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Research Article

Potential of extract leaf cherry (*Muntingia calabura L*) on production macrophage mice *(Mus musculus)*

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ABSTRACT

The cherry plant (Muntingia calabura L.) is a shrub or small tree only up to 12 meters high, although generally only around 3-6m. The purpose of this study was to see the application of cherry leaf extract to mice infected with Salmonella typimirium. This research is a laboratory experimental study with a post test-only control group design using Balb/c mice as the research object. This research was conducted from 10 February to 15 April 2022. The extract of cherry leaves was used at the basic biology laboratory of Pattimura University. The mice used were adult male mice aged 3 months and weighing about 20 grams. Macrophage analysis was measured from the spleen of mice using the SPPS 20 One Way Anova test and then continued with the LSD follow-up test. The results of the study showed that in the control group the average weight of the spleen was 0.126 lighter because it was not infected with Salmonella typimerium compared to the treatment of cherry leaf extract infected with Salmonella typimerium) dose of 15 mg/kgbw (0.556), dose of 75 mg/kgbw (0.516) and a dose of 150 mg/kgbw (0.510). While there was no significant difference in macrophages. Salmonella typimurium infection will initiate an inflammatory response at the site of spread of the bacteria, including reaching the spleen and causing enlargement of the spleen.

Keywords: cherry, macrophages, lymphocytes.

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INTRODUCTION

The cherry (Muntingia calabura L) is a type of tree that is evergreen and can grow to a height of 5-12 m. Cherry leaves have health benefits because they contain various compounds or secondary metabolites. Cherry leaf compounds include 5-hydroxy-3,7,8-trimethoxyflavone, 3,7-dimethoxy-5-hydroflavone, and 2,4-hydroxy-3-methoxysalcone as well as derivatives of flavonoids, phenols, alkoloids and terpenoids (Ragasa, 2015). These compounds are known to be used as antiproliferative, antioxidant, anti-inflammatory, antibacterial, and cytotoxic (Zakaria, 2013). Research on the immunomodulatory activity of cherry leaf plants showed that the extract was able to increase the phagocytic activity of macrophages. NADPH oxidase will catalyze molecular oxygen into O_2- (superoxide) and be metabolized into reactive oxygen intermediated such as H_2O_2. This molecule plays a very important role in bacterial killing by macrophages of salmonella because it is bactericidal. In the face of these intracellular facultative pathogenic bacteria, T cells activate macrophages and destroy cells infected with viruses or bacteria. T cells consist of CD4+, CD8+, and NK cells. Naive T cells exposed to APC-presented MHC-bound antigens or specific cytokine stimuli will develop into CD4+ and CD8+ T cell subsets with different effector functions. CD4+ naïve T cells recognize antigen co-

presented with MHC-II by APCs and develop into subsets of Th1 or Th2 cells that are dependent on environmental cytokines. CD8+ recognizes antigens presented with MHC-I molecules. The MHC-I molecule is found in all nucleated cells of the body. The main function of CD8+ cells is to get rid of virus-infected cells and also cells infected with intracellular bacteria (Bratawidjaja K.G. 2006).

The ability of bacteria to thrive in macrophages is determined by the germ's resistance to the killing power of activated macrophages and T cells (Shaghayegh et al, 2014). In general, the function of the spleen and its response to antigens is the same as that of the lymph nodes, the most important difference is that the spleen is the site of an immune response to antigens that enter through the blood circulation, while the lymph nodes respond to antigens that enter through the lymph vessels. (Chaieb et al, 2007). Cherry leaves as a traditional medicine meet the criteria of efficacy and safety. Kersen leaf extract can be used as an immune response through the cytokine pathway. For example interleukin 12 (IL-12), and interleukin 10 (IL-10). Cytokines mediate and regulate the function of cells of innate and adaptive immunity. The immune response of experimental animals can be induced by bacterial infection. Mice immune response induction in this study was carried out with Salmonella typhimurium. Salmonella typhimurium is a facultative intracellular bacterium that will induce innate and adaptive immune responses. The innate immune response against intracellular bacteria is carried out by phagocytes and NK cells. The interactions between these cells are mediated by cytokines. The adaptive immune response against these bacteria is cell-mediated immunity, in which T cells activate phagocytes to eliminate bacteria. Salmonella typhimurium has been widely used as a model to study innate and adaptive immune responses, especially macrophage activation (Amanda L et al, 2009).

In fighting intracellular microorganisms such as salmonella, the immune response that occurs is a cellular immune response. One of the characteristics of intracellular microorganisms is their ability to live and even reproduce in phagocytes. In the cellular immune system, antigen-stimulated T cells activate macrophages to destroy phagocytized microorganisms. Macrophages carry out most of their effector functions only after they have been activated by macrophage activating factors (MAF). This activation can convert molecular oxygen into reactive oxygen intermediate (ROI) and produce Nitric Oxide (NO) which plays an important role as a cytotoxic mediator against tumor cells and intracellular microorganisms. NO produced by macrophages is the result of synthesis from L-arginine through the action of the enzyme inducible nitric oxide synthase (iNOS). iNOS can arise in response to lipopolysaccharide (LPS) and antigens, especially in combination with IFN. In addition, iNOS expression is also stimulated by cytokines (Mancinelli N.E et al, 2007). Kersen leaves can stimulate cellular immune function, namely lymphocyte proliferation and ROI production of macrophages. Research on macrophage production from cherry leaf extract is new. The results of this study can provide scientific evidence of cherry leaf extract as macrophage production.

METHODS

This research is a laboratory experimental study with a post test-only control group design using mice balb/c as the research object. Cherry leaf extract is used in the Basic Biology Laboratory of Pattimura University. This research was conducted from 10 February to 15 April 2022. The mice used were adult male aged 3 months and weighing around 20 grams. Macrophage analysis was measured from the spleen of mice using SPPS 20 with the One Way Anova program and then continued with the LSD follow-up test.

Materials and Tools

The materials used in this study were cherry leaves which were still in good condition. The chemicals used for extraction were methanol, filter paper, aluminum foil, distilled water, dragendorf, celsium sulfate, RPMI medium, FBS, PBS, penicillin-streptomicin, (Sigma Chem. CO. St. Louis. USA), trypsin (Sigma Chem . CO. St. Louis. USA). Giemsa, PMA, NBT, alcohol, Neutral red solution, glutamine, physiologically sterile Nacl, sterile aquades, NH4Cl, Salmonella typhimirium, microtubes, mice. The tools used for extraction are blenders, analytical scales, beakers, porcelain cups, funnels, spatulas, fan, gloves, masks, plastic bags, boxes, digital cameras, maceration cups, beakers, stirrers, measuring cups, funnel, Erlenmeyer, evaporatory, soxhletation, water pump, conical tube, microplate well 96, centrifuge, microscope, hemocytometer, laminar airflow, vortex, tissue, micropipette, tip.

Procedure

1. Twelve male mice weighing 20 grams were divided into 4 small groups where each group consisted of 3 mice and then kept in cages per group.
2. Balb/c were infected with 0.2 ml of Salmonella typhimurium on the 7th day after administration of the leaf extract.
3. Each group was given the same standard feed and weighed the mice with a weight of 20 grams before treatment, the first to the sixth day, they were given cherry leaf extract to increase the cellular immune response to antigens, so they are called immunomodulators. Therefore, on the 7th day, they were infected with Salmonella typhimurium and on the 11th day, they were examined due to systemic infection of mice by Salmonella typhimurium intra

peritoneally. After 3-7 days, Salmonella typhimurium infection in the liver and spleen becomes a plateau under the influence of activated macrophages.

a. Control group (K): Mice received a standard diet and aquadest/day orally for 11 days. From day 11 to 14, mice were killed as needed to check macrophage production.
b. Group 1: Mice received a standard diet and cherry leaf extract at a dose of 15 mg/kgBW orally every day for 12 days, on the 7th day infected with Salmonella typhimurium intra peritoneal. On the 12th day, mice were killed for examination.
c. Group 2: Mice received a standard diet and dosed cherry leaf extract 75 mg/kg BW orally every day for 13 days, on the 7th day infected with Salmonella typhimurium intra peritoneal. On the 13th day, the mice were killed for examination.
d. Group 3: Mice received a standard diet and cherry leaf extract at a dose of 150 mg/kgBW orally every day for 14 days, on the 7th day infected with Salmonella typhimurium intra peritoneal. On the 14th day, the mice were killed for examination.
e. Peritoneal macrophage preparation by opening the abdomen of the mice was put in 3 ml of PBS intraperitoneal then massaged slowly to get macrophage cells. Intraperitoneal fluid was taken with a syringe and put into a sterile tube, centrifuged at 500 rpm for 15 minutes at 4^{o}C twice in a row.

Macrophage examination

Examination of macrophages by NBT reduction. Macrophages are stimulated with PMA so that they secrete superoxide anions (O_2-) which will oxidize NBT to formazan precipitate (insoluble) which appears blue in color. Macrophage suspension (PEC) was cultured on a 24-well microplate, incubated CO_2 a 5% for 300 minutes, 1 l/well of complete medium was added, incubated for 2 hours. Cells were washed with RPMI twice, then 1 ml/well of complete medium was added and incubated for 24 hours. After that, 500 ml of NBT solution containing 125 ng/ml PMA was added. Incubated in a 5% CO_2 incubator for 60 minutes. Cells were washed with PBS three times, dried at room temperature. Fixation with absolute methanol for 2-3 minutes, then dried, stained with Giemsa, washed for 15 minutes with aquades and dried at room temperature.

RESULTS AND DISCUSSION

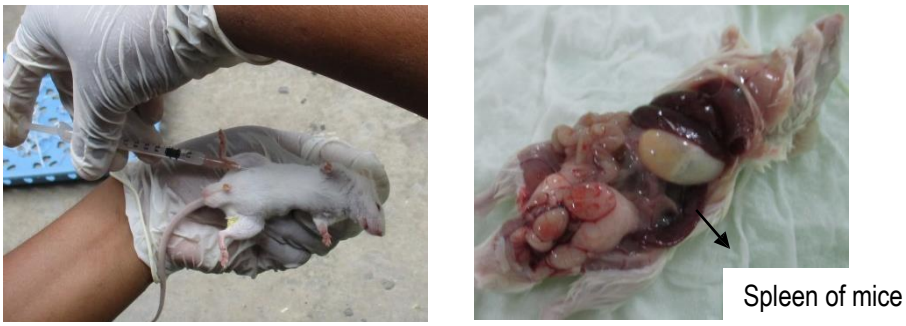
The cherry (Muntingia calabura L.) is a shrub or small tree only up to 12 meters high, although generally only about 3-6 meters. Always green and continuously flowering and fruiting throughout the year, the branches are flat, hanging at the ends to form a shady shade. Twigs with fine hairs mixed with glandular hairs, as well as leaves, leaves lying flat, alternate, leaf blade asymmetrical, egg-lanceolate, serrated edges and pointed tip 1-4 X 4-14cm on the underside of dense gray hair, stalked short (Kokasih et al. 2013). The following is a classification of cherry plants.

Picture 1. Cherry plants (Kokasih et al, 2013)

Classification of cherry plants

Kingdom : Plantae
Devisi : Magnoliophyta
Class : Magnoliopsid
Ordo : Malvales
Family : Muntingiaceae
Genus : Muntingia
Spesies : *Muntingia calabura L*

Cherry (muntingia calabura L). Is a plant that can grow and bear fruit quickly throughout the year. The fruit is round in diameter (1-1.25 cm) with a red or sometimes yellow color, the skin is thin and smooth. When eaten, this fruit is runny with a very sweet taste, has a distinctive but not sharp aroma, the seeds are very fine and yellowish in color (Trieha, 2015). Every 100g of cherry fruit contains several kinds of substances needed by the body water (77.8 grams), protein (0.384 grams), fat (1.56 grams), carbohydrates (17.9 grams), fiber (4.6 grams), ash (1.14 gram), calcium(12.6 mg), phosphorus (84 mg), iron (1.18 mg), carotene (0.19g), tianin (0.065g), ribofalin(0.037g), niacin (0.554g) and the content of vitamin C (80.5 Mg) energy value is 380KJ/100 gram (Seddiqua et al, 2010). In this study, mice were injected with salmonella tipymirium and then treated with cherry leaf extract. The control group after injection of Salmonella typimirium on the 7th day was only able to survive until the 10th day, possibly due to the Salmonella typimirium dilution being too concentrated and not given cherry leaf extract so in this study used control without Salmonella typimirium induction as a comparison. After the treatment of cherry leaf extract in the treatment group, the mice were dissected to determine the weight of the spleen as an indicator of increased proliferation. Injection of Salmonella typimirium and spleen organs of mice can be seen in the image below.



Picture 2. Injection of Salmonella typimirium and dissection of the spleen in mice

Infection of Salmonella typhimurium does not only affect innate immune cells during the initial infection. Reduction of splenic T cells during primary Salmonella typhimurium infection may be due to cell death or recruitment to the circulation and periphery. Induction of splenic T-cell release to the periphery as a result of inflammation elsewhere also contributes to T-cell loss during primary infection. However, splenic T cells were activated at the end of Salmonella typhimurium infection and IFN-γ-producing T cells increased 5 days after primary infection (Farizal J, 2013). Antibodies and T cells are both required to induce immunity against Salmonella typhimurium and it is believed that the ability to elicit antibody opsonization in response is essential for optimal protection against the bacterium (Kumar P et al, 2006). The most important surface molecule that sends costimulatory signals to T cells is CD28. CD28 is expressed on T cells and NK cells, and the ligands for CD28 and the structures associated with CTLA4 are the molecules CD80 and CD86. Both molecules are expressed on professional APCs, reduced cytokine production, and altered formation of the CD4 Th cell subset. CD28 is also important in T-B cell cooperation (Bratawidjaja KG, 2006). In response to Salmonella typhimurium, specific IgM and IgG3 were reduced and IgGl and IgG2a were absent in CD28-deficient mice. In contrast to IgM and IgG3, IgGl and IgG2a are T cell dependent, indicating that loss of CD28 expression thwarts T-B cell cooperation during infection. (Mancinelli NE et al, 2007). T cells that encounter antigens will proliferate and differentiate into antigen-specific effector cells (Kumar P et al, 2006).

T cells are required for the full expression of Salmonella typhimurium immunity to mice infection, clearance of these bacteria from tissues requires functional CD4+ T cells and the development of ThI-type T-cell immunity. CD4+ T cells play an important role in assisting B cell activation and differentiation. The function of these CD4+ T cells besides helping B cells to produce antibodies, also helps the formation of Salmonella typhimurium specific CD8+ T cells and regulates granuloma formation to limit bacterial growth (Farizal J, 2013). Cytotoxic CD8+ T cells can lyse infected cells and can produce cytokines required for phagocyte activation. When stimulated, CD4+ T cells produce IL-2 as their main lymphokine. Initially, these cells develop into cells that produce IFN-γ TNF-β and IL-2 or IL-4. IL-2 is required by cells to develop into Th1 or Th2-like cells but does not determine their differentiation. If IL-4 is also present during the early period, the resulting CD4+ T cells produce IL-4 upon stimulation. The development of IFN-γ-producing cells is inhibited by IL-4. No initial emergence of IL-4 to produce IFN-γ occurs, but it is characterized by increased IL-12 in some in vitro systems, IFN-γ reacting together with IL-12 to increase its production. Anti IFN-γ inhibits the start for IFN-γ production in vivo (Mancinelli NE et al, 2007).

The cellular immune response will be activated to eliminate infection by intra-cellular bacteria such as Salmonella typimurium, including by the lymphocyte proliferation response. The lymphocyte proliferation response is microscopically seen by the difference in the increase in the size and weight of the spleen (Farizal J, 2013). In this study there was a significant increase in spleen weight compared to the control in all groups given cherry leaf extract. In the control group the average value of spleen weight was 0.126 lighter because it was not infected with Salmonella typimerium compared to the treatment of cherry leaf extract infected with Salmonella typimerium) dose of 15 mg/kgbw (0.556), dose of 75 mg/kgbw (0.516) and dose of 150 mg /kgbw (0.510). Salmonella typimurium infection will initiate an inflammatory response at the site of spread of the bacteria, including reaching the spleen and causing enlargement of the spleen (Farizal J, 2013). The results of statistical analysis of cherry leaf extract administration can be seen in the following table.

Table 1. Statistical test of cherry leaf extract administration

Group	Dose	Sig.
Control	15 mg/kgbw	0.000*
	75 mg/kgbw	0.000*
	150 mg/kgbw	0.000*
15 mg/kgbw	75 mg/kgbw	0.12
	150 mg/kgbw	0.09
75 mg/kgbw	150 mg/kgbw	0.70

Statistical test of Anova shows (p=0.00). This administration of cherry leaf extract can affect cell proliferation by observing the weight of the spleen. The spleen is the main site of the immune response to antigens of blood origin. The weight and size of the spleen varies with life span, individuals and conditions. In a healthy adult, the spleen generally weighs about 200 grams. It is composed of a zone of T cells and a zone of B cells. It is also a filter for blood. Microbes in the blood are cleared by macrophages in the spleen. The spleen is the main site for phagocytes to feed on antibody-coated microbes. Individuals without a spleen will be susceptible to bacterial infections (Baratawidjaja, 2006). Histologically, the spleen is divided into red pulp and white pulp, the red pulp is very rich in red blood cells. The red pulp color is due to the large number of erythrocytes in the lumen of the venous sinuses and invading the surrounding splenic cords. The red pulp makes up about 80% of the entire healthy spleen and functions to filter the blood and destroy old and damaged erythrocytes. The white pulp is inside the red pulp, appears as a white nodule called the malpighian corpuscle, and consists of a small portion of lymphoid tissue (Farizal J, 2013). Antibodies are produced microscopically, the malpighian corpuscles are associated with lymphoid follicles which are rich in B cells, and the periarteolar lymphocyte sheaths contain germinal centers of T cells (De Saint Jean M et al, 2009).

Several disease conditions can cause enlargement of the size of the spleen, including hemolytic anemia, cancer and infectious diseases. To separate spleen cells for research or diagnostic purposes and other purposes,

pure lymphocyte suspension is often required, both at the population and subpopulation levels. Obtaining these lymphocytes can be obtained from the spleen tissue or directly from the peripheral blood. To separate lymphocytes from unwanted cells, the presence of marker molecules that are characteristic of the lymphocyte cell surface is used. Based on this, there are various methods of centrifugation. If it comes from spleen tissue, cell suspension in an impure PBS solution is rotated by centrifugation, to separate it from the tissue components (Farizal J, 2013). The LSD test on the administration of cherry leaf extract showed that the control group with the treatment had a significant level value. While in the treatment group there was no significant. To see the number of macrophages in the administration of cherry leaf extract can be seen in the following table.

Table 2. Analysis of macrophages

Treatment	mean ± st deviasi
15 mg/kg	5.20 ± 0.44
75 mg/kg	6.80 ± 1.78
150 mg/kg	7.00 ± 3.00

The table shows that the lowest number of macrophages was in the 15 mg/kg treatment group (5.20 ± 0.44), followed by the 75 mg/kg treatment (6.80 ± 1.78) and 150 mg/kg (7.00 ± 3.00). The results of Shapiro Wilk's statistical analysis on the number of macrophages had an abnormal data distribution. Testing data with abnormal data distribution used the Kruskal Walis test (0.253), indicating that there was no significant difference in the number of macrophages. Although there was no significant difference, there was an increase in macrophages in the administration of kesen leaf extract at a dose of 150 mg/kgbb compared to doses of 75 mg/kgbb and 15 mg/kgbb. Administration cherry leaf extract in this study can increase the number of lymphocytes and lymphoblasts significantly between treatment groups. This is because there is stimulation of cherry leaf extract compounds which are the main effector molecules in modulating lymphocyte increase and are responsible for antioxidant activity (Dibazar et al, 2014). Cherry leaf extract compounds are the main components of the role of immunomodulators and anti-inflammatories. Kersen leaf extract administration to balb/c mice can produce cytokines Th1 (IFNγ and IL-2) and Th2 (IL-4 and IL-10).

Macrophages carry out most of their effector functions only after they have been activated by bacteria, cytokines, and other stimuli known as MAF. Macrophages are long living and have few granules and release various substances such as lysozyme, complement, interferons and cytokines which contribute to nonspecific and specific defense (Baratawidjaja, 2006). Intracellular microorganisms activate NK cells either directly or through stimulation of macrophages that produce IL-12, a potent NK cell activating cytokine. Then NK cells produce IFN-2 which reactivates macrophages and increases the elimination of phagocytic bacteria. Besides IFN, IL-3 can also act as a MAF (Mancinelli NE et al, 2007). Activated macrophages will increase the number of lysosomal granules, more mitochondria and a greater capacity to phagocytize the presented particles. Fusion of the phagocytic vacuole (phagosome) with the lysosome produces the phagolysosome, where the microbial killing mechanisms are concentrated. Activated macrophages kill phagocytosed microbes by producing microbial killer molecules in phagolysosomes. This activation converts molecular oxygen into ROI which is a highly reactive oxidizing agent that destroys microbes. In addition to ROI, macrophages also produce RNI, especially NO (Mancinelli NE et al, 2007).

CONCLUSION

The administration of cherry leaf extract to mice infected with Salmonella typimirium showed a significant difference between the doses of cherry leaf extract and the weight of the spleen, whereas there was no significant increase in macrophages between doses.

REFERENCES

Baratawidjaja, K. G. 2006. Imunomogi dasar. *edisi ke-7*. Fakultas Kedokteran Universitas Indonesia.

De, Saint, Jean, M., Nakamura, T, Wang, Y., Mircheff, A, K. 2009. Suppression of lymphocyte proliferation and regulation of dendritic cell phenotype by soluble mediators from rat lacrimal epithelial cells. *J Immunol* 70; p53-62.

Farizal, J., Lisyani, B, S., Winarto. 2014. Effect of ethanol extract bidara upas tuber (merremia mammosa) to lymphocytes proliferation And ROI macrophage production. *Media medika indonesiana*.

Kresno, S, B. 2001. Imunologi. *Diagnosis dan prosedur laboratorium*. Kedokteran

Kumar, P., Singh, V, K., Singh, D, K. 2009. Kinetics of enzyme inhibition by active molluscicidal agents Ferulic acid, Umbelliferone, Eugenol and Limonene in the nervous tissue of snail *Lymnaea acuminata*. *Phytother Res*. 23: 172-177.

Mancinelli, N, E., Campisi, L., Bassand, D., Cazareth, J. 2007. *Memory CD8+ T* cells mediate antibacterial immunity via ccl3 activation of tnf/roi. *Phagocytes*.

Akter, S., Rahman, M.M., Abe, S.K. & Sultana, P. 2014, Prevalence of diabetes and prediabetes and their risk factors among bangladeshi adults*: A Nationwide Survey, Bull World Health Organ.* 92: 204–213.

Arisman. 2011. Obesitas, diabetes mellitus, dan dislipidemia*: Konsep, Teori dan Penanganan Aplikasi*, 51-53, Jakarta, EGC.

Chen, J., Lin, R., Duh, C., Huang, H. & Chen, I. 2004. Flavones and cytotoxic constituents from the stem bark of *muntingia calabura, Journal of the Chinese Chemical Society*, *51*: 665-670.

Dipiro, J. T., Talbert, R. L., Yee, G. C., Matzke, G. R., Wells, B. G. & Poey, L. M. 2008. *Pharmacotherapy: a pathophysiologic approach, chapter 77 diabetes mellitus,* 1205-1225, USA, In Mc Graw Hill Medical.

Indradevi, S., Ilavenil, S., Kaleeswaran, B., Srigopalram, S. & Ravikumar, S. 2012. Ethanolic extract of *crinum asiaticum* attenuates hyperglycemiamediated oxidative stress and protects hepatocytes in alloxan induced experimental diabetic rats, *Journal of King Saud University Science*, 24: 171–177.

Krishnaveni, Marimuthu & Ravi Dhanalakshmi. 2014. Qualitative and quantitative study of phytochemicals in *muntingia calabura* l. leaf and fruit. *Journal of Pharmaceutical Research*, 3(6): 1687-1696.

Lenzen, S. 2008. The mechanism of alloxan and sterpotozotocin induced diabetes.

Lucacinova. 2008. preventive effect of flavonoids on alloxan-induced diabetes melitus in rats. *Acta Vet, brno*, 77: 175-182.

Malviya, N., Jain, S., & Malviya. S. 2010. Review antidiabetic potential of medicinal plants. *Acta Poloniae Pharmaceutica-Drug Research*, 67(2), 113-118.

Singhe, J., Cumming, E., Manohara, G., Adeghate, E. 2011. Medicinal chemistry of the anti-diabetic effects of actions. The open momordica charantina: active constituens and modes of medicinal chemistry. Journal. 2011; 5 (2):70-77.

Sindhe, A., Yadav, D. B. & Chanrashekar, A. 2013. Antioxidant and in vivo anti-hyperglycemic activity of *muntingia calabura* leafves extracts. *Der Pharmacia Lettre,* 5 (3): 427-435.

Sridhar, M., Thirupathi, K., Chaitanya, G., Krishna, G., M. & Kumar, B. R. 2011. Antidiabetic effect of leaves of *muntingia calabura l.,* in normal and alloxan-induced diabetic rats. *Pharmacologyonline* 2: 626-632.

Surjowardojo, P., Sarwiyono, Thohari, I. & Ridhowi, A. 2014. Quantitative and qualitative phytochemicals analysis of *muntingia calabur, Journal of Biology, Agriculture and Healthcare*, 4(16): 84-89.

Szkudelski, T. 2001. The mechanism of alloxan and streptozotocin action in b cells of the rat pancreas. *Physiological Research,* 50: 536-546.

Tepas, A. R., Sakarkar, D. M. & Kakde, R. B. 2008. Flavonoids as nutraccuticlas: a. review, *Tropical journal Of Pharmaceutical Research,* 7(3): 1089-1099.

Zakaria, Z.A., Mat, A., Mastura, M., Mat, S.H., Mohamed, A.M., Moch, Jamil, N. S., Roflee, M.S., Sulaiman, M.R., Abdullah, F.C. 2007. The antinocieptive action of aqueous extract from muntigia calabura leaves: the role of opioid receptors. *Med Princ Pracyt.* 16:130-136.

Siddiqua, A., Premakumari, K. B. M., Sultana, R.,Vithya. 2010. *Antioxidant activity and estimation of total phenolic contentn of muntingia calabura by colorimetry. International journal of ChemTech Research,* 2(1): 205-208.