in vitro* model system, and assessed outcome. Abbreviations should be avoided. The ideal length is between 10 to 20 words. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| A Structured Abstract | Objective | A2-1 | | Present the gap(s) in research based on which the study was designed. Explain the main objective of the work, indicating its novelty and/or difference compared to previous such studies | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Methods | A2-2 | | Briefly describe the natural product preparation by indicating the appropriate tools and methods used for its extraction as well as identification/quantification, *in vitro* model system, and anticancer assay method | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Result | A2-3 | | Report all main outcomes | |  |  | | --- | --- | | Pg |  | | Ph |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Conclusion | A2-4 | | Give a qualitative assessment of the anticancer effect of the natural compound and highlight the message of the work | |  |  | | --- | --- | | Pg |  | | Ph |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| **Introduction** | | | | | |  |  | |  |
| Background  /rationale | | I1 | | Introduce the natural product and describe the background information about its phytochemical profiling and ethnopharmacological relevance | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| I2 | | Justify the rationale of the selection of the test agent as a probable candidate for cancer prevention or treatment based on available literature and evidence | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Objectives | | I3 | | Outline the purpose and state the specific objectives of the research, pointing to the novelty of the work | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| **Material and Methods** | | | | | |  |  | |  |
| Natural product characteristics | | M1 | | Indicate the geographical location and time of specimen collection | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M2 | | Indicate the identification of the specimens from authentic resources i.e. taxonomists, herbarium, plant information centers, and experts in the field | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M3 | | Indicate which parts of the natural entity were used for bioassay (e.g., leaves, twigs, bark, flowers, fruits, roots, etc.) | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M4 | | Describe the extraction method (e.g., Soxhlet, microwave-assisted extraction, ultrasound-based extraction, etc.), indicating the name and concentration of solvents, extraction temperature and time, and the percentage yield of dried extract | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M5 | | Describe the quality evaluation and standardization of the natural product according to the “quality control methods for herbal materials” released by World Health Organization. The proper methods for phytochemical profiling with respect to major active components should be indicated. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Materials, reagents and software | | M6 | | Indicate the name of all reagents and chemicals with all vendor details, including company/institution, city and country | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M7 | | If commercial antibodies are used, report the code number in addition to the information mentioned above. For academic antibodies, report the source laboratory and relevant references. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
|  | | M8 | | Provide the details of any software used in the experiment (name, code etc.) |  |  |  | |  |
| *In vitro* model system characteristics | | M9 | | Indicate the category of *in vitro* model system (cell line, tumoroid, tissue model, etc.), including host origin (human, mouse, etc.) and the relevant histopathologic type of cancer | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M10 | | Provide the source of commercially available cell lines. Indicate the ethical approval and consent for cell lines, tumoroids or tissue models derived from patients. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M11 | | Describe the culture conditions of *in vitro* model (media, serum, growth factors, incubation characteristics, the vehicle used to dissolve the natural product in the medium, etc.) | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M12 | | Indicate the authentication of *in vitro* model system and state what method was used for authentication | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M13 | | Confirm that mycoplasma testing has been done for *in vitro* model system | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Experimental outcomes | | M14 | | Clearly define the primary and secondary experimental outcomes assessed (e.g., survival fraction, growth inhibition, cell migration, angiogenesis, etc.) | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Design of experiment | | M15 | | Specify the number of biological replications (n) per each intervention. Explain how the number of replications decided. Provide details of any sample size calculation used | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M16 | | Indicate the use of multiple biological entities (more than one cell line, organoid, etc.) from biologically independent sources as experimental units. Otherwise, authors need to justify their use of a single entity | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M17 | | Indicate the random assignment of experimental units to the various groups. Report the method of randomization | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M18 | | Report the allocation concealment, blinded conduct of the experiment, and blinded assessment of outcomes | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M19 | | Indicate the assessment method of outcomes | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M20 | | Report the concentrations of the test product and exposure or treatment times | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M21 | | If variables such as IC50 (GI50) or EC50 are outcomes of interest, indicate the use of the four-parametric logistic model. Indicate the use of at least five concentrations of the test product to calculate the variables mentioned above. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M22 | | Indicate the use of vehicle as the negative control |  |  |  | |  |
| M23 | | Indicate the use of an appropriate positive control | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M24 | | Indicate the use of normal biological entities (normal cell lines, normal organoids, etc.) beside neoplastic models if selective cytotoxicity has been assessed | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M25 | | Express the use of the appropriate method of drug interaction analysis if synergism/antagonism has been assessed | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Statistical analysis | | M26 | | Provide details of the statistical methods used for each analysis | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M27 | | Specify the unit of analysis for each dataset | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M28 | | Report any methods used to assess whether the data met the assumptions of the statistical approach. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| M29 | | Name the statistical software used. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Ethics code | | M30 | | Report protocol approval by the ethics committee. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| **Results** | | | | | |  |  | |  |
| Natural product characteristics | | R1 | | Report the results of phytochemical profiling of the test entity. Including a figure that represents the profiling of the extracted compound is mandatory. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Baseline data | | R2 | | For each experimental group, report relevant characteristics of the *in vitro* model before treatment | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| R3 | | Report the effect of vehicle on *in vitro* model system | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Numbers analyzed | | R4 | | Report the number of experimental units in each group included in each analysis. Report absolute numbers (e.g., 2/4, not 50%) | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| R5 | | If any data has not been included in the analysis, explain why. Attrition information for each group should be reported. | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Outcomes and estimation | | R6 | | Report the results for each analysis carried out, with a measure of precision (e.g., standard error or confidence interval) | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Figures and tables | | R7 | | Should be referred to in the text, should be express only essential information, and should be legible, easy to read, and easy to understand | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| **Discussion** | | | | | | | | |  |
| Key results | | D1 | | Summarize key results with reference to study objectives | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Interpretation/scientific implications | | D2 | | Interpret the results, considering the study objectives and hypothesis, current theory, and other relevant studies in the literature | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| D3 | | For antiproliferative natural products, interpret that the test agent has selective cytotoxicity against neoplastic cells and is not anti-life | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| D4 | | Interpret that the concentrations showed the favorable outcomes *in vitro* are suitable for further pharmaceutical development | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| D5 | | Discuss about the mechanism of action of natural product |  |  |  | |  |
| Limitations | | D6 | | Explain the limitations of the study in methodology or findings | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| Generalizability/translation | | D7 | | Comment on whether and how this study's findings are likely to translate to other biological systems, including any relevance to human cancers | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  | |  |
| **Acknowledgment section** | |  | | | | | | | |
| How and if the study was financed | | Ak1 | List all funding sources (including grant number) and the funder(s) role in the study. | | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  |  | |
| Is the experimental protocol registered in any registry system? | | Ak2 | Report if the experimental protocol has been registered in the journals or online resources | | |  |  | | --- | --- | | Yes |  | | No |  | | |  |  | | --- | --- | | Pg |  | | Ph |  | |  |  | |

The tools supporting documents can be find at:

Ahmad, R., Riaz, M., Aldholmi, M., Qureshi, M., Uddin, S., Bhat, A., Poyil, P., Baig, M., Pourahmad, J., Ganesan, T., Khan, A., Siddiqui, Z., El-Demellawy, M., Gholamalizadeh, M., Purnomosari, D., Salim, E., Mousavi Jarrahi, S., Zhang, J., Mohammadnejad, S., Jarrahi, A. Development of a Critical Appraisal Tool (AIMRDA) for the Peer-Review of Studies Assessing the Anticancer Activity of Natural Products: A Step towards Reproducibility. Asian Pacific Journal of Cancer Prevention, 2022; 22(12): 3735-3740. doi: 10.31557/APJCP.2021.22.12.3735