ANTIOXIDANT TEST AND IN-SILICO TOXICITY ANALYSIS OF INDICATED COMPOUNDS FROM THE ETHANOL EXTRACT OF PURPLE EGGPLANT (SOLANUM MELONGENA L) SKIN THROUGH USING THE LC-MS/MS METHOD

Muhammad Irfan[1], Ali Napiah Nasution[1], Evelyn Angie[1], Chairul Radjab Nasution[1], Yensuari[1], Anita Rosari[1]

Article History: **Received:** 08.02.2023 **Revised:** 23.03.2023 **Accepted:** 18.05.2023

Abstract

Eggplant, which is known by the scientific name Solanum melongena L. is a popular vegetabel commodity that is liked by many people because of its delicious taste and high nutritional content, so it has the potential to be cultivated. It is necessary to carry out further research to prove the efficacy of its content by conducting phytopharmaceutical testing and ADMET testing (Absorption, distribution, metabolism, excretion, and toxicity) of the compounds contained in eggplant skin using the Lc-ms/ms method, by comparing pKCSM, SwissAdme, and Protox-II with the ChemDraw software application. The results of phytochemical screening of eggplant peel ethanol extract (EEKT) found the presence of flavonoid compounds, alkaloids, saponins, tannins, and glycosides, but steroids/triterpenoids were not found. The LC-MS/MS examination on EEKT found the presence of flavonoid compounds; luteolin 5-O-glucoside, rutin, gardenin B, rosmarinic acid, ursolic acid, cosmosiin, eugenol, apigenin, ocimarin, xanthomicrol, salvigenin, but no compound kaemperol 3-O- maonylglucoside was found. The prediction results of ADMET for pulsed pk-CSM, SwissAdme, and Protox-II compounds, found relatively the same results, among others in absorption, the assessment of the molecular weight of the compounds was significantly the same, based on the results of the analysis concluded that the average molecular weight of flavonoid compounds in EEKT is below 500 g/mol, then the compound is categorized as easily absorbed, this can be supported by tests of water solubility, caco2 permeability, intestinal absorption human. P-glycoprotein is a biological barrier that can remove toxins and xenobiotics from cells, the flavonoid compounds in EEKT do not function as a biological barrier and even function as an inhibitor of the creation of toxins in the body. In distribution assessment, EEKT flavonoid compounds tend to be normally distributed, this can be concluded from the assessment of VDss, fraction unbound, BBB permeability, and CNS permeability. Substances that become substrates indicate that these compounds can be metabolized by CYP 450 which is an important detoxifying enzyme in the body, while compounds that can act as inhibitors can suppress their metabolic activity. All EEKT flavonoid compounds were not affected by metabolism in the presence of CYP2D6 and could not act as inhibitors for CYP2D6. Excretion assessment, namely by measuring total clearance and renal OCT2 substrate levels, EEKT flavonoid compounds tend not to burden the kidneys or be toxic to the kidneys. In the assessment of toxicity, through testing Ames toxicity, Max tolerated dose of human, hERG I/II, LD50, the flavonoid compounds in EEKT tend to be non-toxic.

***Keywords**: Antioxidants, Toxicity, In-silico, Purple Eggplant Skin, LC-MS/MS*

[1]Master of Biomedical Science, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia

Corresponding Email: alinapiahnasution@unprimdn.ac.id

1. Introduction

Indonesia is a country with the second highest terrestrial biodiversity wealth in the world. If combined with marine biodiversity, Indonesia will be the first. According to the Minister of Research and Technology/Head of the National Research and Innovation Agency, Bambang Permadi Soemantri Brodjonegoro, biodiversity is a long-term asset that needs to be studied, studied and researched. The efforts of the Indonesian Institute of Sciences (LIPI) which have utilized biodiversity for bioproducts through bioprospecting in the form of herbal immunomodulators for Covid-19 and bioeconomics through products made

from biological materials need to be continuously developed. Laksana Tri Handoko, chairman of LIPI stated that LIPI's mission is to develop knowledge that is useful for conservation and sustainable use of resources, in line with the United Nations program and the Aichi Target 2050, and LIPI as the coordinator of the national biodiversity research program, continues to make efforts to explore to reveal Indonesia's biodiversity (Handoko, 2020), the WHO-GCTM (Global center for traditional medicine) in 2022 noted that 88% of the world's population still depends on traditional medicine systems, the majority of which involve plants to cure diseases (WHO, 2022). Yahia et al, (2018) stated nutritional habits have changed a lot, and fruit and vegetable consumption has increased thanks to the many health-promoting compounds found in them. They provide a wide variety of minerals, protein, fiber and antioxidants. However, the appreciation of fruits and vegetables has especially increased due to the beneficial effects associated with dietary antioxidants (Martinez-Espizua et al, 2021).

Eggplant, known by the scientific name Solanum melongena L., is a popular vegetable commodity that is liked by many people because it tastes good and contains high enough nutrition, so it has the potential to be cultivated. Every 100 grams of raw eggplant contains 26 calories; 1 gram of protein; 0.2 gram of charcoal hydrate; 25 IU of vitamin A; 0.04 grams of B vitamins; and 5 grams of vitamin C, as well as the content of alkaloids, solanine, and solasodin make eggplant efficacious as medicine. The content of trypsin (protease) can fight cancer-promoting substances (Muldiana and Rosdiana, 2017), the content of steroid saponins, steroid alkaloids, terpenes, flavonoids, lignans, sterols, phenolic compounds, and coumarins, among other compounds, provides a large number of pharmacological activities such as cytotoxicity against different tumors such as breast cancer (4T1 and EMT), colorectal cancer (HCT116, HT29, and SW480), and prostate cancer cell line (DU145) (Kaunda and Zhang, 2019). Many parts of the eggplant plant have also been proven to have medicinal properties, Fadiani (2017) proved eggplant leaves with 1.5 g/kg BW water extract have antidepressant activity comparable to Imipramin Hcl 20 mg/kg (Fadiani, 2017), fruit powder eggplant is useful as a hepatoprotective (Widyana et al, 2017), Dong et al, (2020) stated that eggplant does not only contain protein, minerals, dietary fiber, interesting minerals such as potassium, calcium, magnesium, sodium, iron, but is also enriched with polyphenols , including phenolic acids such as chlorogenic. acids, caffeic acid, and p-coumaric acid, and flavonoids, including small amounts of flavonols and a high content of a variety of acylated and non-acylated anthocyanins especially in the purple varieties. It is also valued for its content in other antioxidants such as ascorbic acid and vitamins, although it has a low content of provitamin A carotenoids compared to other Solanaceous plants such as tomatoes and peppers. These bioactive compounds are responsible for the higher functional properties of eggplant, because they neutralize reactive oxygen species (ROS) by reducing lipid peroxidation and damage to cellular organelles, and provide antibacterial, anti-inflammatory, antiallergic, antithrombotic, antiviral, anticarcinogenic, vasodilating, and anti-inflammatory properties. hepatoprotective in humans (Martinez-Ispizua et al, 2021).

Eggplant or eggplant is a member of the Solanaceae or nightshade family which also includes tomatoes, potatoes, and peppers. It is grown mainly as a food crop. The genus name Solanum comes from the Latin word solamen, which means comforting and soothing. Eggplant is also known by other names, such as Aubergine, Brinjal, Eggplant, Mad apple, Raging apple. The species name, melongena, refers to the shape of the melon. Other sources state that the species name, melongena, comes from the Italian name "melanzane," which comes from "mela insane" or "mad apple."

Eggplants grow best in full sun. It prefers moist, well-drained, fertile, sandy and loamy soil with a pH range of 5.5 to 6.8. It is propagated by seed and germination occurs in 8 -12 days. Fruit can be harvested in about 105-133 days. Plant flowers attract bees, eggplant fruit and seeds are edible, but the flowers, leaves and roots of the plant are poisonous and should not be consumed, due to the content of alkaloids including solanine, if

ingested it can cause a burning throat, nausea, vomiting and heart rhythm irregular which can be fatal (Nc State, 2021). Eggplant (Solanum melongena L.) is a herbaceous, vegetable plant with leathery lobed leaves, white to purple flowers, berries and grown worldwide primarily for food representing one of the best dietary sources of biologically active polyphenolic compounds, vitamins, antioxidants, and drug needs. This plant contains flavonoids, tropane, glycoalkaloids, arginine, lanosterol, gramisterol, and aspartic acid as important constituents.

In order for traditional medicine to be accepted in society and in health services, it must be scientifically supported for its efficacy, safety and quality for its use in humans. Therefore it is necessary to broaden the knowledge base, and provide guidance on regulatory standards, quality assurance, and increase availability and affordability (Lestari, 2020). Drug discovery and development is a long, high-risk journey and the average cost of developing a new drug is very high. Most of the drugs evaluated in clinical trials do not reach the market due to lack of efficacy or unacceptable side effects. To maximize the efficacy, safety and pharmacokinetics of a substance, it is necessary to identify the pharmacokinetic properties and molecular toxicity of a sample, so that therapeutic effectiveness and success can be achieved. The interaction between pharmacokinetics, toxicity, and potency is necessary for the effectiveness of a drug. In-vitro and in-vivo tests are performed to investigate drug safety, including various drug toxicities and side effects, and in recent years there have been efforts to develop in-vitro models such as "organ on a chip", but these approaches are still expensive and time-consuming. Finally, a computational method was developed which provides greater advantages over the experimental approach because it is environmentally friendly, the process is fast, the cost is low, it is very accurate, and most importantly it can be carried out before the compounds are synthesized (Yang et al, 2018).

LC-MS/MS is an effective qualitative and quantitative analytical technique with a wide range of applications, such as clinical applications, including therapeutic drug monitoring (TDM), toxicology, endocrinology, pediatrics, microbiology, and proteomics. The principle of Tandem Mass Spectrometry is based on using two mass spectrometers together to analyze a mixture of samples. This method uses two mass analyzers arranged sequentially with a collision cell between them. The mass analyzer is used to select a specific mass to charge (m/z) ratio. The first mass analyzer analyzes the m/z ratio of the parent ion, then in the collision cell the parent ion collides with gas molecules and is fragmented into smaller ions and the m/z ratio is obtained in the second mass analyzer as product ions. The advantage of KCKUT-SM/SM cannot be separated from the advantages of tandem mass spectrometry which has high selectivity because it is able to recognize the two physical properties of the analyte being analyzed, namely the m/z ratio of parent ions and product ions. Combining with liquid chromatography is able to accurately identify analytes based on retention time so as to increase specificity. The sensitivity of tandem mass spectrometry shows flexibility in developing the analysis of new compounds or biomarkers, because it is able to produce a lower limit of detection than other methods. Another benefit of LC-MS/MS is the ability to perform multicomponent analysis simultaneously; identify and measure multiple analytics that are analyzed concurrently. The ability to analyze multicomponents can reduce costs, especially in the process of preparing samples in biological matrices. Sample preparation can be simplified by liquid-liquid extraction or protein precipitation methods, compared to time-consuming and expensive sample preparation methods such as solid-phase extraction or privatization (Harmita et al, 2019).

Drug discovery and development is a highly complex and expensive endeavor, which includes disease selection, target identification and validation, lead discovery and optimization, and preclinical and clinical trials. With the development of in silico methods in recent years, the number of new molecular entities (NMEs = new molecular entities) approved by the US Food and Drug Administration (FDA = Food and Drug Administration) has obviously increased. However, there are still many drug candidates

that fail to become drugs. Lack of efficacy and safety are two of the leading causes of drug failure, meaning absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of chemicals play a critical role at every stage of drug discovery and development. Therefore, it is necessary to find efficacious molecules with better ADMET properties. Chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET), play a key role in drug discovery and development. A high-quality drug candidate should not only have sufficient efficacy against therapeutic targets but also demonstrate appropriate ADMET properties at therapeutic doses. Therefore, many in silico models were developed for the prediction of the chemical properties of ADMET. However, it is still not easy to evaluate the drug similarity of the compounds in terms of so many properties of ADMET. In this case the scoring function is called the ADMET score to evaluate the drug similarity of a compound. The scoring function is determined based on 18 ADMET properties predicted via the web server admetSAR. The weight of each property in the ADMET score is determined by three parameters: the accuracy of the model, the importance of the end point in the pharmacokinetic process, and the usability index. FDA-approved drugs from DrugBank, small molecules from ChEMBL, and older drugs withdrawn from the market due to safety concerns were used to evaluate the performance of the ADMET score. The arithmetic mean index and p-value can show the ADMET score between several data sets that differ significantly, besides that it can tell whether there is a clear linear correlation between the ADMED and QED scores (quantitative estimate of drug-likeness). These results indicate that the ADMET score will be a comprehensive index to evaluate chemical drug similarity, and may be useful for users to select appropriate drug candidates for further development (Guan et al, 2019).

The in silico toxicity prediction method (PTI= Prediction of toxicity In silico) is a computational approach to analyze, simulate, visualize, and predict the toxicity of chemicals. PTI includes all methodologies for analyzing the chemical and biological properties of the chemical structures they represent whether actual or proposed (i.e., virtual) chemicals. Currently, the in silico approach is often used in combination with other toxicity assays, it is beginning to be used to assess toxicity information with little need for in vitro or in vivo studies. IST (In silico toxicology) uses models that can be coded in software to predict the potential toxicity of chemicals and in some situations to quantitatively predict toxic doses. This model is based on experimental data, activity structure relationships, and scientific knowledge (such as warning structures reported in the literature) (Kar and Leszczynski, 2019).

Based on the background of technological advances in LC-MS/MS automation, miniaturization, detectors and LC repairs, as well as future standardization and regulations greatly affect the role of LC-MS/MS in the toxicology laboratory, this makes researchers interested in conducting research on eggplants. by making it into the form of an ethanol extract, then identifying the ethanol extract of eggplant fruit by LC-MS/MS, then the identified chemical compounds were carried out in silico toxicity prediction (PTI= Prediction of toxicity In silico) by analyzing the ADMET prediction parameters (absorption, distribution, metabolism, and toxicity), using the concept of "Graph-based structural singnatures" with the pkCSM "Cutoff Scanning algorithm" approach to extract distance patterns from protein structure graphs by condensing them into "Signature vectors" and then comparing them with Swiss-ADME from the Swiss Institute of Bioinformatics , for protein structure classification, function prediction, receptor-based ligand prediction, as a component of a structure-based mutation analysis approach, so that the prediction and pharmacokinetic properties and toxicity of small molecules can be optimized. This research method uses Software ChemDraw 2016 (http://scistore.cambridgesoft.com/) (License Code: 338-284099-4415), Openbabel GUI 2.4.1 (https://sourceforge.net), pkCSM (http://biosig.unimelb.edu.au/pkcsm), SwissAdme, and Protox-II. This study analyzed the compounds in the oral systemic EEKT for absorption rates and six descriptors consisting of molecular weight (MW), logP, hydrogen

bond acceptors (HBA), hydrogen bond donors (HBD), and analyzed the absorption of compounds in the body using the in silico method. with the hope that this research can be a consideration for the development of oral systemic drugs. The purpose of this study was to determine the content of biomolecular compounds in eggplant ethanol extract using LC-MS/MS.

2. Methods

The method of this research is experimental, which includes several steps, namely preparation of samples, preparation of hydro-alcoholic extracts, identification of chemical compounds using LC-MS/MS then predicting the toxicity of compounds identified in silico. Extract preparation was carried out at the Faculty of Pharmacy, University of North Sumatra, Identification of chemical compounds using LC-MS/MS was carried out at the National Police Laboratory Center, Toxicity Prediction Analysis of chemical compounds that had been identified was carried out at the Pharmacology Laboratory of the USU Faculty of Pharmacy. The tools used are drying cabinets, rotary evaporators, water baths, a set of instruments for determining moisture content, desiccators, balances, electric balances, blenders, microscopes, glass objects, spatulas, 100 mL beaker glass, 500 mL beaker glass, aluminum foil, paper. filter, oral sonde, syringe, hose, LC-MS/MS, centrifuge, microtube, Asus Core i5 Laptop, Ultrasonic homogenizer UP100H with MS14 Probe (hielscher). The material used in this study was purple eggplant skin, the chemical used was 96% ethanol, standard internal TMS LC-MS/MS, Bouchardat reagent, Dragendorff, Mayer, iron (III) chloride, Molisch, lead (II) acetate, sulfuric acid, hydrochloric acid, methanol, chloroform-isopropanol, Liebermann Burchard, n-hexane, toluene, chloroform, distilled water, and CMC, ABTS (sigma), PBS (Phosphate Buffer Saline), K2S2O8 (Potassium persulfate) and aquadest pro injection.

Prediction of the pharmacokinetic properties (ADMET: absorption, distribution, metabolism, excretion, and toxicity) of the identified compounds was carried out using the pkCSM online tool. First the tested compound and the reference compound are drawn as 2D molecular structures with ChemBio Ultra Image and copy to ChemBio 3D Ultra to create 3D structures, and then saved as *.sdf or *.pdb files. Second, all tested compounds and comparative compounds were translated into the SMILES format using the SMILES Translator Online Help. In SMILES format, compounds were processed using the online tool pkCSM to predict ADMET and compound toxicity. The parameters analyzed included the Lipinski rules of five calculations. Lipinski's rule of five calculations are carried out to determine the rate of absorption or permeability of a compound to the lipid bilayer in the human body. The Lipinski rule is a parameter indicating the oral bioavailability of a compound. Good bioavailability will comply with the Lipinski rule where the maximum molecular weight of a compound is 500, the logP is not more than 5, the hydrogen bond donors are less than 5, and the hydrogen bond acceptors are less than 10.

The software used in this research was ChemDraw 2016. (http: //scistore.cambridgesoft.com/) (License Code: 338-284099-4415), Openbabel GUI 2.4.1 (https://sourceforge.net), pkCSM (http://biosig.unimelb.edu.au/pkcsm).

Identification of chemical compounds from ethanol extract of eggplant shell was carried out by TSQ Exactive (Thermo) (Puslab, Biology University of Brawijaya) with mobile phase A (0.1% formic acid in water) and phase B (0.1% formic acid in acetonitrile). follow the gradient method. A flow rate of 40 μL/min was used for the Hypersil GOLD aQ column, 50 1 mm 1.9 μm, and the time for analysis was 70 minutes. The results were analyzed using Compound Discoverer software with mzCloud (Satria, 2020).

3. Results and Discussion

Phytochemical Screening of Purple Eggplant Ethanol Extract

The results of the phytochemical screening of eggplant skin ethanol extract can be seen in table 1 below:

Table 1. Phytochemical scring

No.	Classes of compounds	Result
1.	Flavonoid	+
2	Alkaloid	+
3.	Saponin	+
4.	Tannin	+
5.	Glycosides	+
6.	Steroid/triterpenoid	-

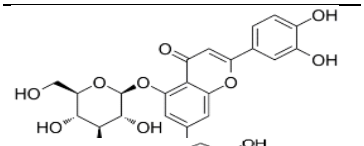
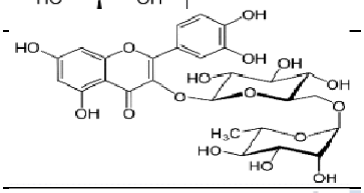
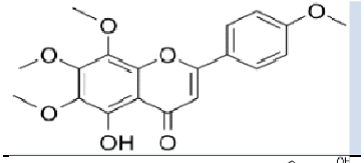
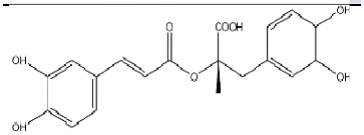
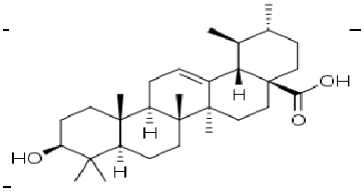
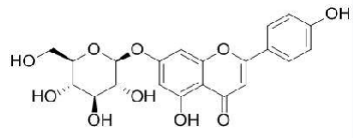
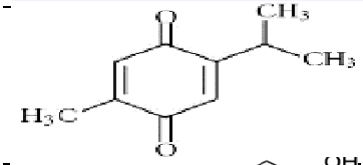
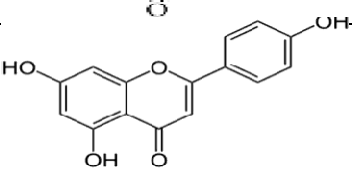
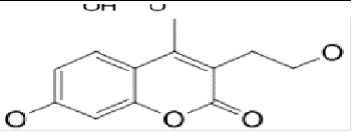
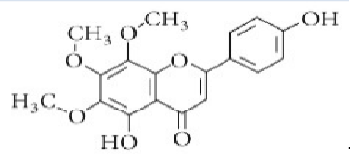
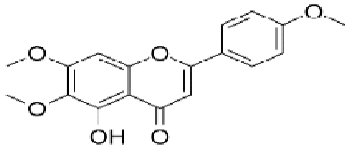
Information (+): positive, (-): negative

The results of the phytochemical screening of eggplant skin ethanol extract (EEKT) contained flavonoids, alkaloids, saponins, tannins, and glycosides.

Compounds Found in EEKT Using LC-MS/MS

The chemical compounds contained in the ethanol extract of eggplant shells can be seen in table 2. below this:

Table 2. Chemical compound

No.	Compound	MM SwissAdme	MM Pro-ToxII	MM pK- CSM	ChemicalFormula	Chemical structure
1.	Luteolin 5-O-glucosida	448.38 g/mol	437.29 g/mol	448.38 g/mol	C 21 H 20 O 11	
2	Rutin	610.52 g/mol	610.52 g/mol	610.521 g/mol	C 27 H 30 O 16	
3.	Gardenin B	358.34 g/mol	358.34 g/mol	358.346 g/mol	C 19 H 18 0 7	
4.	Rosmarinic acid	360.31 g/mol	360.34 g/mol	360.318 g/mol	C 18 H 16 O 8	
5.	Ursolic acid	456.70 g/mol	456.7 g/mol	456.711 g/mol	C 30 H 48 O 3	
6.	Cosmosiin	432.38 g/mol	421.29 g/mol	432.381 g/mol	C 21 H 20 O 10	
7.	Eugenol	164.20 g/mol	164.2 g/mol	164.204 g/mol	C 10 H 12 O 2	
8.	Apigenin	270.24 g/mol	270.24 g/mol	270.24 g/mol	C 15 H 10 O 5	
9.	Ocimarin	220.22 g/mol	220.22 g/mol	220.224 g/mol	C 12 H 12 O 4	
10.	Xanthomicrol	344.32 g/mol	344.32 g/mol	344.319 g/mol	C 18 H 16 O 7	
						

11.	Salvigenin	328.32 g/mol	328.32 g/mol	328.32 g/mol	$C_{18}H_{16}O_6$

Luteolin 5-O-glucoside is a class of flavonoids, Jung et al, (2017) reported the anti-inflammatory activity of luteolin 5-O-glucoside in lipopolysaccharide (LPS) stimulated RAW 264.7 cells. In this study, Jung et al determined the anti-inflammatory mechanism of luteolin 5-O-glucoside through inhibition of nitric oxide (NO) production in vitro and in vivo. Results revealed that dose-dependent luteolin 5-O-glucoside inhibited NO production and expression of iNOS and COX-2 in LPS-induced RAW 264.7 cells. Luteolin 5-O-glucoside also significantly inhibited NF-κB translocation, MAPK activation, and ROS generation in LPS-induced RAW 264,7 cells. In addition, the expression of Nrf-2 and HO-1 proteins was also regulated by luteolin 5-O-glucoside treatment. In addition, luteolin 5-O-glucoside inhibited carrageenan-induced rat leg edema by 65.34% and 48.31% at doses of 50 and 100 mg/kg body weight, respectively. These findings indicate the potential anti-inflammatory effect of luteolin 5-O-glucoside, especially by downregulating NF-κB and increasing the HO-1/Nrf-2 pathway (Jung et al, 2017).

The contemporary scientific community has now recognized flavonoids as a unique class of therapeutic molecules because of their diverse therapeutic properties. Of these, rutin, also known as vitamin P or rutoside, has been explored for a number of pharmacological effects, and has been investigated for its nutraceutical effects. Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonol that is widely found in plants such as passion fruit, buckwheat, tea, and apples. It is an important nutritional component of foodstuffs. Rutin, also referred to as rutoside, quercetin-3-rutinoside, and sophorin is a flavonoid glycoside consisting of the flavonol aglycone quercetin along with the disaccharide rutinose. It has demonstrated a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective, analgesic, antipyretic, antidiabetic, anticholesterolemic, antiasthmatic, anticataract, antihypertensive, and cardioprotective activity. In addition, rutin also has sedative, anticonvulsant, antidepressant activity. The anti-inflammatory activity of rutin is to suppress the activity of pro-inflammatory cytokines by reducing the production of TNF-α and IL-1β in microglia. Such an effect appears to be useful in the treatment of Alzheimer's disease as evidenced by the prevention of oligomeric –amyloid cytotoxicity (Ganeshpurkar and Saluja, 2017).

Gardenin B is beneficial in inducing cell death in human leukemia cells involving multiple caspases, by MedChemExpress, (2022) informing that Gardenin B is only traded for research, but not for sale to patients (MedChemExpress, 2022). Flavonoids have attracted great interest because of their possible anticancer activity, gardenin B was the most cytotoxic compound against HL-60 and U-937 cells, showing IC50 values between 1.6 and 3.0 M, but had no significant cytotoxic effect against mononuclear cells. quiescent or proliferating human peripheral blood. This effect on viability is accompanied by concentration- and time-dependent apoptotic appearance as evidenced by DNA fragmentation, formation of apoptotic bodies, and increased sub-G1 ratio. Comparative studies with the most studied bioflavonoid quercetin demonstrated that gardenin B is a more cytotoxic and more apoptotic inducer than quercetin. Gardenin B-induced cell death was associated with: (i) significant induction of caspase-2, -3, -8, and -9 activity; (ii) cleavage of initiator caspase (caspase-2, -8 and -9), from executioner caspase-3, and poly(ADP-ribose) polymerase; and (iii) concentration-dependent generation of reactive oxygen species. In conclusion, gardenin B-induced apoptosis is associated with activation of both extrinsic and intrinsic apoptotic pathways of cell death and occurs through a mechanism that is independent of the generation of reactive oxygen species (Cabrera et al, 2016).

Rosmarinic acid (RosA) is a water-soluble phenolic compound that is an ester of caffeic

acid and 3,4-dihydroxyphenyl lactic acid. It is found in many plants, such as the Boraginaceae and Lamiaceae families. RosA has various pharmacological effects, including anti-oxidative, anti-apoptotic, anti-tumorigenic and anti-inflammatory effects. The anti-inflammatory effect of RosA has been revealed through in vitro and in vivo studies of various inflammatory diseases such as arthritis, colitis, and atopic dermatitis. Other biological activities are antiviral, antibacterial, antioxidant, antimutagenic (Luo et al, 2020).

Ursolic acid (UA), a naturally occurring pentacyclic triterpenoid, is widely present in medicinal plants, and has attracted considerable interest in recent years due to its various pharmacological activities, such as anti-inflammatory and antioxidant. Mainly, in vitro experiments show that UA has little or no toxicity to cells. Tan et al, (2017) found that UA was effective in relieving inflammatory diseases as a natural inhibitor of phosphodiesterase-4D (PDE4D). Seow and Lau, (2017) reported that UA plays a therapeutic role in inflammatory enteritis by regulating the expression of the progesterone receptor X (PXR) gene. In addition, UA has been found to inhibit nuclear factor-κB signaling in intestinal epithelial cells and macrophages, and attenuate experimental colitis in mice (Peng et al, 2021). Seo et al, (2018) reported that UA is useful as an alternative treatment and prevention of cancer, obesity, diabetes, cardiovascular disease, brain disease, liver disease, sarcopenia, antitumor. The mechanisms underlying the anticancer effects of UA were reported as prevention of tumorigenesis inhibition, cancer cell proliferation, modulation of apoptosis, prevention of cell cycle arrest, and promotion of autophagy (Seo et al, 2018).

Cosmosiin is a class of flavonoids that are beneficial for diabetes complications through increasing adiponectin secretion, insulin-β receptor tyrosine phosphorylation, and GLUT4 translocation (ChemFaces, 2021).

Eugenol is an allylic guaiacol which is a pale-yellow oily liquid. It is one of the most important compounds present in cloves and has a pleasant, spicy, clove-like smell. Eugenol (4-allyl-2- methoxyphenol) exhibits antioxidant, antiseptic and antimicrobial properties. Eugenol is one of the reduced phenylpropanoids, the main component of clove oil (Byju's, 2022).

Apigenin, chemically known as 4′, 5, 7, -trihydroxyflavone is a yellow crystalline powder belonging to the class of flavones, which are aglycones of several naturally occurring glycosides. It is insoluble in water but soluble in organic solvents. Many pharmacological activities, including anti-inflammatory, anti-toxicant, anti-cancer, etc., are associated with apigenin. Research by Ali et al, (2017) has shown that apigenin has many molecular targets involved in inflammation. Based on in vivo, in vitro, and clinical trials studies show that apigenin is a powerful therapeutic agent for treating diseases such as rheumatoid arthritis, autoimmune disorders, Parkinson's disease, Alzheimer's disease, and various types of cancer. Delayed plasma clearance and slow decomposition in the liver enhance its systemic bioavailability and make it a powerful therapeutic agent in pharmaceutical studies (Ali et al, 2017).

Salvigenin is a natural polyphenolic compound, with neuroprotective effects. Salvigenin has antitumor cytotoxic and immunomodulatory properties. Salvigenin inhibits H2O2-induced cell apoptosis (MedChemExpress, 2022).

Ocimarin is useful for increasing dopamine levels in the frontal cortex and decreasing them in the striatum and hippocampus, increasing the selective metabolites of dopamine, and serotonin and their metabolites increasing significantly, and decreasing noradrenaline in the brain region. Ocimarin is also useful for increasing the activity of superoxide dismutase and glutathione peroxidase in the frontal cortex and striatum, so that it can be used as an antistress (PubChem, 2022).

Xanthomicrol is a trimethoxylated hydroxyflavone (trimethoxyflavone) which is a flavone substituted by methoxy groups at positions 6, 7 and 8 and hydroxy groups at positions 5 and 4'. It has a role as a potential antineoplastic agent (PubChem, 2022), antiangiogenic, anticancer (Ghazizadeh et al, 2020).

Conclusion

The results of the phytochemical screening of eggplant skin ethanol extract (EEKT) found the presence of flavonoids, alkaloids, saponins, tannins, glycosides, but steroids/triterpenoids were not found. In the LC-MS/MS examination on the EEKT, the presence of flavonoids was found; luteolin 5-O-glucoside, rutin, gardenin B, rosmarinic acid, ursolic acid, cosmosiin, eugenol, apigenin, ocimarin, xanthomicrol, salvigenin, but no kaemperol 3-O-maonylglucoside was found.

References

Ali, F., Rahul., F. Naz., S. Jyoti., Y. H. Siddique. 2017. Health functionality of apigenin: A review. *International Journal of Food Properties. 20*(6):1197- 1238.

Cabrera, J., E.Saavedra., H.del-Rosario., J.Perdomo. (2016). Gardenin B-induced cell death in human leukemia cells involves multiple caspases but is independent of the generation of reactive oxygen species. *Chemico- biological Interaction*. 256: 220-227.

Fadiani,. (2017). Efektivitas Alat "Black and White Box" Pada Uji Antidepresan Daun dan Buah Terung Ungu (Solanum melongena L) Terhadap Mencit Jantan (Mus musculus). *Jurnal Ilmiah Mahasiswa Universitas Surabaya. 6*(2): 525-538.

Handoko, L.T. (2020). Potensi Keanekaragaman Hayati Indonesia Untuk Bioprospeksi dan Bioekonomi. Lembaga Ilmu Pengetahuan Indonesia. 17 Sep 2020 http://lipi.go.id/berita/potensi-keanekaragaman-hayati-indonesia-untuk-bioprospeksi-dan-bioekonomi-/22154.

Harmita,A.A.K.,Y.Harahap.,Supandi. (2019). *Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Cetakan pertama. Penerbit PT.ISFI Penerbitan*. Jakarta Barat. Halaman: 64-85.

Ganeshpurkar,A.,A.K. Saluja. (2017). The Pharmacological Potential of Rutin. *Saudi Pharm Journal-Elsevier. 25*(2): 149–164.

Ghazizadeh, F., M.Shafiei., R.Falak., M.Panahi., NRakhshani., S.A.Ebrahimi., P.Rahi mi-Moghaddam. 2020. Xanthomicrol Exerts Antiangiogenic and Antitumor Effects in a Mouse Melanoma (B16F10) Allograft Model. Evidence-Based Complementary and Alternative Medicine-Hindawi. Volume 2022: 1-12.

Guan, L.F., H.B. Yang.,Y.C.Cai., L.X.Sun., P.W.Di.,W.H.Li.,G.X.Liu.,Y.Tang. 2019. ADMED score – A Comprehensive Scoring Function For Evaluation of Chemical Drug-Likeness. *Medchemcomm. 10*(1): 148-157.

Harmita, A.A.,Y. Harahap., Supandi. 2019. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Jakarta. P.T ISFI Penerbitan. Hal: 64-107.

Jung,H.A.,A.Roy.,O.A.Abdul.,H.R.Kim.,H.J.Park.,J.S.Choi. 2017. Luteolin 5-O-glucoside from Korean milk thistle, Cirsium maackii, exhibits anti-inflammatory activity via activation of the Nrf2/HO-1 pathway. *Natural Product Sciences. 23*(3):183-191.

Kaunda, J.S., Zhang, Y.J. 2019. The genus Solanum: an ethnopharmacological, phytochemical and biological properties review. Nat Prod Bioprospect. (2019) 9:77–137. 10.1007/s13659-019-0201-6.

Kar, S., & Leszczynski, J. (2019). Exploration of computational approaches to predict the toxicity of chemical mixtures. *Toxics*, *7*(1), 15.

Lestari, T.R.P. 2020. Penyelenggaraan Keamanan Pangan sebagai Salah Satu Upaya Perlindungan Hak Masyarakat sebagai Konsumen. *Jurnal Masalah- Masalah Sosial. 11*(1): 57-72.

Li,A.W.,X.Q.Xiang.,P.S.Ong.,M.E.Q.Ying.,N. Syn.,L.Wee.,A.P.Kumar.,W.P.Yong. ,G.Sethi.,B.C.Goh.,P.C.L.Ho.,L.Z.W ang. 2018. A Review on Liquid Chromatography-Tandem Mass Spectrometry Methods for Rapid Quantification of Oncology Drugs. *Pharmaceutics. 10*(221): 1-20.

Luo, C., Zou, L., Sun, H., Peng, J., Gao, C., Bao, L., Ji, R., Jin, Y., and Sun, S. 2020. A Review of the Anti-Inflammatory Effects of Rosmarinic Acid on Inflammatory Diseases. *Front in Pharmacology*. 11:153. doi: 10.3389/fphar.2020.00153.

Muldiana, S., Rosdiana. 2017. Respon Tanaman Terong (Solanum malongena L.) Terhadap Interval Pemberian Pupuk Organik Cair Dengan Interval Waktu Yang Berbeda. *Prosiding Seminar Nasional 2017 Fakultas Pertanian UMJ*. 8 November 2017 pp: 155-162.

Seo, D.Y., S.R.Lee., J.W.Heo., M.H.No., B.D.Rhee., K.S.Ko., H.B.Kwak., J.Han. 2018. Ursolic acid in Health and Disease. *The Korean Journal of Physiology and Pharmacology. 22*(3): 235-248.

Supandi., Yeni., F. Merdekawati. (2018). In Silico Study of Pyrazolylaminoquinazoline Toxicity by Lazar, Protox, and Admet Predictor. *Journal of Applied Pharmaceutical Science. 8*(9): 119-129.

Widyana, B.L.,P. Srianto., M.G.A. Yuliani. (2017). The Effect of Eggplant's (Solanum melongena L) Powder on Serum Level Glutamate Pyruvate Transaminase (SGPT) and Glutamate Oxaloacetate Transaminase (SGOT) of White Rat (Rattus norvegicus) Blood That Given High Fatty Diet. *Journal of Basic Medical Vaterinary. 6*(1): 30-35.

Yang,H.B.,L.X.Sun.,W.H.Li.,G.X.Liu.,Y.Tan .2018. In Silico Prediction of Chemical Toxicity for Drug Design Using Machine Learning Methods and Structural Alerts. *Frontiers in Chemistry. 6*(30): 1-12.

Zeomic. (2022). Terminology: NOEL (Non Observed Effect Level). Zeomic Sinanen Zeomic Co.,Ltd. https://www.zeomic.co.jp/en/glossar y/ antibacterial/40.