Eleutherine americana Merr. extract regulates mitochondrial calcium homeostasis in intra-abdominal adhesion: A computational study

by Hery Poerwosusanta

Submission date: 30-Jan-2021 09:51AM (UTC+0700)
Submission ID: 1497486078
File name: Jurnal_Drug_Invention_Today_copy.pdf (1,011.24K)
Word count: 3025
Character count: 16856

ISSN: 0975-7619

ELSEVIER

Drug Invention Today

JPR Solutions

Research Article

Eleutherine americana Merr. extract regulates mitochondrial calcium homeostasis in intra-abdominal adhesion: A computational study

Hery Poerwosusanta[1,3]*, Didik Huswo Utomo[2], Zairin Noor[3], Ika Kustiyah Oktaviyanti[4], Karyono Mintaroem[5], Bambang Pardjianto[5], Moch Aris Widodo[5], Edi Widjajanto[5]

ABSTRACT

Aim: Intra-abdominal adhesion (IAA) is the most significant complication after surgery. This study explores the active compound from *Eleutherine americana* Merr. a regulator of mitochondrial calcium homeostasis in IAA. This study aimed to evaluate computationally the potential of *E. americana* Merr. extract as a mitochondrial calcium activator in IAA cases. **Materials and Methods:** The sample was 14 active compounds from *E. americana* Merr. extract that was collected from the PubChem database. Prediction as a calcium channel activator was conducted using Prediction of Activity Spectra for Substances SERVER. Human intestinal absorption (HIA) and lethal dose 50 (LD50) were evaluated using SERVER. Protein target and pathway were evaluated using STITCH, and computational analysis was done using Cytoscape. **Results:** The results showed that 10 bioactive om *E. americana* Merr. extract have potential as calcium activators (Pa >0.3). The active compound of *E. americana* Merr. can be easily absorbed in the intestine based on its HIA+ score (HIA >0.9). **Conclusion:** The application of this extract was possible due to LD50 evaluation that showed that it was less toxic (LD50 <3 mol/kg-BW). *E. americana* Merr. extract regulates mitochondrial calcium homeostasis, by modulation of two types of the mitochondrial calcium channels, VDAC1 and MARCK (mRyR).

KEY WORDS: Calcium activator, *Eleutherine americana* Merr., MARCK, VDAC1

INTRODUCTION

Intra-abdominal adhesion (IAA) is the most common serious and significant complication after surgery, with the incidence rate reaching 90–95%.[1,2] Approximately 15% of patients have to reoperate due to intestinal obstruction, where adhesions result in bowel obstructions and require lysis. Reoperated patients have a high rate of recurrence and 5–20% mortality.[3] Now becoming a standard procedure, laparoscopy can lower the incidence of adhesion to 12–1.3%, but adhesions may still ensue after this procedure.[4,5] No agent or definitive method has been developed that reliably prevents IAA.[4]

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

Mast cell (MC) degranulation is suggested to be a major factor in adhesion formation. Laparoscopies cause MC degranulation and secretion of histamine, tryptase, and chymase. Fibrosis of connective tissue is affected by the MCs tryptase and chymase. Histamine results in vasodilatation and aggravates reperfusion injury.[6]

Calcium homeostasis is very important to the MCs. Injuries and cell death are caused by insufficient calcium for homeostasis.[7] Ischemia and reperfusion injuries during laparoscopy can open calcium channels in the MC membranes.[8] The cytoplasmic calcium increase causes MC degranulation[9] Evaluating the molecular mechanism could benefit drug development. Activating calcium channels in membrane mitochondria are a possible solution for stabilizing intracellular calcium levels. Calcium

[1]Biomedical Science Doctoral Study Program, Faculty of Medicine Brawijaya University, Jalan Veteran Malang - 65145, East Java, Indonesia, [2]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Brawijaya, Jalan Veteran Malang - 65145, East Java, Indonesia, [3]Department of Surgery, Universitas Lambung Mangkurat Jalan Brigjen H. Hasan Basri, Kayu Tangi, Banjarmasin, Indonesia, [4]Department of Pathology Anatomy, Universitas Lambung Mangkurat, Jalan Brigjen H. Hasan Basri, Kayu Tangi, Banjarmasin, Indonesia, [5]Department of Biomedical Science, Faculty of Medicine, Universitas Brawijaya, Jalan Veteran Malang - 65145, East Java, Indonesia

***Corresponding author:** Hery Poerwosusanta, Biomedical Science Doctoral Study Program, Faculty of Medicine Universitas Brawijaya, Jalan Veteran Malang - 65145, East Java, Indonesia. E-mail: herypo@gmail.com

Received on: 17-11-2018; Revised on: 14-12-2018; Accepted on: 11-01-2019

homeostasis is important for preventing apoptosis and necrosis of the MCs.

This study explores the active compound from *Eleutherine americana* Merr. as a regulator of mitochondrial calcium homeostasis in IAA. This plant is well known as traditional medicine in Borneo, Indonesia. *E. americana* Merr. has savor as an immune stimulant, anti-inflammatory, antitumor, antioxidant, and antibleeding agent.[10,11] Like methlut and cromolyn, *E. americana* Merr. consists of flavones. Its mechanism(s) of action involve decreasing intracellular calcium levels.[12] It is promising that *E. americana* Merr. is a MC stabilizer. The biological activity and pathways of *E. americana* Merr. in humans could be explored computationally based on the structure–activity relationship (SAR) approach. SAR is a well-established method for biological process prediction.

MATERIALS AND METHODS

Ligand Preparation

Structure of simplified molecular-input line-entry system and 3D components of bioactive compound *E. americana* Merr. were obtained from the PubChem Open Chemistry Database. These 14 bioactive compounds are triterpenoid (CID 451674), anthraquinone (CID 6780), eleutherinol/eleutherinone (CID 15559106), elecanacin (CID 102091822), eleutheroside-a (CID 101855622), eleutherine (CID 10166), isoeleutherine (CID 10445924), eleutherol (CID 120697), eleuthoside-b (CID 95224384), isoeleutherol (CID 10800314), dihydroeleutherinol (CID 102473740), hongconin (CID 110108147), naphthol (CID 8663), and naphthoquinone (CID 8530).[10,11,13-16]

Biological Activity Prediction

Biological activity was predicted using the Prediction of Activity Spectra for Substances (PASS) SERVER http://www.pharmaexpert.ru/passonline/based on SAR approach. The PASS SERVER score uses a constant probability activity (Pa) with a threshold of 0.7. If Pa more than 0.7, the laboratory tested will not much be different with the computer prediction results.[17] The compounds were predicted for human intestinal absorption (HIA) for evaluating oral use using Laboratory of Molecular Modeling and Design server (http://lmmd.ecust.edu.cn/). The lethal dose (LD50) of each compound was evaluated using Advanced Chemistry Development iLab (https://ilab.acdlabs.com/iLab2/) to predict the lethal dose when applied *in vivo* in an animal model.

Protein Target and Pathway Analysis

The protein target of the active compound was evaluated using hit identification and target prediction using HITPICK (http://mips.helmholtz-muenchen.de/hitpick). Analysis of the molecular pathway prediction of this protein target using STRINGdb. The pathway analysis was used for describing the molecular mechanism of the active compound in the cell. The direct pathway analysis was conducted using the STITCH web (http://stitch.embl.de) server for determining the chemical and protein interaction. All pathways were downloaded and merged to get comprehensive pathway. The shortest path analysis was done in Cytoscape software.

RESULTS

The Biological Activity of Active Compound from *E. americana* Merr. Extract

The compound screening for biological activity is the first step to exploring herbal potency computationally. The calcium channel inhibitor in the membrane cell and calcium channel activator in the mitochondria has a role in stabilizing MCs. The screening was based on the Pa score. A Pa of 0.3 means that the active compound has minimum potency for the specific activity. If Pa

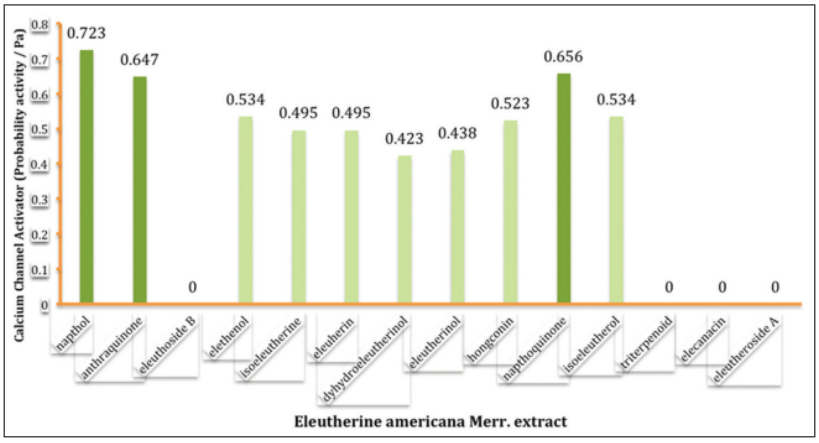

Figure 1: Probability activity of *Eleutherine americana* Merr. extract as calcium channel activator

Table 1: *E. americana* Merr. active compound based on canonical SMILES, HIA and LD50 analysis

Name of compound	CID	Canonical SMILES	HIA+	LD50 (Mol/kg)
Triterpenoid	451674	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC (C5(C) COS(=O)(=O) O) O) C) C) C2C1) C) C(=O) O) C	0.9840	2.4620
Naphthol	8663	C1=CC=C2C=C (C=CC2=C1) O	1.0000	1.8556
Anthraquinone	6780	C1=CC=C2C(=C1) C(=O) C3=CC=CC=C3C2=O	1.0000	2.7726
Eleuthoside B	95224384	CC1C2=C (C=C3C=CC=C (C3=C2OC4C (C(C (C(O4) COC5C (C(C (C(O5) CO) O) O) O) O) O) O) OC) C(=O) O1	0.5342	2.5761
Eleutheroside A	101855622	CC1=CC(=O) C2=C (C=C3C=C (C=C (C3=C2O1) O) OC4C (C(C (C(O4) CO) O) O) O) C	0.6505	2.3027
Eleutherol	120697	CC1C2=C (C=C3C=CC=C (C3=C2O) OC) C(=O) O1	0.9838	3.0288
Isoeleutherine	10445924	CC1CC2=C (C(O1) C) C(=O) C3=C (C2=O) C=CC=C3OC	0.9920	2.8741
Elecanacin	10291822	CC1CC23C (CC2O1) C(=O) C4=C (C3=O) C=CC=C4OC	0.9963	2.6281
Eleutherine	10166	CC1CC2=C (C(O1) C) C(=O) C3=C (C2=O) C=CC=C3OC	0.9920	2.8741
Dihydroeleutherinol	102473740	CC1CC(=O) C2=C (C=C3C=C (C=C (C3=C2O1) O) O) C	0.9637	2.3861
Eleutherinol/ Eleutherinone	15559106	CC1=CC(=O) C2=C (C=C3C=C (C=C (C3=C2O1) O) O) C	0.9722	2.1674
Hongconin	110108147	CC1C2=C (C3=C (C=CC=C3OC) C(=C2C(=O) C (O1) C) O) O	0.9524	2.9636
Naphthoquinone	8530	C1=CC=C2C(=O) C=CC(=O) C2=C1	1.0000	2.8888
Isoeleutherol	10800314	CC1C2=C (C=C3C=CC=C (C3=C2O) OC) C(=O) O1	0.9838	3.0288

SMILES: Simplified molecular-input line-entry system

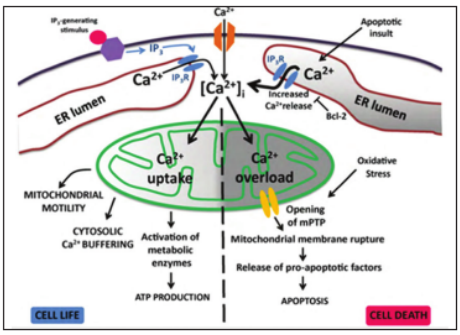

Figure 2: Calcium homeostasis by regulating mitochondrial calcium channel has an essential role in cell survival

is more than 0.7, the laboratory research results will be similar to the computer prediction results.[17] There are 10 compounds that have a Pa score above 0.3 as a calcium channel activator. However, we screened the best three compounds that have high probability, which are naphthol (0.723), naphthoquinone (0.656), and anthraquinone (0.647) [Figure 1].

HIA analysis was conducted for evaluating the pharmacokinetic properties of the active compound. The results showed that these active compounds could apply orally. 98% of active compounds from *E. americana* Merr. extract has an HIA score above 0.9. This means that the extract could be easily absorbed in the human intestine. The lethal dose parameter is necessary before conducting the *in vivo* experiment. LD50 analysis showed that most compounds have an LD50 below 3.0 Mol/Kg as a lethal dose prediction [Table 1].

Activation of calcium channels in mitochondria will be a solution to IAA. The extract has a Pa score for calcium channel activation above 0.3. This means that it has to be shown experimentally to confirm that hypothesis. Laparoscopies make intracellular calcium higher in MCs. The sudden increase of calcium in the cytoplasm causes loss of calcium homeostasis and triggers injury and cell death. Injuries due to calcium toxicity may be reversible or irreversible. In physiologic condition, normal levels of calcium in the cytosol are between 10 and 100 nMol, in contrast to the extracellular level of 1–2 mMol (approximately 10,000 times).[7] Preserving calcium homeostasis is essential in life[15,18] [Figure 2].

E. americana Merr. Extract as the Regulator of Mitochondrial Calcium Homeostasis

Pathway analysis revealed that naphthol, naphthoquinone, and anthraquinone are able to interact with protein calcium channel activators (VDAC1, MCU, and MARCKS/mMyR) through the intermediate protein. Naphthol interacts with SULTIA1 and PTGES3, then with HSP90AA1 and finally with the calcium activator protein [Figure 3]. Anthraquinone interacts with the calcium activator protein through TOP1 and HSP90AA1. NR1H4 is a target protein of naphthoquinone obtained the target HITPICK protein analysis. NR1H4 can activate the expression of the ABCB11 protein and bind to the HSP90AA1 protein to interact with the calcium protein activator. This molecular mechanism was categorized based on the type of action in the stitch database. VCAM1 is a calcium channel protein in the membrane which will be inhibited by calcium channel mitochondria (VDAC1) to stabilize the cytosol calcium level [Figure 3].

DISCUSSION

Average shortest path length analysis was done to determine the fastest and the most effective pathway.

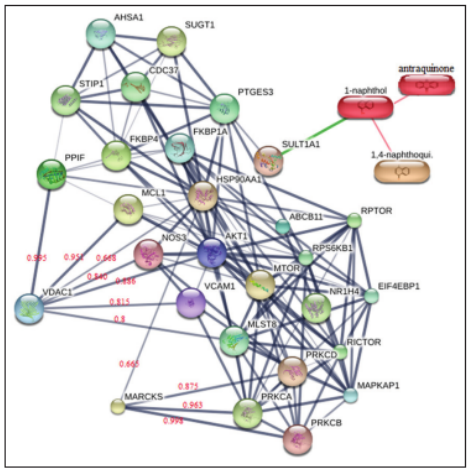

Figure 3: Naphthol, anthraquinone, and naphthoquinone play a role in the regulation of the mitochondrial calcium channel (VDAC and MARCKS)

Table 2: Average shortest path length analysis

Protein target	Average shortest path length
VDAC1	2.05
MARCKS/mMyR	2.15
MCU	2.25

VDAC1 is predicted to be the fastest protein pathway to interact with bioactive of *E. americana* Merr. followed by MARCKS [Table 2]. The lowest score indicates the most effective path for the regulatory process.

Calcium entry into the mitochondria is regulated by several channels including VDAC.[19] The calcium entry mechanism is regulated by calcium signaling in the subcellular region. Like both sides of a coin, VDAC1 has a multifunctional mitochondrial protein action.[20] Metabolic and survival pathways interact with the VDAC1 protein in regulation of the mitochondrial calcium homeostasis.[21] VDCA1 involved in mitochondrial calcium uptake induced an apoptosis/anti-apoptosis balance. Early activation of VDAC1 may optimize calcium homeostasis because VDAC is able to regulate the influx and efflux of calcium.[19,20] In hypoxia and anoxia, VDAC has excessive closure and leads to downregulation of mitochondrial function.[22] Regulation of VDAC is expected to improve mitochondrial function. In addition, MARCKS (mRyR) that are located at the inner membrane mitochondria may play a critical role in mitochondrial calcium-mediated functions such as energy metabolism, the mitochondria transition pore opening, and reactive oxygen species forming.[23,24] This unique property makes mRyR an ideal candidate for sequestering calcium quickly and transiently during physiological activity in cells that mRyR may function to sequester calcium in mitochondria in response to the elevation of calcium level in intracellular.[25]

It can be concluded that *E. americana* Merr. extract has calcium channel activator activity. The computational study revealed that naphthol, naphthoquinone, and anthraquinone were the main components. They can regulate mitochondrial calcium homeostasis by modulating VDAC and MARCKS/mRyR in mitochondrial membranes. Both proteins could uptake high intracellular calcium in injuries. This study could be used as basic information for further experimental analysis of *E. americana* Merr. extract.

ACKNOWLEDGMENTS

The author thanks to Anggia Noor Ramadhani S.Si. from Indonesia Institute for Bioinformatics for her technical support to this study.

AUTHORS' CONTRIBUTIONS STATEMENT

H.P. contributes to conception and design of study. E. D. and Z. N contribute to acquisition of data. I.K.O and K.M contribute to analysis and/or interpretation of data. B.P. and M.A.W. contribute to drafting the manuscript, and H.P. and D.H. U revising the manuscript critically for important intellectual content.

REFERENCES

1. Bi J, Zhang S, Du Z, Zhang J, Deng Y, Liu C, *et al*. Peripheral serotonin regulates postoperative intra-abdominal adhesion formation in mice. Sci Rep 2017;7:10001.
2. Şahin H, Toman H, Kiraz HA, Şimşek T, Erbaş M, Özkul F, *et al*. Effects of sugammadex on the prevention of postoperative peritoneal adhesions. Kaohsiung J Med Sci 2015;31:463-7.
3. Wei G, Chen X, Wang G, Jia P, Xu Q, Ping G, et al. Inhibition of cyclooxygenase-2 prevents intra-abdominal adhesions by decreasing activity of peritoneal fibroblasts. Drug Des Devel Ther 2015;9:3083-98.
4. Altınel Y, Taşpınar E, Ozgüç H, Oztürk E, Ulker Akyıldız E, Bağdaş D, *et al*. The protective effect of clinOleic against post-surgical adhesions. Ulus Travma Acil Cerrahi Derg 2014;20:1-6.
5. Bolnick A, Bolnick J, Diamond MP. Postoperative adhesions as a consequence of pelvic surgery. J Minim Invasive Gynecol 2015;22:549-63.
6. Hermanowicz A, Debek W, Oksiuta M, Matuszczak E, Chyczewski L, Dzienis-Koronkiewicz E, *et al*. Mast cells in peritoneal fluid in rats with experimentally induced peritoneal adhesions. Folia Histochem Cytobiol 2010;48:153-6.
7. Dong Z, Saikumar P, Weinberg JM, Venkatachalam MA. Calcium in cell injury and death. Annu Rev Pathol 2006;1:405-34.
8. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 8th ed. Saunders, Philadelphia, PA: Elsevier Health Sciences; 2015. p. 97.
9. Cuong DV, Kim HK, Marquez J, Kim N, Ko KS, Rhee BD, *et al*. Mitochondrial calcium uniporter inhibition attenuates mouse bone marrow-derived mast cell degranulation induced by beta-1,3-glucan. Korean J Physiol Pharmacol 2016;20:213-20.
10. Amelia T, Pratiwi D, Romsiah X, Daryono HT. *In silico* Study of the Component of *Eleutherine americana* MERR. On Human Estrogen Receptor Alpha as Potential Anti-Breast Cancer. 3rd International Conference on Computation for Science and

Technology (ICCST-3); 2015.
11. Kuntorini EM, Nugroho LH. Structural development and bioactive content of red bulb plant (*Eleutherine americana*); A tradisional medicines for local Kalimantan people. Biodiversitas 2010;11:102-6.
12. Weng Z, Patel AB, Panagiotidou S, Theoharides TC. The novel flavone tetramethoxyluteolin is a potent inhibitor of human mast cells. J Allergy Clin Immunol 2015;135:1044-5200000.
13. Han AR, Min HY, Nam JW, Lee NY, Wiryawan A, Suprapto W, *et al*. Identification of a new naphthalene and its derivatives from the bulb of *Eleutherine americana* with inhibitory activity on lipopolysaccharide-induced nitric oxide production. Chem Pharm Bull (Tokyo) 2008;56:1314-6.
14. Ifesan BO, Joycharat N, Voravuthikunchai SP. The mode of antistaphylococcal action of *Eleutherine americana.* FEMS Immunol Med Microbiol 2009;57:193-201.
15. Hung CH, Ho YS, Chang RC. Modulation of mitochondrial calcium as a pharmacological target for Alzheimer's disease. Ageing Res Rev 2010;9:447-56.
16. Xu J, Qiu F, Duan W, Qu G, Wang N, Yao X. New bioactive constituents from *Eleutherine americana*. Front Chem China 2006;1:320-3.
17. Goel RK, Singh D, Lagunin A, Poroikov V. PASS-assisted exploration of new therapeutic potential of natural products. Med Chem Res 2010;20:1509-14.
18. Viola HM, Hool LC. Cross-talk between L-type ca2+ channels and mitochondria. Clin Exp Pharmacol Physiol 2010;37:229-35.
19. Shoshan-Barmatz V, Maldonado EN, Krelin Y. VDAC1 at the crossroads of cell metabolism, apoptosis and cell stress. Cell Stress 2017;1:11-36.
20. Gincel D, Zaid H, Shoshan-Barmatz V. Calcium binding and translocation by the voltage-dependent anion channel: A possible regulatory mechanism in mitochondrial function. Biochem J 2001;358:147-55.
21. Roos D, Seeger R, Puntel R, Vargas Barbosa N. Role of calcium and mitochondria in meHg-mediated cytotoxicity. J Biomed Biotechnol 2012;2012:248764.
22. Lemasters JJ, Holmuhamedov E. Voltage-dependent anion channel (VDAC) as mitochondrial governator-thinking outside the box. Biochim Biophys Acta Mol Basis Dis 2006;1762:181-90.
23. O-Uchi J, Pan S, Sheu SS. Perspectives on: SGP symposium on mitochondrial physiology and medicine: Molecular identities of mitochondrial ca2+ influx mechanism: Updated passwords for accessing mitochondrial ca2+-linked health and disease. J Gen Physiol 2012;139:435-43.
24. Ryu SY, Beutner G, Dirksen RT, Kinnally KW, Sheu SS. Mitochondrial ryanodine receptors and other mitochondrial ca2+ permeable channels. FEBS Lett 2010;584:1948-55.
25. Finkel T, Menazza S, Holmström KM, Parks RJ, Liu J, Sun J, *et al.* The ins and outs of mitochondrial calcium. Circ Res 2015;116:1810-9.

Source of support: Nil; Conflict of interest: None Declared

Eleutherine americana Merr. extract regulates mitochondrial calcium homeostasis in intra-abdominal adhesion: A computational study

ORIGINALITY REPORT

13%	12%	9%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	repo-dosen.ulm.ac.id Internet Source	3%
2	etheses.uin-malang.ac.id Internet Source	3%
3	Jakob, Regina, Gisela Beutner, Virendra K. Sharma, Yuntao Duan, Robert A. Gross, Stephen Hurst, Bong Sook Jhun, Jin O-Uchi, and Shey-Shing Sheu. "Molecular and functional identification of a mitochondrial ryanodine receptor in neurons", Neuroscience Letters, 2014. Publication	2%
4	Submitted to Universitas Airlangga Student Paper	1%
5	fkg.ulm.ac.id Internet Source	1%
6	www.veterinaryworld.org Internet Source	1%

7	Dharma P.T.R. Maluegha, M. Aris Widodo, Bambang Pardjianto, Edi Widjajanto. "Endothelial progenitor cells lowering effect and compensative mechanism in electrical burn injury models of rat", Biomarkers and Genomic Medicine, 2015 Publication	1%
8	Aida Fitriah, Kholifah Holil, Umaiyatus Syarifah, Fitriyah, Didik Huswo Utomo. "In silico approach for revealing the anti-breast cancer and estrogen receptor alpha inhibitory activity of Artocarpus altilis", AIP Publishing, 2018 Publication	1%

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		