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## Actinodaphnine and Rutacridone as New T-Cell Protein Tyrosine Phosphatase Inhibitors for Drug Development of Obesity

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# Actinodaphnine and Rutacridone as New T-Cell Protein Tyrosine Phosphatase Inhibitors for Drug Development of Obesity

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**Abstract.** T-Cell Protein Tyrosine Phosphatase (TCPTP) is an obesogenic enzyme that inactivates a Signal Transducer and Activator Transcription 3 (STAT3) protein, leading to inhibition of leptin and insulin signalling. This protein can be a target for development of anti-obesity drugs. This study aimed to identify Indonesian phytochemicals as *in silico* TCPTP inhibitor. This bioinformatics study used a molecular docking method with AutoDock Vina software version 1.1.2. Three-dimensional structure of TCPTP protein and its Inhibitor (XIX, standard ligand) was obtained from Protein Data Bank (PDB) database with code 1L8K and PubChem database with code 9926586. Indonesian phytochemicals in this study were registered in the HerbalDB database and met criteria of Lipinski's rule. The three-dimensional structure of phytochemicals was obtained from PubChem National Center for Biotechnology Information (NCBI). Binding affinity and molecular conformation of selected Indonesian phytochemicals were assessed and compared to the standard ligand. PyMol version 1.3 software was used to visualize molecular docking results. Inhibitor XIX interacted with TCPTP protein at Gln125 and Thr129 residues with -6.30 kcal/mol binding affinity. Actinodaphnine and Rutacridone had lower binding affinity (-6.40 kcal / mol) than the standard ligand. Actinodaphnine interacted with the TCPTP protein at Thr129 and Asp130 residues while Rutacridone had interaction at Thr129 only. However, both phytochemicals had different conformation from PTP inhibitor XIX. Actinodaphnine is more potential to become a TCPTP inhibitor *in silico* for treatment of obesity than Rutacridone. Further investigation is required to evaluate the inhibitory effect of both phytochemicals towards the TCPTP protein.

**Keywords:** T-Cell Protein Tyrosine Phosphatase, Molecular docking, Phytochemicals, Obesity treatment.



## 1. Introduction

The prevalence of overweight and obesity has become a major challenge in order to prevent chronic diseases across the human life around the world. Multi factors have contributed to obesity such as genetics, behaviour, social economics, and environments, which raises its morbidity and mortality rates [1]. The worldwide prevalence of obesity nearly tripled between 1975 and 2016. Body mass index (BMI) has an important role in assessment of overweight and obesity in community basis [2]. Increased BMI is a major risk factor for noncommunicable diseases such as cardiovascular diseases, diabetes, musculoskeletal disorders, and some cancers [3-5].

The arcuate nucleus (ARC) in the hypothalamus contains two opposing neuronal population that helps integrate complex peripheral signals. Activation of proopiomelanocortin (POMC) neurons suppress appetite and increase energy expenditure while the orexigenic agouti-related peptide (AgRP) neurons express neuropeptide Y (NPY) and  $\gamma$ -aminobutyric acid to inhibit the POMC neuronal activation and function [6-8]. Leptin hormone, which is produced by white adipocytes in proportion to fat mass and insulin, activates POMC neurons and inhibits AgRP neurons to repress feeding and to increase energy expenditure [9]. On the other hand, the gut-derived ghrelin hormone activates AgRP/NPY neurons to promote feeding and repress energy expenditure. POMC and AgRP neurons may partly responsible for conversion of white adipose tissue (WAT) browning [6,10].

Beige adipocytes can reversibly convert white into brown-like states and switch on-off energy storage versus energy expenditure, which are regulated by the hypothalamus [11,12]. Expression of TCPTP protein is induced by fasting and glucocorticoid hormone, which leads to inhibition of leptin and insulin signalling in AgRP/NPY neurons and repression of the beige adipocytes formation [13]. Conversely, downregulation of hypothalamic TCPTP expression is induced by feeding, leading to increase of AgRP/NPY neurons and of WAT browning. Recent evidence has indicated that mice lacking TCPTP in AgRP/NPY neurons were resistant to diet-induced obesity and had increased beige fat activity and energy expenditure. The deletion of hypothalamic TCPTP in obese mice restored feeding-induced browning and increased energy expenditure to promote weight loss [14]. Therefore, TCPTP may become a potential target for drug development of obesity.

A new drug for obesity can be developed by identification of new molecules that inhibit the TCPTP protein. Active compounds derived from herbal plants are a promising drug since they have social values to community because of easily obtained, cheap, and few side effects [15]. In addition, development of new drugs using high throughput screening is time consuming and expensive. Clinical trials for new drugs spend much more money, approximately \$3.4 million for phase 1, \$8.6 million for phase 2 and \$21.4 million for phase 3 [16]. Virtual screening (*in silico*) is an alternative approach for development of new drugs, which needs a short time frame and low cost [17]. A biocomputational study using molecular docking is often used to predict the preferred orientation between ligands and macromolecules efficiently [18]. This study therefore aimed to find the TCPTP inhibitor from Indonesian herbal plants using the molecular docking method.

## 2. Materials and Methods

### 2.1. Preparation of Standard Ligand, Target Protein and Phytochemicals

The standard ligand in this study was the PTP inhibitor XIX, which was obtained from the PubChem database with an access code 9926586, and the target protein was the TCPTP, which was downloaded from a Protein Data Bank (PDB; [www.rscb.org](http://www.rscb.org)) with the PDB code 1L8K. The TCPTP protein was modified using the AutoDock Tools 1.5.6 (<http://mgltools.scripps.edu/downloads>) by which removed water molecules and added hydrogen atoms in order to increase its polarity in the binding pockets. Phytochemicals in the Indonesian Herbal Plants Database (HerbalDB), Faculty of Pharmacy, University of Indonesia (<http://herbaldb.farmasi.ui.ac.id/>) were used as research samples in this study that fulfilled Lipinski's rule of five to evaluate drug-like properties [18]. These phytochemicals had to have 3D structure that was taken from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>).

## 2.2. Ligand Validation

Before validation of standard ligand and the TCPTP protein, amino acids at Thr129, Asp130, Asp131, Gln132, Glu133, Leu135, Leu145, Leu146, Ser147, Glu148, Val150, Thr155, Leu158, dan His176 were surrounded by a centre grid box with  $x=47.645$ ,  $y=53.799$ , and  $z=72.29.19$ . The next step was to dock molecularly the standard ligand and TCPTP protein at least three times using the PyRx software (<https://pyrx.sourceforge.io/downloads>) in order to find residual interaction and to have root mean square deviation (RMSD)  $< 2\text{\AA}$ .

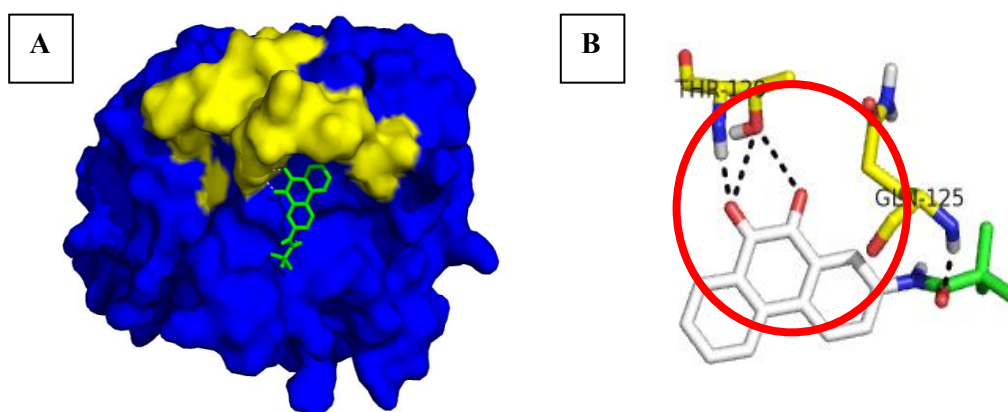
## 2.3. Molecular Docking of Phytochemicals and TCPTP

Selected phytochemicals were molecularly docked with TCPTP three times using the PyRx software and the mean docking scores were compared with the mean docking score of the standard ligand. Data of docking scores were presented as mean  $\pm$  standard deviation. Interaction between selected phytochemicals and the TCPTP protein were then visualized using the Pymol 2.0.6 (<https://pymol.org/2/>) to obtain binding sites, bond types, and molecular conformation. All collected data were then compared with the standard ligand.

## 3. Results and Discussions

### 3.1. Validation of Standard Ligand

Figure 1A and B showed that binding sites of TCPTP and standard ligand at Gln125 and Thr129 residues were different from reference residues of TCPTP active site. From 3D structure, the TCPTP protein has two binding pockets that 129–135 amino acids are located in outer surface of binding pocket and 145–150 amino acids are in the inner surface of binding pocket, the major part of the inhibitor patch [19]. In terms of binding energy, PTP inhibitor XIX interacted with the TCPTP protein with  $-6.3$  kcal/mol binding score (Table 1). This validation is accurate because of the RMSD  $< 2\text{\AA}$ . We could not compare our result of binding energy to another study because we got only a study conducted by Le et al. (2017) that the PTP inhibitor XIX inhibited the TCPTP protein *in vitro*.



**Figure 1.** TCPTP-PTP inhibitor XIX binding complexes were visualized using the Pymol software. A: 3D structure of TCPTP protein was blue and yellow colour indicated active sites of TCPTP protein. Molecular structure of PTP inhibitor XIX was green and closely located to the TCPTP active sites. B: The main interaction between TCPTP and PTP inhibitor XIX was in red circle; Green: Carbon atoms (C), Red: Oxygen atoms (O), Blue: Nitrogen atoms (N), Yellow: Sulfur, Dotted lines: interaction between atoms. Gln-125: Glutamine at the 125 protein sequence, Thr-129: Threonine at the 129 protein sequence

**Table 1.** Docking score between PTP inhibitor XIX and TCPTP protein

Standard Ligand (ID Pubmed)	Docking score (kcal/mol)			Mean docking score (kcal/mol)	RMSD	Binding site
	1	2	3			
PTP Inhibitor XIX (9926586)	-6.3	-6.3	-6.3	-6.3	0 Å	Gln125 and Thr129

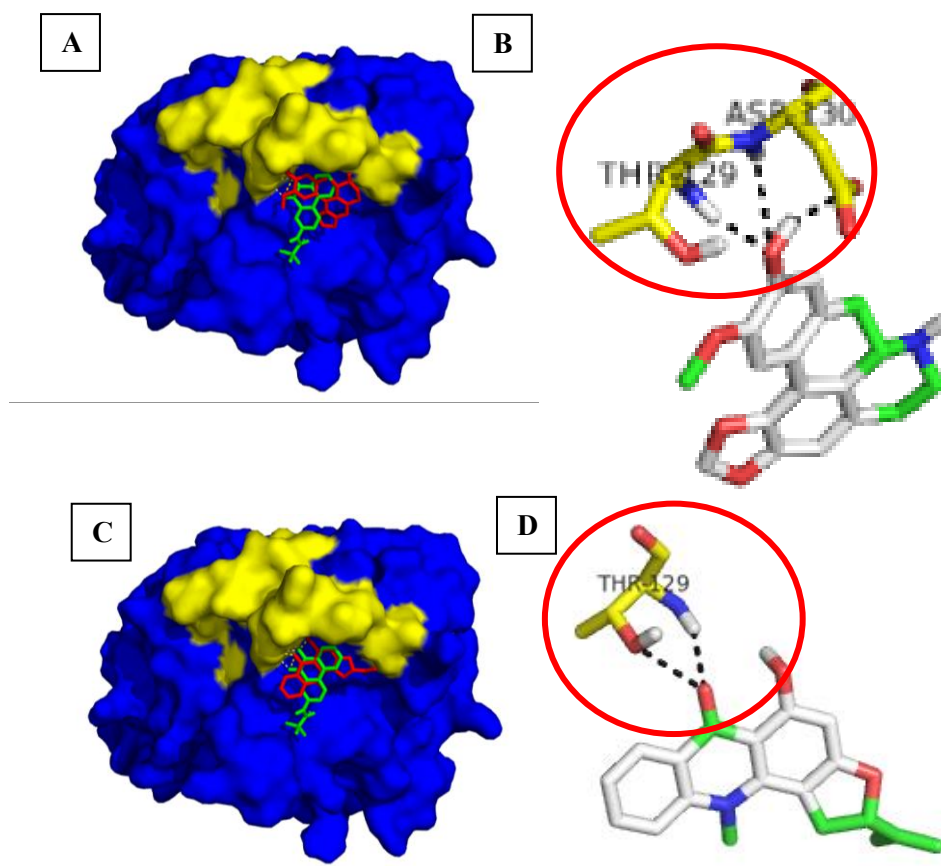
### 3.2. TCPTP Inhibitor from Phytochemicals of Indonesian Herbal Plants

From 510 phytochemicals which full filled Lipinski's criteria, Actinodaphnine and Rutacridone had similarity to the standard ligand regarding to docking score, binding site, and conformation (Table 2 and Figure 2). In Table 2, the mean docking score of Actinodaphnine and Rutacridone (-6.40 kcal/mol) was lower than the mean docking score of PTP inhibitor XIX. The docking score is an important value that indicates the ability of ligand to interact with its cognate receptor. A lower docking score of ligand-receptor binding complexes indicates a stronger affinity and more stable bond [20]. As a result, Actinodaphnine and Rutacridone have stronger affinity and more stable bond to TCPTP protein than the standard ligand.

In terms of binding site, Actinodaphnine and Rutacridone had as same binding sites as the standard ligand (Thr129 with hydrogen bond) but an additional binding site at Asp130 residue was only found in Actinodaphnine (Figure 2). According to Iversen et al., (2017), Asp130 also belongs to residues in the active site of TCPTP protein. Therefore, Actinodaphnine is able to occupy the binding site of TCPTP protein better than standard ligand. Further investigation is required to verify the inhibition of Actinodaphnine against TCPTP protein. In contrast to actinodaphnine, Rutacridone had an additional bond between O and O atoms (van der Waals) in the Thr129 residue. For drug design, the hydrogen bond is preferred than the van der Waals bond since hydrogen atom will interact with electronegative atoms such as F, O, or N and their interactions become reversible [21]. Consequently, Rutacridone maybe not ideal to be developed as a TCPTP inhibitor because its molecular interaction is stronger than the standard ligand, which results in less reversible.

Based on the Lipinski's rule of five criteria, PTP inhibitor, Actinodaphnine and Rutacridone fulfilled the Lipinski's criteria for drug development (Table 2). PTP inhibitor and Rutacridone had the same molecular weight while Actinodaphnine had higher molecular weight than the standard ligand. The same pattern was also observed in H-bond donor and acceptor between PTP inhibitor XIX, Actinodaphnine and Rutacridone. However, better lipophilicity was found in Actinodaphnine, compared to PTP inhibitor XIX and Rutacridone. Although Actinodaphnine has more hydrogen bond donor and acceptor than PTP inhibitor XIX and Rutacridone, it potentially becomes a new TCPTP inhibitor because lower lipophilicity determines higher ability of a compound to pass through lipid bilayer of cell membrane. The recent study also proposes that the most optimal range of drug lipophilicity is log P between 1 – 3 because the lipophilicity of a compound tends to increase during drug testing in clinical trials phase I, II, and III [22].

Molecular conformation between ligand and target protein is very important in structure-based drug design [23]. The conformation of the standard ligand and phytochemicals in this study was assessed through superposition. Molecular orientation of Actinodaphnine and Rutacridone differed from the standard ligand orientation. However, Actinodaphnine and Rutacridone had comparable molecular orientation. For example, *in silico* and *in vitro* studies have documented that ellagic acid has similar binding sites and molecular conformation to Sodium Glucose Co-transporter 2 (SGLT-2) inhibitor [24]. Further analysis also indicates that ethanol extract of pomegranate seeds and peels is able to inhibit glucose uptake in normal kidney cell line [25]. Growing evidence reveals that administration of pomegranate peel extract has the same beneficial effects on lowering blood glucose level and increasing insulin level in Wistar rat with type 2 diabetes mellitus. Overall, it suggests that molecular conformation of Actinodaphnine may have an important role in inhibition of TCPTP activity.



**Figure 2.** Visualization of ligand standards/phytochemicals and Ngb using the Pymol software. (A-B) Conformation of Actinodaphnine and standard ligand. (C-D) Conformation of Rutacridone and standard ligand. Main interaction was showed in red circles; Green: Carbon atoms (C), Red: Oxygen atoms (O), Blue: Nitrogen atoms (N), Yellow: Sulfur atoms (S), Dotted lines: interaction between atoms. Thr-129: Threonine at the 129 protein sequence, Asp-130: Aspartate at the 130 protein sequence.

**Table 2.** Result of docking between phytochemical of Indonesian herbal plant and standard ligand with TCPTP.

ID Pubchem Code access	Ligand	Mean docking score (kcal/mol)	Binding Site	Lipinski's Rule of Five			
				Molecular weight (<500 Da)	H Bond Donor (<5)	H Bond Acceptor (<10)	Log P (<5)
<b>9926586</b>	<b>PTP Inhibitor XIX</b>	<b>-6.30</b>	Gln125, Thr129	<b>307.349</b>	<b>1</b>	<b>3</b>	<b>3.1</b>
160502	<i>Actinodaphnine</i>	-6.40	Thr129, Asp130	311.337	2	5	2.4
5281849	<i>Rutacridone</i>	-6.40	Thr129	307.349	1	4	4.6

### 3.3. Properties of Actinodaphnine and Rutacridone

Actinodaphnine is a phytochemical that belongs to alkaloid class from *Lauraceae Juss.* family and can be found in *Cassytha filiformis*, *Cylicodaphne sebifera*, *Litsea sebifera* and *Litsea glutinosa* plants [26,27]. Alkaloid extract and fraction of *Annona hypoglauca* which contain Actinodaphnine have antibacterial effects. In addition, Ethanol extract of *C. australis* can inhibit DPP-4 activity, comparable to sitagliptin (DPP-4 inhibitor) [28,29]. Meanwhile, Rutacridone is a secondary metabolite that belongs to alkaloid class from *Rutaceae Juss.* family and can be found in *Ruta angustifolia* and *Ruta graveolens* plants [30]. Alkaloid extract of *R. graveolens* and crude extract of *Asteraceae* plants containing Rutacridone has antiproliferative effect on human breast cancer cell lines [31,32]. Pro-apoptotic effects are also detected in alkaloid extract of *R. graveolens* [31].

### 4. Conclusion

Actinodaphnine is better TCPTP inhibitor than Rutacridone, based on lower binding affinity, similar binding sites, and similar conformation to PTP inhibitor XIX. *Lauraceae* family plants have rich sources of Actinodaphnine and have extracted using different solvents for treatment of some human diseases. Further investigation of these family extracts is needed to confirm anti obesogenic activity *in vitro*.

### References

- [1] Hruby A, Hu FB. The epidemiology of obesity: A big picture. *Pharmacoeconomics*, 33(7): 673-689 (2015).
- [2] World Health Organization. Overweight and obesity Fact Sheet. Available from: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight> (2019).
- [3] Chen Y, Copeland WK, Vedanthan R, Grant E, Lee JE, Gu D, et al. Association between body mass index and cardiovascular disease mortality in east Asians and south Asians: pooled analysis of prospective data from the Asia Cohort Consortium. *BMJ* (2013).
- [4] Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, Obesity, and Mortality from Cancer in a Prospectively Studied Cohort of U.S. Adults. *N Engl J Med*. 348(17):1625–38 (2003).
- [5] American Diabetes Association Standards of Medical Care in Diabetes—2012. *Diabetes Care*. 35(Supplement 1): S11–63 (2012).
- [6] Waterson MJ, and Horvath TL. Neuronal Regulation of Energy Homeostasis: Beyond the Hypothalamus and Feeding. *Cell Metab*. 22, 962–970 (2015).
- [7] Andrews ZB, Liu ZW, Wallingford N, Erion DM, Borok E, Friedman JM, Tschöp MH, Shanabrough M, Cline G, Shulman GI, et al. UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. *Nature* 454, 846–851 (2008).
- [8] Atasoy D, Betley JN, Su HH, and Sternson SM. Deconstruction of a neural circuit for hunger. *Nature* 488, 172–177 (2012).
- [9] Park HK, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. *Metabolism*, 64(1):24-34 (2014).
- [10] Betley JN, Xu S, Cao ZF, Gong R, Magnus CJ, Yu Y, and Sternson SM. Neurons for hunger and thirst transmit a negative-valence teaching signal. *Nature* 521, 180–185 (2015).
- [11] Wang W, and Seale P. Control of brown and beige fat development. *Nat. Rev. Mol. Cell Biol*. 17, 691–702 (2016).
- [12] Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 150, 366–376 (2012).
- [13] Dodd GT, Decherf S, Loh K, Simonds SE, Wiede F, Balland E, et al. Leptin and insulin act on POMC neurons to promote the browning of white fat. *Cell*, 160: 88–104 (2015).
- [14] Dodd GT, Andrews ZB, Simonds SE, Michael NJ, DeVeer M, Bruning JC, Spanswick D, et al. A hypothalamic phosphatase switch coordinates energy expenditure with feeding. *Cell*

- Metab, 26: 375–393 (2017).
- [15] Yadav R, Agarwala M. Phytochemical analysis of some medicinal plants. *J. phytol.*, 3(12): 10–14 (2011).
  - [16] Martin L, Hutchens M, Hawkins C, Radnov A. How much do clinical trial cost? *Nat. Rev. Drug Discov.*, 16: 381–382 (2017).
  - [17] Meng X, Zhan H, Meze M, Cui M. molecular docking: A powerful approach for structure-based drug discovery. Institute of Theoretical Chemistry (2011).
  - [18] Nogara, PA, Saraiva R de A, Caeran BD, Lissner LJ, Lenz Dalla Corte C, Braga MM, Rosemberg DB, Rocha JBT. Virtual Screening of Acetylcholinesterase Inhibitors Using the Lipinski's Rule of Five and ZINC Databank. *BioMed Res. Int.*, 2015, 1–8 (2015).
  - [19] Iversen LF, MØller KB, Pedersen AK, Peters GH, Petersen AS, Andersen HS, Branners S, et al. Structure determination of T cell protein-tyrosine phosphatase. *J. Biol. Chem.*, 277: 19982–19990 (2002).
  - [20] Yanuar A, Suhartanto H, Munim A, Anugraha BH, Syahdi RR. Virtual Screening of Indonesian Herbal Database as HIV-1 Protease Inhibitor. *Bioinformation*, 10(2): 52–55 (2014).
  - [21] Chen D, Oezguen N, Urvil P, Ferguson C, Dann SM, and Savidge TC. Regulation of protein-ligand binding affinity by hydrogen bond pairing. *Science Advances*, 2(3), e1501240–e1501240 (2016).
  - [22] Palopoli N, Monzon AM, Parisi G, & Fornasari MS. Addressing the Role of Conformational Diversity in Protein Structure Prediction. *PLOS ONE*, 11(5), e0154923 (2016).
  - [23] Palopoli N, Monzon AM, Parisi G, and Fornasari MS. Addressing the Role of Conformational Diversity in Protein Structure Prediction. *PLOS ONE*, 11(5), e0154923 (2016).
  - [24] Amradani RAR. Molecular docking : exploration of sodium glucose co-transporter 2 inhibitors of Indonesian herbal plant compounds as a type 2 diabetes mellitus therapy. Bachelor thesis. Sebelas Maret University (2015).
  - [25] Utami SM, Ulfia M, Putrinadia AV, Amradani RAR and Dono Indarto, Pomela (Pomegranate Derived Ellagic Acid): A Natural Sodium Glucose Co-Transporter 2 Inhibitor For Type 2 Diabetes Treatment. Bachelor thesis. Sebelas Maret University (2017).
  - [26] Zhou J, Wu L, and Wu, S. The identity of *Actinodaphne sessilifructa* (Lauraceae). *Phytotaxa*, 374(2), 162 (2018).
  - [27] Farmasi UI. Basis Data Tanaman Obat Indonesia. <http://www.herbaldb.farmasi.ui.ac.id/v3/> - Accessed on August 2018
  - [28] Rinaldi MVN, Díaz IEC, Suffredini IB, Moreno PRH. Alkaloids and biological activity of beribá (*Annona hypoglauca*). *Rev. Bras. Farmacogn.*, 27(1), 77–83 (2017).
  - [29] Sukma BAR, Indarto D, Suselo YH. Inhibition effect of *Cuscuta australis* ethanol extract containing actinodaphnine on dipeptidyl peptidase-4 enzyme activity in the MCF-7 cell line. *AIP Conference Proceedings*, 2021(1) (2018).
  - [30] Maier W, Schumann B, and Gröger D. Biosynthesis of acridone alkaloids formation of rutacridone by cell-free extracts of *Ruta graveolens* cell suspension cultures. *FEBS Letters*, 263(2), 289–291 (1990).
  - [31] Schelz Z, Ocsosvski I, Bózsity N, Hohmann J, and Zupkó I. Antiproliferative Effects of Various Furanoacridones Isolated from *Ruta graveolens* on Human Breast Cancer Cell Lines. *Anticancer Res.* 36(6):2751–8 (2016).
  - [32] Réthy B. Antitumor Effect Of Plant Extracts And Their Constituents On Cancer Cell Lines. PhD Thesis. University of Szeged (2007).