

# Cytotoxic Activity and Selectivity Index of *Solanum Torvum* Fruit on T47D Breast

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## Abstract

*Solanum torvum* has antioksidan. This study aimed to analyse cytotoxic activity and selectivity index of *Solanum torvum* on T47D breast cancer cells invitro. Identification of *Solanum torvum* fruit chemical compounds with LCMS containing Clorogenic acid, 4-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, 3-O-feruloylquinic acid, 5-O-feruloylquinic acid, 3-O-feruloylquinic acid, 3,4 - O dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 3-O-feruloylquinic acid, 5-O-feruloylquinic acid, putative dicaffeoylquinic lactone. Cytotoxic assays showed that the *Solanum torvum* Without Fermentation were IC<sub>50</sub> (IGT) 1111.62 µg/mL, IC<sub>50</sub> (MGT) 1889.20 µg/mL, IC<sub>50</sub> (EGT) 1171.90 µg/mL, IC<sub>50</sub> (FEGT) 305.98 µg/mL. Cytotoxic assays showed that the treatment of fermentation was IC<sub>50</sub> (IBT) 1760.36 µg/mL, IC<sub>50</sub> (MBT) 1631.06 µg/mL, IC<sub>50</sub> (EBT) of 1111.58 µg/mL, IC<sub>50</sub> (FEBT) 39.17 µg/mL, IC<sub>50</sub> (FHBT) 85.58 µg/mL. Index of selectivity (IS) *Solanum torvum* (without fermentation) were IS (IGT) 5.23, IS (MGT) 0.61, IS (EGT) 1.06, IS (FEGT) 1.45. Index of selectivity of *Solanum torvum* (fermentation treatment) were IS (IBT) 2.41, IS (MBT) 0.78, IS (EBT) 0.18, IS (FEBT) 4.64, IS (FHBT) 1.67. The best results are treatment of ethyl acetate fraction of *Solanum torvum* (FEBT) has IC<sub>50</sub> was 39.17 µg/mL and Index selectivity was 4.64, declared safe/not toxic to normal cells, thus potentially developed as T47D breast anticancer cells.

**Keywords:** Cytotoxic, T47D Breast Cells, Selectivity Index, *Solanum torvum*.

## Introduction

*Solanum torvum* in Indonesia in fertile soils has antioksidan. *Solanum torvum* contains steroids, terpenoids, saponins, tannins, alkaloids, fatty acids, 3-o-acetyl-stigmasta-5,25-diene-2,3-diol, methyl stearate, 21,25-dimethylmelianodiol<sup>(1)</sup>. *Solanum torvum* extraction on seeds and fruit peel has compounds of flavonoids, sterols and saponins<sup>(2)</sup>. *Solanum torvum* has alkaloids, flavonoids, saponins, tannins, glycosides<sup>(3)</sup>. *Solanum torvum* has constituent chemicals such as neochlorogenin 6-O-β-D-quinovo-pyranoside, neochlorogenin 6-O-β-D-xylopyranosyl- (1 → 3)

-β-D-quinovopyranoside, 6-O-α-L -rhamnopyranosyl- (1 → 3) -Dbeta quinovopyranoside, solagenin 6-O-β-D-quinovopyranoside, solagenin 6-O-α-L-rhamnopyranosyl (1 → 3) -β-D-quinovopyranoside, isoque-rctin, routine, kaempferol and quercetin<sup>(4)</sup>. The wild eggplant fruit (*Solanum torvum*) in Indonesia was relatively used by the population because it has not been cultivated. *Solanum torvum* containing sesquiterpen functions immunosuppressive<sup>(5)</sup>. Clorogenic acid serves to prevent and treat anti-inflammatory diseases<sup>(6)</sup>. Chlorogenic acid is a polyphenol compound proven to stimulate anti-inflammatory, antibacterial and antioxidant activities as agents for clinical treatment of hepatic I/R injuries<sup>(7)</sup>. Carcinogenesis is the process of the occurrence of multi-stage cancer that undergoes genetic changes and progressive transformation of normal cells into malignant cells<sup>(8)</sup>. The biggest cancer causes of death in the world are breast, cervix, colon, lung and stomach cancer<sup>(9)</sup>. The most common causes of death for women are cervical cancer and breast cancer. T47D breast cancer cells used in this study

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have unlimited replication ability, high homogeneity and was easily replaced with frozen stock in the event of contamination<sup>(10)</sup>. This was supported by research that states that the methyl caffeoyl compound in *Solanum torvum* fruit functions as an anti-cancer<sup>(11)</sup>. The study aimed to analyze antioxidant compounds found in *Solanum torvum* fruit extracted with various solvents to function as cytotoxic and selectivity index for T47D breast cancer cells.

## Materials and Method

**Solanum Torvum:** *Solanum torvum* was obtained from Sumber Manjing village Malang. Fruit wild eggplant was sorted, washed, drained. In the next stage processing into dry powder using 2 types of treatment. Treatment 1 was the fruit that has been cleaned in a small chunk and then vacuum dryer temperature 50<sup>o</sup> C for 14 hours. The two treatments were fermented, namely 5 days curing, 6.18 hours fermentation time, 50<sup>o</sup> C drying temperature and 14 hours drying time, thus there stored on main raw material.

**Chemical Materials:** Materials include, water, aquabides, methanol pa, ethanol pa, n hexane pa, ethyl acetate (p.a.), DMSO, alcohol, T47D cells and vero cells, RPMI, M199 media, FBS, PS, trypsin-EDTA, SDS in 0.01 N HCL, MTT, and PBS, Rnase, ethanol, 2 N hydrochloric acid, lead (II) acetate 0.4 M, isopropanol, chloroform, Molish, 2 N hydrochloric acid, iron (III) chloride reagent, Liebermann-Burchard, n-hexan, DPPH 0.1 mM, methanol, Folin-Ciocalteai, Gallic Acid, AICI<sub>3</sub>, Quercetin, Sodium Nitrite, Diethyl ether, Na<sub>2</sub>CO<sub>3</sub>. Chemicals obtained from the parasitology laboratory of the UGM medical faculty jogjakarta.

**Analyzing Solanum Torvum using LCMS:** UHPLC The brand of ACCELLA type 1250 made by Thermo Scientific which consists of vacuum degassers. Solvents A = 0.1% wet format in water and B = formic acid in Acetonitrile. A mobile phase gradient with a speed of 300 µl/minute with the following settings: 0.0-0.6.00 minutes 5% B, 0.6 - 3.0. is 2 µL at 160<sup>o</sup>C. The column is controlled at 30<sup>o</sup>C, and the autosampler compartment is set to 16<sup>o</sup>C. Use LCMS/MS Triple minutes 75% B, 3.0 - 3.5 minutes 75% B, 4.0- 5.5 minutes 5%. The injection volume in the TSQ Quantum Access Max spectromethemic acid LC from Thermo Finnigan with an ESI ionization source is controlled by TSQ Tune software which is operated in negative mode. The ionization conditions of ESI are as follows:

3.0 kV spray voltage; evaporation temperature of 250<sup>o</sup>C; capillary temperature of 300<sup>o</sup>C; nitrogen as sheath gas pressure 40 psi, and 10 psi pressure aux gas with argon gas (12)

### Preparation of breast cancer cells T47D (13):

**Ethanol extraction:** Samples weighed 10 grams plus 80% ethanol as much as 100 ml then placed on elenmeyer diprasi for 24 hours while being shaken. The results of the extract were filtered put in an impermeable container, the sample was again macerated with 80% ethanol solvent which was repeated until 3 times. The results of the ethanol extract put together are concentrated with a rotary evaporator vacuum temperature of 40<sup>o</sup>C until concentrated, then stored in bottles that are impermeable to drying with nitrogen gas until the solvent evaporates, stored in a safe place.

**Methanol Extraction:** 10 grams of the test sample were extracted with 100 ml of methanol concentration of 95% with 24-hour maceration. The extract was filtered with Whatman no 1 filter paper. Then evaporator until thick and with nitrogen gas until dry, stored in a safe place.

**Water Extraction:** 10 grams of the test sample were each extracted with 100 ml of hot water in an infusion pan for 15 minutes. The extract was filtered with Whatman filter paper No. 1. The dirotarievapore extract until thick and the results stored in a bottle then dried with freeze drying, stored in a safe place.

**Hexane Fractionation:** Ethanol extract of *Solanum torvum* fruit powder was inserted in a separating funnel, added hexane and water each 50 ml (1: 1) shaken so that 2 layers were formed. The hexane layer is collected, the water layer is extracted again with hexane up to 3 times. The results are applied with a vacuum rotary evaporator until thick and dried with nitrogen gas so that stored in a safe place.

**Ethyl Acetate Fractionation:** Ethanol extract of the test sample was put in a separating funnel added with ethyl acetate and 50 ml (1: 1) of water each shaken to form 2 layers. Ethyl acetate layer is accommodated, the water layer is extracted again with ethyl acetate up to 3 times. The results are applied with a vacuum evaporator until thick and dried with nitrogen gas, thus stored in a safe place.

**Citotoxic analysis via MTT:** Citotoxic analysis

inT47D breast cells with 9 samples treatment *Solanum torvum* were 31,25 ppm, 62,5 ppm, 125 ppm, 250 ppm, 1000 ppm with ELISA reader (Benchmark Bio Rad), wavelength 595<sup>(13)</sup>.

**Index Selectivity Analysis:** The principle works the same as cytotoxic analysis but the complete media uses M199. The test sample concentrations were 1000ppm, 500ppm, 250ppm, 125 ppm, 6.25 ppm, 31.125 ppm. The

results of Selectivity Index formula was (13).

$$\text{Selectivity Index} = \text{IC}_{50} \text{ Cells Vero} / \text{IC}_{50} \text{ Cells T47D}$$

## Result

**Identification of chromatographic chemical compounds with LCMS:** The results of the identification of chemical compounds in the *Solanum torvum* fruit (without fermentation treatment) can be seen in Figure 2 and Table 1.

**Table 1: Chemical components of *Solanum torvum***

No	TR	(M- H)- (m/z)	MS <sup>2</sup> (m/z)	Compounds
1	3,72	353	191,127	chlorogenic acid
2	4,46	353	173,179	4-O- caffeoylquinic
3	3,9	353	191,179	3-O- caffeoylquinic
4	5,00	367	193,191	3-O-feruloylquinic acid
5	5,54	367	191	5-O-feruloylquinic acid
6	5,03	367	193	3,4-O-dicaffeoylquinic acid
7	7,01	515	353	3,4-O-dicaffeoylquinic acid
8	7,19	515	353	3,5-O-dicaffeoylquinic acid
9	7,37	529	367	3-O-feruloylquinic acid
10	7,54	529	367	5-O-feruloylquinic acid
11	7,87	497	335	putative dicaffeoylquinic lactone

In Table 3, it shows the identification of chemical compounds using the LCMS (With Fermentation treatment)

**Table 2: Chemical components of fruit *Solanum torvum***

No	TR	(M- H)- (m/z)	MS <sup>2</sup> (m/z)	Compounds
1	3,69	353	191	Clorogenic acid
2	4,46	353	179	4-O- caffeoylquinic acid
3	3,9	353	179	3-O-caffeoylquinic acid
4	5,03	367	191	3-O-feruloylquinic acid
5	5,54	367	191	5-O-feruloyquinic acid
6	4,67	367	193	3-O-feruloylquinic acid
7	7,01	515	353	3,4,O- dicaffeoylquinic acid
8	7,34	515	353	3,5-O-dicaffeoylquinic acid
9	7,31	529	367	3- O- feruloylquinic acid
10	2,8	529	367	5-O- feruloylquinic acid
11	7,34	497	355	putative dicaffeoylquinic lactone

**Cytotoxic Analysis Results of *Solanum torvum*:** In the cytotoxic test samples on T47D breast cancer cells in Table 4.

**Table 3: Cytotoxic Test Data on T47D breast cancer cells**

Sample testing		IC 50 (µg/mL)
1.	Infusion of Solanum torvum fruit powder (without fermentation) IGT	1111,62
2.	Methanol extract of Solanum torvum fruit (without fermentation) MGT	1889,20
3.	Ethanol extract of Solanum torvum fruit (without fermentation) EGT	1171,90
4.	Ethyl acetate fraction of Solanum torvum fruit (without fermentation) FEGT	305,98
5.	Solanum torvum fruit infusion (fermentation treatment)IBT	1760,36
6.	Methanol Extract of Solanum torvum Fruit (Fermentation Treatment) MBT	1631,06
7.	Ethanol Extract of Solanum torvum Fruit (Fermentation Treatment) EBT	1111,58
8.	Ethyl Acetate Fraction of Solanum torvum Fruit (Fermentation Treatment) FEBT	39,17
9.	Solanum torvum Fruit Hexane Fraction (Fermentation Treatment) FHBT	85,58
10.	Doxorubicin (Cancer Drug)	36,76

**Solanum torvum Selectivity Index Results:** The results of a good and non-toxic objectivity test for normal cells were infusion of Solanum torvum without fermentation treatment with index selectivity of 5.23 and

ethyl acetate fraction of Solanum torvum fermentation treatment having a selectivity index of 4.64, can be seen in Table 5.

**Table 4: Index test data on the selectivity**

Sample Testing		Selectivity Index	Remarks
1	Infusion of Solanum torvum (without fermentation) IGT	5,23	Selective
2	Methanol extract of Solanum torvum (without fermentation) MGT	0,61	Non-Selective
3	Ethanol extract of Solanum torvum fruit (without fermentation) EGT	1,06	Non-Selective
4	Ethyl acetate fraction of Solanum torvum fruit (without fermentation) FEGT	1,45	Non-Selective
5	Infusion Solanum torvum fruit (fermentation treatment) IBT	2,41	Non-Selective
6	Methanol Extract of Solanum torvum Fruit (Fermentation Treatment) MBT	0,78	Non-Selective
7	Ethanol Extract of Solanum torvum Fruit (Fermentation Treatment) EGT	0,18	Non-Selective
8	Ethyl Acetate Fraction of Solanum torvum Fruit (Fermentation Treatment) FEBT	4,64	Selective
9	Solanum torvum Fruit Hexane Fraction (Fermentation Treatment) FHBT	1,67	Non-Selective

## Discussion

Identification of *Solanum torvum* fruit chemical compounds with LCMS containing Chlorogenic acid, 4-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, 3-O-feruloylquinic acid, 5-O-feruloylquinic acid, 3-O-feruloylquinic acid, 3,4 - O dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 3-O-feruloylquinic acid, 5-O-feruloylquinic acid, putative dicaffeoylquinic lactone. This was supported by opinions<sup>(14)</sup> The results of phytochemical analysis of *Solanum torvum* fruit extraction with organic solvents contained alkaloids, indole alkaloids, saponins glycosides, flavonoids, phenols, sterols, proteins, carbohydrates, alkaloid lepac, vitamin A, Vitamin C, Vitamin E, polyphenols. The

results of research that have a good selectivity index are samples IGT and FEBT (Table 5).The selectivity index value greater than 3 indicates that extract is selective and not toxic, and vice versa if the selectivity index is less than 3, meaning that the extract used is not selective and has a toxicity to normal cells<sup>(15)</sup>. Antioxidants in small concentrations can prevent or slow down the rate of free radical oxidation or lipid oxidation. These antioxidant compounds can function as free radical scavengers, forming complexes with metal elements as antioxidants and functioning as reducing compounds<sup>(16)</sup>. Antioxidants were useful in preventing tumors and cancer, premature aging<sup>(17)</sup>. The best cytotoxic activity in this research was ethyl acetate fraction of *Solanum torvum* fruit

(Fermentation Treatment) IC<sub>50</sub> value of 39,17 µg/mL. Doxorubicin as a positive control has an IC<sub>50</sub> value of 36.76 µg/mL<sup>(18)</sup>. A very effective anticancer agent for breast cancer metastasis was doxorubicin<sup>(19)</sup>. The criteria for compounds that have antiproliferative potential are IC<sub>50</sub> ≤ 100 µg/ml<sup>(20)</sup>. The research Chromatographic analysis of *Solanum torvum* containing Chlorogenic acid and derivative. That chlorogenic acid functions to prevent and treat anti-inflammatory diseases<sup>(21)</sup> A new chlorogenic oxide called oxovanadium complex has the potential for antioxidant agents and anti cancer (human breast cancer)<sup>(22)</sup>.

### Conclusion

The results of Chromatographic analysis containing Chlorogenic acid, and derivative. So the best research results as cytotoxic activity of *Solanum torvum* ethyl acetate fraction having IC<sub>50</sub> which was 39.17 µg/mL and index selectivity of 4.64 which means it was not toxic to normal cells, thus potentially developed as T47D breast anticancer

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**Conflict of Interest Statement:** Herevly we declare that the entire authors of this manuscript have no conflict of interest.

**Ethical Clearance:** This study was approved by the Department of Food Technology Brawijaya University. According to the research ethics, this research has no ethical violation and the process of collecting data was in accordance with experimental ethics.

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