Evaluation form of 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.

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 1 Ph 2-3		
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 2 Ph 27-28		
	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 2 Ph 29-33		
	Result	A2-3	Report all main outcomes	Yes / No	Pg **2** Ph **34-41**		
	Conclusion	A2-4	Give a qualitative assessment of the anticancer effect of the natural compound and highlight the message	Yes / No	Pg **2** Ph **42-43**		

			of the work				
Introduction							
Background /rationale		I1	Introduce the natural product and describe the background information about its phytochemical profiling and ethnopharmacological relevance	Yes / No	Pg **4** Ph **88-100**		
		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 **4** Ph **91-100**		
Objectives		I3	Outline the purpose and state the specific objectives of the research, pointing to the novelty of the work	Yes / No	Pg **4** Ph **88-106**		
Material and Methods							
Natural product characteristics		M1	Indicate the geographical location and time of specimen collection	Yes / No /	Pg Ph	This study used a commercial pure compound	
		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	This study used a commercial pure compound	
		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	This study used a commercial pure compound	
		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	This study used a commercial pure compound	
		M5	Describe the quality evaluation and standardization of the natural product according to the "quality control methods for herbal materials" released by	Yes / No /	Pg Ph	This study used a commercial pure	

		World Health Organization. The proper methods for phytochemical profiling with respect to major active components should be indicated.			compound	
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 **4-5** Ph **109-120**		
	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 **4-5** Ph **114-120**		
	M8	Provide the details of any software used in the experiment (name, code etc.)	Yes / No	Pg **5-7** Ph **143,148,154,165**		
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 **5** Ph **121-127**		
	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 **5** Ph **121-127**		
	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 **5** Ph **121-127**		
	M12	Indicate the authentication of *in vitro* model system and state what method was used for authentication	Yes / No	Pg **5** Ph **121-127**		
	M13	Confirm that mycoplasma testing has been done for *in vitro* model system	Yes / No	Pg **5** Ph **122-**		

					128		
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 **7-9** Ph **174-245**		
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 **7** Ph **167-172**		
	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 **7** Ph **174-185**		
	M17	Indicate the random assignment of experimental units to the various groups. Report the method of randomization	Yes / No	Pg Ph	The studies were compared between 2 groups (treatment, non-treatment)	
	M18	Report the allocation concealment, blinded conduct of the experiment, and blinded assessment of outcomes	Yes / No	Pg Ph	The studies were conducted in *in vitro*	
	M19	Indicate the assessment method of outcomes	Yes / No	Pg **5-7** Ph **129-172**		
	M20	Report the concentrations of the test product and exposure or treatment times	Yes / No	Pg **5-7** Ph **129-172**		
	M21	If variables such as IC_{50} (GI_{50}) or EC_{50} 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 **7** Ph **174-185**		

	M22	Indicate the use of vehicle as the negative control	Yes / No	Pg **7-9** Ph **174-243**		
	M23	Indicate the use of an appropriate positive control	Yes No /	Pg Ph	We used standard methods to assess the experiment outcome and compare effect between experimental group and control.	
	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	This study did not focus on selective toxicity of compound	
	M25	Express the use of the appropriate method of drug interaction analysis if synergism/antagonism has been assessed	Yes No /	Pg Ph	This study did not focus on synergism/antagonism effect of compound	
Statistical analysis	M26	Provide details of the statistical methods used for each analysis	Yes / No	Pg **7** Ph **167-172**		
	M27	Specify the unit of analysis for each dataset	Yes / No	Pg **7-9** Ph **174-243**		
	M28	Report any methods used to assess whether the data met the assumptions of the statistical approach.	Yes / No	Pg **7,8,9** Ph **167-172, 207-214,228-233**		
	M29	Name the statistical software used.	Yes / No	Pg **7** Ph **168-172**		

Ethics code	M30	Report protocol approval by the ethics committee.	Yes No /	Pg Ph	This study did not use animal, human samples	
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	This study used a commercial pure compound	
Baseline data	R2	For each experimental group, report relevant characteristics of the *in vitro* model before treatment	Yes / No	Pg **7-9** Ph **174-243**		
	R3	Report the effect of vehicle on *in vitro* model system	Yes / No	Pg **7-9** Ph **174-243**		
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 **7-9** Ph **174-243**		
	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	Every data have been included in the analysis	
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 **7-9** Ph **174-243**		
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 **7-9** Ph **174-243**		
Discussion						
Key results	D1	Summarize key results with reference to study objectives	Yes / No	Pg **9-12** Ph **246-318**		

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 **9-12** Ph **246-318**		
	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	This study did not focus on selective toxicity of compound	
	D4	Interpret that the concentrations showed the favorable outcomes *in vitro* are suitable for further pharmaceutical development	Yes / No	Pg **9-12** Ph **246-318**		
	D5	Discuss about the mechanism of action of natural product	Yes / No	Pg **9-12** Ph **246-318**		
Limitations	D6	Explain the limitations of the study in methodology or findings	Yes / No	Pg **12** Ph **314-318**		
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 **12** Ph **314-318**		
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 **15** Ph **410-411**		
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 study used standard protocols that have been published	