IN VITRO ANTI-INFLAMMATORY ACTIVITY TEST OF TINOCRISPOSIDE AND FREEZE-DRIED AQUEOUS EXTRACT OF TINOSPORA CRISPA STEMS ON HUMAN RED BLOOD CELL BY INCREASING MEMBRANE STABILITY EXPERIMENT

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ABSTRACT

Objective: This study was aimed to evaluate the anti-inflammatory effect of isolated tinocrisposide and freeze-dried aqueous extract of Tinospora crispa stems on human red blood cell (HRBC) by increasing membrane stability in vitro models.

Methods: Anti-inflammatory effect of tinocrisposide and FDAETCS was evaluated by in vitro HRBC membrane stabilization method. The study was separated into two steps which were a hemolytic and a membrane stabilization experiment. The hemoglobin that was released throughout the damaged erythrocytes membrane was then quantified at the wavelength of (λ) 560 nm.

Results: The hemoglobin in the HRBC supernatant that treated with tincrisposide at concentration of 100, 200, 400, 600, 800, and 1000 μg/ml showed an absorbance at λ 560 nm of 0.060, 0.061, 0.071, 0.072, 0.075, and 0.0793, respectively, and the calculated hemolysis percentage was 0.032, 0.097, 1.203, 1.236, 1.641, and 2.079%, respectively. We found a linear correlation between concentration and hemolytic activity of tinocrisposide, with regression equation, y=0.0023x−0.1312 (r=0.929). Meanwhile, the positive control ibuprofen 25 μg/ml only exerted the membrane stability of 5.620%. It was found a linear correlation between tinocrisposide concentration and its hemolytic percentage showed a parabolic relationship, which gave a maximum at a concentration of the extract of 400 mg/ml with membrane stabilizing of 10.944%.

Conclusion: It can be concluded that tinocrisposide and FDAETCS have an anti-inflammatory activity by increase the membrane stability of lysosome cell that has equal physiological properties with erythrocytes membrane cell and it has no hemolytic activity.

Keywords: Anti-inflammatory, Hemolytic, Human red blood cell, Membrane stabilization, Tinocrisposide, Tinospora crispa.

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INTRODUCTION

Tinospora crispa is a climber plant widely distributed from the Southwestern part of China to Southeast Asia including Indonesia, Malaysia, Vietnam, Thailand, and India. It naturally occurs in primary rainforests or mixed deciduous forests up to 1000 m above sea level [1]. T. crispa is well known as a bitter medicinal plant called Brotowalli, Akar Seruntun, or Andawali in Indonesia, Akar Patawali in Malaysia, and Makabuhay in Philippine. A decoction of the stems has been traditionally used for the treatment of goit, diabetes, fever, wound healing, and hypertension, and scientifically reported as an analgesic and anti-inflammatory agents [2].

T. crispa has been demonstrated to possess antioxidant, antiprofiterative [3], antinociceptive [4], anti-inflammatory [5], antibacterial [6], antifilarial, antimalarial, antipyretic [7], and antihyperglycemic activities [8]. In our previous study from methanol extract of dried pulverized T. crispa stems, tinocrisposide (C_{18}H_{26}O_{5}) has been isolated, a furanoditerpene glycoside with a very bitter taste (Fig. 1) [9]. Furthermore, in our previous research, various concentrations of tinocrisposide were tested against the LPS-stimulated RAW 264.7 cells, and the results showed the decrease of NO level production in concentration activity-dependent manner, with half-maximal inhibition concentration of 46.92 μM. Thus, it can be developed as anti-inflammatory candidate drug because NO is a reactive nitrogen species which is produced by NO synthase [10]. S. Chanda and A.R. Juvkar have tested in vitro anti-inflammatory activity of various concentrations of syringic acid by protein denaturation and human red blood cell (HRBC) membrane stabilization assay. The reference drugs used in experiments were aspirin and diclofenac sodium [11]. Meanwhile, Shamsi et al. studied anti-inflammatory activity of Joshanda, a polyherbal Unani formulation consists of seven plant ingredients by albumin denaturation inhibition experiment used aspirin as a reference compound [12]. In the current research, we are trying to prove the anti-inflammatory activity of tincrisposide that can exert the increasing of HRBC membrane stability.

Inflammation is a normal protective response to tissue injury caused by physical trauma, destructive chemicals, or microbiological
agents [13]. It is characterized by an alteration of immune system [14]. The inflammatory process is very closely related to wound healing. Inflammation and repair are a continuous process in wound healing involving inflammatory cells and enzymes. Cyclooxygenase (COX) family enzyme – COX-1 and COX-2 – is widely studied in inflammation disorder and critically responsible in the illness incident [15].

The inflammation, in general, is treated by nonsteroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs which are useful for reducing swelling and pain from inflammation. NSAIDs inhibit the COX enzyme which caused the conversion of arachidonic acid to prostaglandin become disrupted [16]. These drugs have a risk of gastrointestinal toxicity, cardiac toxicity, and others for prolonged use [17] so that traditional medicine or treatment from nature can be used as an alternative.

MATERIALS AND METHODS

Plant materials

*T. crispa* was collected from Padang, Indonesia. The plant specimens were properly identified and authenticated by Dr. Nurainas at the Department of Biology, Faculty of Science, Andalas University.

Instruments

UV lamp (Merck, Germany), pH meter, column chromatography (Pyrex), micropipette (Socorex), rotary evaporator (Büchi), UV-visible spectrophotometer (Hitachi U-2910), Fourier-transform infrared spectroscopy (FTIR) Spectrum One (PerkinElmer), freeze dryer (Christ, Germany), water bath (Eyela SB-1000), centrifuge (Universal 32 R, Hettich Zentrifugen, USA), incubator (Thermo Scientific), microplate reader (BioRad × Mark’), Falcon tube, Eppendorf tube, 96-well plate (Iwaki), and EDTA vacuum tube were used.

Materials

*T. crispa* stem, dichloromethane (DCM), methanol pro analysis (Merck), ethyl acetate (Merck), hexane (Merck), acetic acid (Merck), silica gel 60 F_{254} (Merck, Germany), TLC sodium dodecyl sulfate (Sigma-Aldrich), isotonic NaCl solution (PT Widatna Bhakti), dimethylsulfoxide (Thermo Fisher Scientific, USA), distilled water, ibuprofen, and human blood were used.

Freeze-dried aqueous extract *Tinospora crispa* stems (FDAETPS) preparation

Freshly collected stems were cut, shade-dried for 2–3 days, and ground into powder. It was then gently boiled with distilled water (1:10) for 30 min, filtered, and allowed to cool at room temperature. The filtrate was then freeze-dried at −50°C and 0.1 atm for 9 h, and stored at 4°C before use.

Tinocrisposide isolation

The stems were cut, shade-dried, ground into powder, and macerated using methanol 3 times for 3 days each at room temperature. The extracts then evaporated under reduced pressure using a rotary evaporator to yield crude extracts. The extracts were dissolved in 5% acetic acid and allowed overnight, decanted, and then partitioned, sequentially, with hexane and DCM. Each fraction was evaporated and dried to yield *n*-hexane, DCM fractions. The DCM fractions then subjected into column chromatography over silica gel 60 and eluted using step gradient elution (3–8%) system with the mixture of DCM and methanol (3–8% methanol) which were combined on the basis of thin-layer chromatography (TLC) evaluation. TLC was carried out using the mixture of MeOH: DCM (1:9) as a solvent system and the fractions with same RF values were combined and further purified by column chromatography to obtain tinocrisposide as white amorphous powder [16]. The powder then identified using TLC, ultraviolet spectrophotometry, and FTIR spectroscopy.

In vitro hemolytic test

Blood from the healthy human volunteer who had not taken any NSAIDs for 2 weeks before the experiment was collected using vuvacutainer tube EDTA, mixed with isotonic NaCl solution, and centrifuged at 3000 rpm for 5 min. The packed cells were washed with isosolaine and a 5% HRBC suspension was made. Tinocrisposide (100, 200, 400, 600, 800, and 1000 µg/ml) and FDAETCS (100, 200, 400, 600, and 800 µg/ml) were prepared in a distilled water. 0.5 ml of sample was mixed with 0.5 ml HRBC suspension in Eppendorf tubes and incubated at 37°C for 1 h [18]. After incubation, the tubes were centrifuged at 1000 rpm for 5 min and the supernatant was transferred into 96-well plate. The absorbance of hemoglobin in the supernatant was measured at a wavelength, λ of 560 nm. As a positive

[Table 1: Absorbance value of tinocrisposide and FDAETCS on hemolytic activity test]

| Sample                | Concentration (µg/ml) | Absorbance 1 | Absorbance 2 | Absorbance 3 | Mean±SD       |
|-----------------------|-----------------------|--------------|--------------|--------------|--------------|
| Tinocrisposide        | 100                   | 0.058        | 0.059        | 0.063        | 0.060±0.002  |
|                       | 200                   | 0.061        | 0.064        | 0.058        | 0.061±0.003  |
|                       | 400                   | 0.061        | 0.071        | 0.074        | 0.071±0.002  |
|                       | 600                   | 0.068        | 0.079        | 0.069        | 0.072±0.007  |
|                       | 800                   | 0.073        | 0.074        | 0.079        | 0.075±0.003  |
|                       | 1000                  | 0.074        | 0.082        | 0.082        | 0.079±0.005  |
| FDAETCS               | 100                   | 0.063        | 0.062        | 0.065        | 0.063±0.002  |
|                       | 200                   | 0.064        | 0.062        | 0.066        | 0.064±0.002  |
|                       | 400                   | 0.066        | 0.070        | 0.063        | 0.066±0.004  |
|                       | 600                   | 0.063        | 0.067        | 0.071        | 0.067±0.002  |
|                       | 800                   | 0.079        | 0.077        | 0.076        | 0.077±0.002  |
| Negative control (0.9% NaCl) | 0.060        | 0.061        | 0.060        | 0.060±0.001  |
| Positive control (5% SDS) | 0.939        | 1.007        | 0.977        | 0.974±0.034  |
control, 5% SDS was employed and an isotonic NaCl solution was used as a negative control. Measurements were replicated 3 times for each concentration [19]. The hemolytic activity was calculated with the following equation:

\[
\text{% Hemolytic} = \frac{A_1 - A_2}{A_3 - A_2} \times 100\%
\]

In vitro anti-inflammatory test

This study was conducted using the HRBC membrane stabilization method. Blood from the healthy human volunteer who had not taken any NSAIDs for 2 weeks before the experiment was collected using vacutainer tube EDTA. Blood samples were mixed with isotonic NaCl solution and centrifuged at 3000 rpm for 5 min. The packed cells were washed with isosaline and a 10% HRBC suspension was prepared [20]. Tinocrisposide (100, 200, 400, 600, 800, and 1000 µg/ml) and freeze-dried aqueous extract (2000, 1000, 800, 600, 400, and 100 µg/ml) were dissolved in a distilled water. Reaction mixtures consisted of 1 ml test sample, 0.5 ml of HRBC suspension, and 1.5 ml distilled water. It was incubated at 37°C for 30 min and centrifuged at 3000 rpm. The hemoglobin content of the supernatant solution was measured spectrophotometrically at a wavelength (λ) of 560 nm. Measurements were replicated 3 times for each concentration. Ibuprofen that used as a standard and a control was made by substituting the test samples with isosaline [21]. The percentage of HRBC membrane stabilization was calculated using the following equation:

\[
\% \text{ Stability} = 100 \times \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \%
\]

Table 2: Hemolytic activity percentage of tinocrisposide and FDAETCS on HRBC

| Sample    | Concentration (µg/ml) | Hemolytic activity (%) |
|-----------|-----------------------|------------------------|
| Tinocrisposide | 100         | 0.032                  |
|           | 200         | 0.097                  |
|           | 400         | 1.203                  |
|           | 600         | 1.236                  |
|           | 800         | 1.641                  |
|           | 1000        | 2.079                  |
| FDAETCS   | 100         | 0.347                  |
|           | 200         | 0.473                  |
|           | 400         | 0.693                  |
|           | 600         | 0.992                  |
|           | 800         | 1.896                  |
| % SDS     | 5          | 100                    |

HRBC: Human red blood cell

Table 3: Absorbance value of tinocrisposide and FDAETCS on HRBC membrane stabilization method

| Sample      | Concentration (µg/ml) | Absorbance 1 | Absorbance 2 | Absorbance 3 | Mean±SD 1 | Mean±SD 2 | Mean±SD 3 |
|-------------|-----------------------|--------------|--------------|--------------|-----------|-----------|-----------|
| Tinocrisposide | 100                  | 0.817        | 0.819        | 0.817        | 0.818±0.001 | 0.836±0.039 | 0.812±0.021 |
|             | 200                  | 0.789        | 0.798        | 0.838        | 0.808±0.033 | 0.821±0.044 | 0.885±0.021 |
|             | 400                  | 0.777        | 0.820        | 0.798        | 0.798±0.022 | 0.845±0.039 | 0.786±0.021 |
|             | 600                  | 0.803        | 0.780        | 0.785        | 0.789±0.012 | 0.852±0.044 | 0.786±0.021 |
|             | 800                  | 0.741        | 0.788        | 0.791        | 0.773±0.028 | 0.870±0.054 | 0.792±0.014 |
|             | 1000                 | 0.734        | 0.752        | 0.796        | 0.761±0.032 | 0.852±0.056 | 0.870±0.021 |
| FDAETCS     | 100                  | 0.799        | 0.800        | 0.807        | 0.802±0.004 | 0.852±0.039 | 0.845±0.021 |
|             | 200                  | 0.781        | 0.792        | 0.808        | 0.794±0.014 | 0.870±0.056 | 0.870±0.021 |
|             | 400                  | 0.784        | 0.789        | 0.804        | 0.777±0.010 | 0.852±0.044 | 0.792±0.014 |
|             | 600                  | 0.724        | 0.795        | 0.855        | 0.791±0.066 | 0.870±0.056 | 0.870±0.021 |
| Ibuprofen   | 6.25                 | 0.841        | 0.812        | 0.852        | 0.835±0.021 | 0.852±0.039 | 0.852±0.021 |
|             | 12.5                 | 0.836        | 0.870        | 0.792        | 0.832±0.039 | 0.870±0.045 | 0.852±0.021 |
|             | 25                   | 0.821        | 0.852        | 0.776        | 0.816±0.038 | 0.865±0.045 | 0.852±0.021 |
| Control     |                       | 0.815        | 0.901        | 0.879        | 0.865±0.045 | 0.852±0.039 | 0.852±0.021 |

Fig. 2: Diagram of hemolytic activity percentage of tinocrisposide and FDAETCS on HRBC
RESULTS AND DISCUSSION

In vitro hemolytic test

The hemoglobin in the HBRC supernatant that treated with tinocrisposide at concentration of 100, 200, 400, 600, 800, and 1000 μg/ml showed an absorbance of 0.060, 0.061, 0.071, 0.072, 0.075, and 0.0793, respectively. Meanwhile, the HBRC supernatant that treated with FDAETCS at concentration of 100, 200, 400, 600, 800, 1000, and 2000 μg/ml showed an absorbance of 0.063, 0.064, 0.066, 0.067, and 0.077, respectively (Table 1).

The percentage of calculated hemolytic activity of various concentrations of tinocrisposide and FDAETCS on HRBC is presented in Table 2.

Data in Table 2 showed that hemolytic activity percentage of tinocrisposide in the concentration of 100, 200, 400, 600, 800, and 1000 μg/ml on HRBC was 0.032, 0.097, 1.203, 1.236, 1.641, and 2.079%, respectively. We found a linear correlation between tinocrisposide concentration and hemolytic activity percentage, with regression equation, \( y = 0.0023x - 0.1312 \), and coefficient correlation, \( R = 0.929 \). The same condition occurs in the FDAETCS that at the concentration of 100, 200, 400, 600, and 800 μg/ml showed the hemolytic percentage of 0.347, 0.473, 0.693, 0.992, and 1.896%, respectively. It also gave a linear correlation between FDAETCS concentration and hemolytic activity percentage, with regression equation, \( y = 0.0072x + 4.8312 \), and coefficient correlation, \( R = 0.9932 \).

Table 4: Membrane stability percentage of various concentrations of tinocrisposide and FDAETCS

| Sample      | Concentrations (μg/ml) | Membrane stability (%) |
|-------------|------------------------|-------------------------|
| Tinocrisposide | 100                    | 5.437                   |
|             | 200                    | 6.533                   |
|             | 400                    | 7.707                   |
|             | 600                    | 8.748                   |
|             | 800                    | 10.597                  |
|             | 1000                   | 12.100                  |
| FDAETCS     | 100                    | 7.283                   |
|             | 200                    | 8.208                   |
|             | 400                    | 10.944                  |
|             | 600                    | 8.555                   |
|             | 800                    | 8.401                   |
| Ibuprofen   | 25                     | 5.620                   |

Fig. 2 showed a clear comparison of hemolytic activity between tinocrisposide and FDAETCS in various concentrations. Hemolytic percentage with a value of <10% was interpreted as non-hemolytic, and a value of >25% was considered showing hemolytic effect. From the experiments, it is showed that tinocrisposide and FDAETCS have no hemolytic effect with a percentage value of <10% and no toxicity on HRBC so that this compound can later be used for drug formulations. Erythrocyte membrane is analogous to the lysosomal membrane, so compounds that caused a damage to erythrocyte membrane will also damage lysosomal membrane [22].

In vitro anti-inflammatory test

Tinocrisposide and FDAETCS were tested if they are able to prevent water-induced lysis of red blood cells using membrane stabilization assay. The result of the HRBC membrane stabilization test is shown in Table 3.

Table 3 showed that by treating tinocrisposide in the concentration of 100, 200, 400, 800, and 100 μg/ml on HRBC membrane stabilization experiment will give the absorption of hemoglobin at wavelength (λ) 560 nm by 0.818, 0.808, 0.798, 0.789, and 0.761, respectively, with stability membrane by 5.437, 6.533, 7.707, 8.748, 10.597, and 12.100%, respectively. Meanwhile, the positive control ibuprofen 25 μg/ml only exerted the membrane stability of 5.620% (Table 4). We found a linear correlation between tinocrisposide concentration and membrane stability percentage, with the regression equation, \( y = 0.0072x + 4.8312 \), and the coefficient correlation, \( R = 0.9932 \) (Fig. 4).

Data in Table 3 showed that by treating tinocrisposide in the concentration of 100, 200, 400, 800, and 1000 μg/ml on HRBC membrane stabilization experiment will give the absorption of hemoglobin at wavelength, \( \lambda \) 560 nm by 0.802, 0.808, 0.798, 0.797, and 0.761, respectively, with stability membrane by 5.437, 6.533, 7.707, 8.748, 10.597, and 12.100%, respectively. Meanwhile, the positive control ibuprofen 25 μg/ml only exerted the membrane stability of 5.620%. This phenomenon is assumed because the extract also contains other compounds that can cause a damage to erythrocyte membrane. It can be seen that the relationship between FDAETCS and its membrane stability percentage is parabolic, which gave a maximum at a concentration of the extract of 400 mg/ml with membrane stabilizing of 10.944%. This phenomenon is assumed because the extract also contains other compounds that can cause inflammatory or hemolysis of membrane erythrocytes. Tinocrisposide at the concentration of 100 μg/ml had HRBC membrane stability of 5.437% that close to membrane stability of the positive control, ibuprofen at the concentration of 25 μg/ml that revealed membrane stability of 5.620%. It means at those concentrations the efficacy of tinocrisposide was 96.74% of Ibuprofen. If membrane stability of tinocrisposide compared to FDAETCS membrane stability, it can be seen an interesting phenomenon, namely, FDAETCS in the level concentration of 100–400 μg/ml showed higher efficacy than tinocrisposide, but in the higher concentration, tinocrisposide revealed evidently higher efficacy than FDAETCS did (Table 4 and Fig. 3).
Anti-inflammatory agents control the biochemical processes involved during the inflammatory response by stabilizing the membranes of lysosomes [18]. The erythrocyte membrane is analogous to the lysosomal membrane, and its stabilization implies that tinocrisposide and FDAETPS may as well stabilize lysosomal membranes [23]. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further inflammation and damage on extracellular release [24].

In this study, distilled water was used as an inducer that triggers hemolysis that can cause the release of inflammatory mediators. By adding 1.5 ml of distilled water into the well plate, it will decrease an osmotic pressure solution outside of the erythrocyte cell, while in the cell, it remains equivalent to 0.9% NaCl solution. Then, the water from outside of the cell will move into the cell, across the erythrocytes cell membrane which functions as a semipermeable membrane, this causes the swelling of the cell, and eventually rupture and hemolysis. In human body, an inflammatory mediator actually plays an important role in preventing the spread of infection and is also needed to repair tissue damage [18]. However, excessive inflammatory mediators can cause tissue damage and chronic inflammation [25].

CONCLUSION
It can be concluded that tinocrisposide and FDAETPS stem have an anti-inflammatory activity by increase the membrane stability of lysosome cell that has equal physiological properties with erythrocytes membrane cell and it has no hemolytic activity; therefore, tinocrisposide can be promoted and developed as a new anti-inflammatory drug candidate.

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AUTHORS’ CONTRIBUTIONS
Conception and design of study: A.Z. Adnan, I.R. Sudji, D.I. Rosema, and M.D. Novida. Acquisition of data: M.D. Novida and H.A. Ali. Analysis and interpretation of data: A.Z. Adnan, M.D. Novida, D.I. Rosema, and A. Fauzana. Drafting the manuscript: F. Armin, M.D. Novida, and A. Fauzana. Revising the manuscript critically for important intellectual content: A.Z. Adnan and I.R. Sudji.

CONFLICTS OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES
1. Dweck AC, Cavin JP, Andawali (Tinospora crispa): A review. Front Pharmacol 2006;7:33-9.
2. Abu MN, Mohd Salleh AM, Mohd Radzaman NH. Insulin sensitivity enhancement of the mixture of Tinospora crispa and gelam (Melaleuca cajuputi) honey and its antiproliferative activity on hepatocellular carcinoma, HepG2: A preliminary study. J Med Res Dev 2013;2:48-54.
3. Zulkhairi A Jr., Abdah MA, Kamal NH, Nursakinah I, Moklas MA, Hasnah B, et al. Biological properties of Tinospora crispa (Akar Patawali) and its antiproliferative activities on selected human cancer cell lines. Malays J Nutr 2008;14:173-87.
4. Sulaiman MR, Zakaria ZA, Liban R. Antiinociceptive and antiinflammatory activities of Tinospora crispa in various animal models. Int J Topical Med 2008;3:66-9.
5. Yokozawa T, Wang TS, Chen CP, Hattori M. Inhibition of nitric oxide release by an aqueous extract of Tinospora tuberculata. Phytother Res 2000;14:51-3.
6. Zakaria ZA, Mat Jais AM, Henie EF, Zaiton H, Somchit MN, Sulaiman MR, et al. The in vitro antibacterial activity of Tinospora crispa extracts. J Biol Sci 2006;6:398-401.
7. Kongkathip N, Dhunmee K, Hipol RL, Hipol RM, Kongkathip B, Chawanararaset K, Sangechomkae P, Hathakiphapanichakul S. Study on cardiac contractility of cycloexedan and cycloexedanol isolated from Tinospora crispa. J Ethnopharmacol 2002;83:95-9.
8. Noor H, Ashcroft SJ. Pharmacological characterisation of the antiinflammation activity of tinocrisposide by inhibiting nitric oxide production in lipopolysaccharides-stimulated raw 264.7 cells. Asian J Pharm Clin Res 2018;11:149-53.
9. Chandra S, Juevkar AR. In vitro anti-inflammatory activity of syruping acid lines. Int J Pharm Pharm Sci 2018;12:173-87.
10. Shamsi TN, Parveen R, Sajida A, Ahmad A, Fatima S. Assessing the therapeutic role of Joshanda: Phytochemical, antioxidant, anti-inflammatory and antimicrobial activities. Int J Pharm Pharm Sci 2018;10:122-8.
11. Nathan C. Points of control in inflammation. Nature 2002;420:846-52.
12. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860-7.
13. Chen C. COX-2’s new role in inflammation. Nat Chem Biol 2010;6:401-2.
14. Katzung BG. Basic and Clinical Pharmacology: 10th ed. San Francisco, USA: McGraw Hill Lange; 2006.
15. Katzung BG. Basic and Clinical Pharmacology. 12th ed. New York: McGraw Hill Lange; 2012.
16. Kumar V, Bhat ZA, Kumar D, Khan NA, Chashoo IA. Evaluation of antiinflammatory potential of leaf extracts of Skimmia anquetilia. Asian Pac J Trop Biomed 2012;2:627-30.
17. Minegishi T, Nakamura K, Yamashita S, Ikeda S, Kobure K. Regulation of human luteinizing hormone receptor in the ovary. Reprod Med Biol 2008;7:11-6.
18. Saleem TK, Azeeem AK, Dilip C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf extracts of Gendarussa vulgaris var. curvata. Int J Trop Biomed 2011;1:147-9.
19. Sudji IR, Suburjai Y, Frenkel N, García-Sáez AJ, Wink M. Membrane disruption caused by the steroid saponin digetinon is related to the presence of cholesterol. Molecules 2011;5:20:20146-60.
20. Hipol RL, Caruaga MF, Hipol RM. Anti-inflammatory activities of the aqueous extract of the stem of Tinospora crispa (Family Menispermaceae). J Nat Stud 2012;11:88-95.
21. Kumar P, Arora S, Yadav YC. Anti-inflammatory activity of coumarin and steroidal fractions from leaves of Moringa oleifera. Int J Drug Discov Med Res 2012;1:22-5.
22. 22. Hipol RL, Caruaga MF, Hipol RM. Anti-inflammatory activities of the aqueous extract of the stem of Tinospora crispa (Family Menispermaceae). J Nat Stud 2012;11:88-95.
23. Kumar P, Arora S, Yadav YC. Anti-inflammatory activity of coumarin and steroidal fractions from leaves of Moringa oleifera. Int J Drug Discov Med Res 2012;1:22-5.
24. Bakkkal F, Averbek S, Averbek D, Idoamoar M. Biological effects of essential oils – a review. Food Chem Toxicol 2008;46:446-75.
25. Serhan CN. System approach to inflammation resolution: Identification of novel anti-inflammatory and pro-resolving mediators. J Thromb Haemost 2009;7 Suppl 1:44-8.