Sys Rev Pharm 2020;11(7):65-70
A multifaceted review journal in the field of pharmacy

Antibacterial and Anti-inflammatory Activities of Ethanol Extract Obtained from The Hooks of *Uncaria tomentosa* (Wild. Ex Schult) DC Originated Kalimantan, Indonesia

Warsidah[1]*, Dzul Fadly[2], Bohari[3]

[1]Department of Marine Science, Faculty of Math and Natural Science, Tanjungpura University, Pontianak, Indonesia
[2]Department of Food Technology, Faculty of Agriculture, Tanjungpura University, Pontianak, Indonesia
[3]Department of Nutrition, Faculty of Public Health, Tadulako University, City of Palu, Indonesia

***Corresponding author: Warsidah**
Department of Marine Science, Faculty of Math and Natural Science, Tanjungpura University, Pontianak, Indonesia. Email: warsidah@fmipa.untan.ac.id

ABSTRACT
Uncaria tomentosa is one natural medicinal plant. The plant parts commonly harvested and utilized are the leaves and twigs. This study aimed to find out the benefit of the hook of *U. tomentosa* regarding the antibacterial and anti-inflammatory produces by phytochemical content. It was a Laboratory-based study. The primary material was the hooks of *U. tomentosa* (Wild. Ex Schult) DC collected from Kapuas Hulu, West Kalimantan, Indonesia, and extracted by Ethanol and Methanol maceration. The antibacterial was determined against *Staphylococcus aureus* and *Salmonella Thypi*; measured the transparent zone after 24 H incubation at 37 ºC. The anti-inflammatory activity was identified on the stability of red blood cells (RBCs) membrane by determining the percentage of hemolysis inhibition. The observation showed that ethanol extract had better phytochemical substances compared to methanol extract, and became a selected sample intended for antibacterial and anti-inflammatory identification. The higher concentration of ethanol extract led to an increased in bacterial growth inhibition. The ethanol extract showed a better impact against RBCs stability at level ≥ 50 ppm compared to 100 ppm aspirin with the EC_{50} was 5.21 ppm. The result indicates that ethanol extract derived from the hooks of *Uncaria tomentosa* (Wild. Ex Schult) DC origin Kalimantan, Indonesia provides the antibacterial and anti-inflammatory at once.

Keywords: Uncaria tomentosa, antibacterial, phytochemicals, anti-inflammatory, red blood cells

Correspondence:
Warsidah[1]*,
Department of Marine Science, Faculty of Math and Natural Science, Tanjungpura University, Pontianak, Indonesia.
Email: warsidah@fmipa.untan.ac.id

INTRODUCTION
Uncaria tomentosa is known well as the cat's claw. This species found in tropical regions, such as Borneo, Southeast Asia. It usually used as one of the traditional medicinal plants and now already commercialized (Valerio and Gonzales, 2005). Commonly, people take the benefit of the cat's claw by the bark and root; also, some utilize the leaves for therapeutic natural materials (Sandoval et al., 2002). People used this liana for treating some diseases associated with inflammation, such as abscesses, infection, fever, wounds (Batiha et al., 2020; Deharo et al., 2004). Besides, it has also been used for non-communicable diseases, i.e., cancer, osteoarthritis, and diabetes mellitus (de Paula et al., 2014; Zhang et al., 2015). These therapeutic effects are caused by some biological substances it has. About 50 bio-compounds identified and successfully isolated from this plant, such as flavalignans, flavan-3-ols monomers, pentacyclic alkaloids, hydroxybenzoic acids, hydroxycinnamic acids, procyanidin dimers and trimmers, and propelargonidin dimers tetracyclic (Navarro Hoyos et al., 2015). Several also isolated, particularly from the leaves, such as carboxyl alkyl esters, Pentacyclic oxindole alkaloid, proanthocyanidins, and catechin monomers (Kośmider et al., 2017).

Uncaria resembles a scandent shrub which used its hooks to climb (Turner, 2018). The hook or the thorn of the stem looks like the claw. Therefore, it's called cat's claw. The hooks or some time called the claws of *U. tomentosa* mainly originate from Kalimantan (Borneo), Southeast Asia, and have not been investigated yet. The purpose of the study was to investigate phytochemical properties, antibacterial, and anti-inflammatory of ethanol extract obtained from the hook of *Uncaria tomentosa* of Kalimantan, Indonesia.

MATERIALS AND METHODS
Collection and Identification of Plants
The primary sample in this study was the hooks of *U. tomentosa* (Wild. Ex Schult) DC gathered from Kapuas Hulu, West Kalimantan (Borneo), Indonesia. The plant identification was executed in the Laboratory of Biology, Department of Biology, Faculty of Math and Natural Science, Tanjungpura University, Pontianak – Indonesia. Preparation of plant extracts, phytochemical analysis, antibacterial determination, and anti-inflammatory activity had been conducted in the Laboratory of Chemistry, Department of Chemistry, Faculty of Math and Natural Science, Tanjungpura University, Pontianak, Indonesia.

Preparation of Plant Extract
After cleaning and sortation, the hooks of *U. tomentosa* were dried through air-dried at room temperature. Dried hooks were crushed to obtain the powder before subjected to the maceration process using ethanol and methanol. After maceration, filtration was done by using the filter paper of Whatman no.1. The filtrate obtained was subjected to a rotary vacuum evaporator to evaporate the residue of the solvent. Then, the extract stored in the freezer for the next analysis.

Analysis of Phytochemicals

Phytochemical screening was determined qualitatively by using colors/foam generated chemical reagent in the extracts from the hooks of *U. tomentosa* (Masriani et al., 2020). The screening was including alkaloid, flavonoid, steroid, and saponin. In alkaloid identification, each extract dissolved in dilute Hydrochloric acid and filtered. The filtrates obtained tested with Mayer's reagent (Potassium Mercuric iodide), yellow cream precipitates indicate the presence of alkaloids; the filtrates tested with Wagner's reagent (Iodine in potassium iodide), brown or reddish-brown precipitates indicate the presence of alkaloids (Somkuwar and Kamble, 2013). Identification of flavonoids was conducted by treating the sample extract with some drops of sodium hydroxide solution, a yellow color which to be colorless after dilute acid addition indicates the presence of flavonoids (Somkuwar and Kamble, 2013). The steroid was identified by Liebermann-Burchard reaction. About 2 ml filtrate was added 2 ml of acetic anhydride and conc. H2SO4, a blue-green ring indicates the presence of terpenoids (Parekh and Chanda, 2007). Detection of saponin carried out by diluting the sample extract into 20 mL distillate water, shaken for about 15 minutes. The formation of a one cm layer of foam indicated the presence of saponin (Somkuwar and Kamble, 2013).

Determination of Antibacterial activity

The antibacterial activity was measured on the best extract, which possessed better phytochemicals properties against two bacteria strains, namely *Staphyloccocus aureus* (a round-shaped Gram-positive bacterium of the family Firmicutes), and *Salmonella thypi* (a rod-shaped Gram-negative bacterium of the family Enterobacteriaceae). The determination of antibacterial activity was following the method of (Orafidiya et al. 2001) with slight modification. About 0.2 mL of a nutrient broth culture of both respective bacteria was seeded into 20 mL liquid and cooled agar medium in two different Petri dishes. For each strain, there were six different treatments, namely tetracycline as a positive control, distilled water as a negative control, ethanol extract of the sample (5%, 10%, 15%, and 20%. Thus, a total of 6 small plates was seeded into each petri dish, which contains one particular microbe. Those small plates added by 0.1 ml of various liquid tests: one plate challenged with tetracycline, one plate of distilled water, and the other three were added by ethanol extract of 5%, 10%, 15%, and 20%, respectively — the plates placed at room temperature for 1 hour before 24 H incubation at 37 ºC. The inhibition zone then measured by the transparent diameter zone.

Determination of anti-inflammatory activity

Solution preparation

Phosphate buffer solution pH 7.4 prepared by firstly 2.671 g Disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) dissolved in distilled water to a volume of 100 mL. About 2 g Sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$) dissolved in distilled water up to a volume of 100 mL. Then, 81 mL $Na_2HPO_4.2H_2O$ solution mixed with 19 mL $NaH_2PO_4.H_2O$ solution at room temperature. Isosaline solution prepared by NaCl of 0.85 g was dissolved in a phosphate buffer solution of pH 7.4, then adjusted to a volume of 100 mL at room temperature (Oyedapo et al., 2010). The hyposaline solution made by dissolved 0.25 g NaCl into a phosphate buffer solution of pH 7.4 up to reach a volume of 100 mL (Oyedapo et al., 2010). Aspirin Solution of 100 µg/mL was prepared by about 5 mg aspirin dissolved into an isosaline solution and adjusted to a volume of 50 mL.

The ethanol extract solution of the hooks was prepared in several concentrations of 100, 50, 10, and 5 µg/mL. A total of 25 mg ethanol extract sample was dissolved in 0.6 ml of 70% ethanol and then adjusted with 25 mL of isosaline solution so that a sample solution of 1000 µg/mL was obtained. The solution was diluted and made with a concentration of 100, 50, 10, and 5 µg/mL.

Preparation of Red Blood Cells (RBCs) (10% v/v)

Red blood cells used were obtained from female experimental rabbits. About 3 mL of the red blood cells put into the EDTA tube, then centrifuged at a speed of 3000 rpm for 15 minutes at room temperature. The supernatant was separated, then the residue was transferred into the centrifugation tube and added enough isosaline solution and again centrifuged. The process was repeated three times until the color of the isosaline solution became clear (Oyedapo et al., 2010). Further, a 10% red blood cell suspension was prepared by mixing 2 mL of red blood cells with 18 mL of isosaline (Saleem et al., 2011).

Anti-inflammatory activity analysis

The preparation of the test solution was carried out according to (Shailesh et al., 2011). The test mixture consisted of 2 mL hyposaline; 1 mL 0.15 M sodium phosphate buffer (pH 7.4); 0.5 mL (10% v/v) RBCs suspension; and 1 mL of test sample and standard solution (aspirin 100 µg/mL). Anti-inflammatory activity was determined using the method of stability of red blood cell membranes (RBCs) with various test solutions used, namely: test solution, test control solution, and standard control solution. The test solution consisted of 1 mL sample solution; 2 mL hyposaline; 1 mL of 0.15 M sodium phosphate buffer (pH 7.4); and 0.5 mL (10% v/v) suspension of RBCs in isosaline. The test control solution consisted of 2 mL hyposaline; 1 mL of 0.15 M sodium phosphate buffer (pH 7.4); 1 mL of isosaline and 0.5 mL (10% v/v) of RBCs suspension in isosaline. The standard test solution consisted of 2 mL hyposaline; 1 mL of 0.15 M sodium phosphate buffer (pH 7.4); 1 mL aspirin (100 µg/mL) and 0.5 mL (10% v/v) suspension of RBCs in isosaline.

Each of the solutions was then incubated in a water bath at 56 ºC for 30 minutes. Then the solution was centrifuged at 3000 rpm for 15 minutes. The absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 560 nm (Juvekar et al., 2009). The percentage of hemolysis inhibition calculated using the below formula (Leelaprakash and Dass, 2011).

$$\%\ hemolysis\ inhibitory = 100 \times \frac{A1 - A2}{A1}$$

A1 = Absorbance of test control solution

A2 = Absorbance of test solution / standard test solution

RESULTS AND DISCUSSION

This study began by extract the preparation of the hook of *U. tomentosa* using ethanol and methanol. The phytochemical analysis was conducted on both extracts. The better phytochemicals content would be selected as

main focused on antibacterial and anti-inflammatory activities.

Phytochemical properties

Phytochemical components were determined on ethanol and methanol extract derived from the hooks of the cat's claw. The effectiveness of the extraction of a compound by a solvent is highly dependent on the solubility of the composition in the solvent according to the principle "like dissolve like", as a compound will dissolve with the same solvent. Polar solvents include ethanol, methanol, acetone, and water. Methanol solvent has the ability to extract various polar and non-polar chemical components in a plant, is universal, contains a higher extract yield compared to ethanol. A compound will dissolve in a solvent that has the same polarity. Flavonoid compounds are divided into several types with a polarity that varies depending on the position and number of the hydroxyl group and will affect the solubility of flavonoids in the solvent (Harborne, 1998).

Flavonoid is one of the polar compounds, so a polar solvent may be needed (Gillespie and Popelier, 2001). A study on *Moringa oleifera* leaves, reported that ethanol solvents were more effective in dissolving flavonoid and phenolic compounds. That works suggests that maceration, along with ethanol 70%, was recommended in preparing a high-quality extract intended for nutraceutical development, which is the final goal in this study on *U. tomentosa* (Vongsak et al., 2013).

Sample extracts	Phytochemicals				
	Alkaloid		Flavonoid	Steroid	Saponin
	Mayer	Wagner			
EtOH	++	++	+++	++	++
MetOH	+	++	++	+	++

Table 1. Phytochemical of the extracts derived from the hook of *Uncaria tomentosa*

The identification was including alkaloid, flavonoid, steroid, and saponin (Table 1). It can be seen that ethanol extract contained better alkaloid, flavonoid, and steroid compared to methanol extract, but both have relatively the same level of saponin.

Several studies have conducted to identify the phytochemical compound in the *Uncaria* genus. Alkaloids, particularly the monoterpene oxindole indole alkaloids, were the most prominent constituents in this genus (Zhang et al., 2015).

(Zhang et al. 2015) declared that Ursan-type pentacyclic triterpenes found as the most triterpenes in *Uncaria.* This genus possesses about 53 species of *Uncaria,* and Quinovic acid glycosides become particularly apparent in *U. tomentosa* and *U. guaianensis.* This Quinovic acid glycoside is nominated as the most prominent anti-inflammatory substance (Aquino et al., 1991). The structure can be seen in Figure 1.

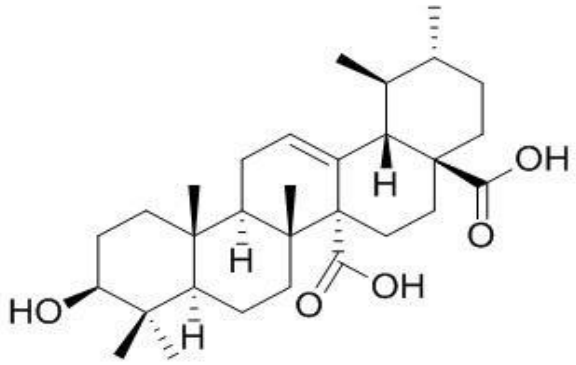

Figure 1. Chemical structure of Quinovic acid glycoside

A study carried out on Peruvian *U. tomentosa* successfully isolated 50 different biochemical substances, including alkaloids (isorhynchophylline, isomitraphylline, rhynchophylline, uncarine, rotundifoline), procyanidins cinchonain, and ursane type pentacyclic triterpenes (ursolic acid and quinovic acid glycosides) (Heitzman et al., 2005). In terms of flavonoids, 40 types of flavonoids have been revealed from this genus. Those are classified as flavonols and flavan-3-ols. The flavonols, flavan-3-ols, and the dimmers and 3-glycosides have dominated the flavonoids in *Uncaria* (Zhang et al., 2015).

Antibacterial activity

Antibacterial activities were observed on two bacteria cultures of Gram-positive bacterium (*Staphylococcus aureus*) and Gram-negative bacterium (*Salmonella thypi).* There were six treatments against both microbes, such as tetracycline as a positive control, distilled water as a negative control, and four EtOH extracts (5%, 10%, 15%, and 20%) (Table 2.)

Table 2. Antibacterial of an ethanol extract derived from the hook of *U. tomentosa*

Bacteria strains	Inhibitory zone diameter (mm)					
	Tetracycline	Distilled water	Ethanol extract of sample			
			5%	10%	15%	20%
Staphylococcus aureus	11.28	-	5.82	6.20	6.70	8.20
Salmonella thypi	12.68	-	6.15	7.00	7.22	9.10

Note: (-) no zone formed.

The observation showed that tetracycline and ethanol extract of samples brought a higher impact on Gram-negative bacteria such as *S. thypi* compared to Gram-positive, such as *S. Aureus.* Also, it was revealed that the higher concentration applied, the inhibitory zone also become broader. It means that the ethanol extract obtained from the hook of *U. tomentosa* has potential as an antibacterial agent. (Kloucek et al. 2005) found that ethanol extract of *U. tomentosa* plant shows good activity in inhibited five bacteria strains *(Streptococcus pyogenes, Bacillus subtilis, Bacillus cereus, Staphylococcus epidermidis, Staphylococcus aureus,* and *Enterococcus*

faecalis) with minimum inhibition concentration (MICs) was from 0.25 to 1 mg/ml. Another works on the bark of cat's claw, reports that three isolated flavonoids isolated, i.e., Artochamin C, 5'-Hydroxycudraflavone A, and Dihydrocudraflavone B show significant antibacterial activities to *Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus*. Artochamin C (1) dedicated as the most active among those three flavonoids constituent with MICs from 4.1 µg/mL to 6.7 µg/mL (White et al., 2011). Besides flavonoids, triterpenes reported also contribute to the antibacterial activity in this plant (Akbar and Malik, 2002; Kloucek et al., 2005; Montoro et al., 2004).

Anti-inflammatory activity on red blood cell membrane stability

Figure 2 shows the effect of ethanol extract on red blood cell membrane stability. This study used 100 ppm of aspirin as a positive control. The ethanol extract of the hook of *U. tomentosa* showed a better impact against RBC stability at level ≥ 50 ppm compared to 100 ppm aspirin.

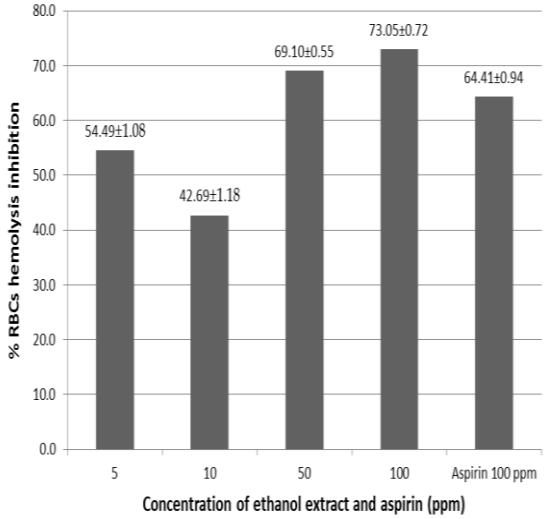

Figure 2. The percentage of hemolysis inhibition of red blood cells

According to the curve shown in Figure 3, it revealed Y=0.2728X+48.579 with R^2=0.7426. Thus, it discovered the half-maximal effective concentration (EC_{50}) of ethanol extract of *U. tomentosa* hook against RBC hemolysis inhibition was 5.21 ppm. RBC's membrane stabilization determination is one method used to determine anti-inflammatory activity in vitro since the membrane of red blood cells is similar to the lysosome membrane. The stabilization of the red blood cell membrane may indicate that there is also a stabilization of the lysosome membrane (Leelaprakash and Dass, 2011). Stabilization of the lysosomal membrane is essential in the limitation of the inflammatory response by preventing the release of enzymes in the lysosome from the activation of neutrophils such as protease enzymes during the inflammatory process in extracellular tissue and fluid (Gupta et al., 2013).

According to (Olchowik et al. 2012), the stabilization mechanism of red blood cell membranes can be identified when treated by hypotonic and oxidative stresses. This process disrupts the stability of the biomembrane. The effect of the hypotonic solution also related to the total fluid entering the erythrocyte membrane, causing hemolysis. When the erythrocyte membrane ruptures, the substances in the red blood cells will come out. Thus, the isosaline solution that enters the red blood cells will inhibit hemolysis triggered by the hypotonic solution induction. The absorbance value of Fe produced would be small, so induction of oxidative stress is needed to lead for optimal lysis.

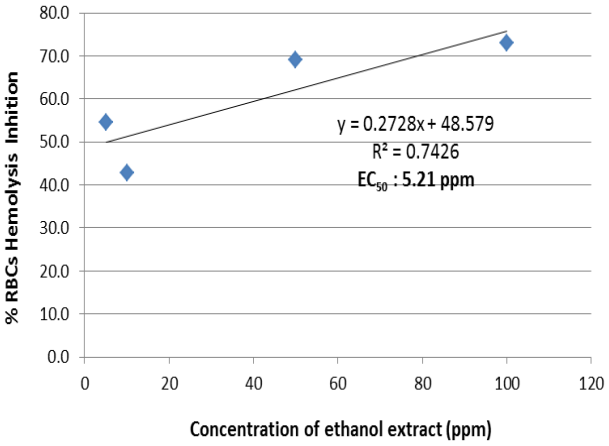

Figure 3. The inhibition curve of an ethanol extract derived **f**rom the hook of *U. tomentosa*

Oxidative stress defined as a condition where the number of free radicals or oxidizing compounds in the body exceeds the body's capacity to neutralize them. Oxidative stress can cause oxidation of lipids and proteins, thereby triggering membrane damage, which is characterized by hemolysis (Kumar et al., 2011). Highly reactive oxygen derivatives are ROS (reactive oxygen species). ROS causes a disturbance in the balance between oxidation and antioxidant activity, which causes damage to biological molecules. One cause of oxidative stress is heat induction. During heat induction, glutathione peroxidase (antioxidant enzymatic) increases significantly. It shows that heat induction causes oxidative stress (Halliwell and Whiteman, 2004; Mujahid et al., 2007).

Following the previous study, the lysis level of red blood cells can be used as a measure to see the anti-inflammatory activity seen from the size of the lysis that occurs due to hypotonic solutions and heat induction. The lysis of red blood cells can be determined from the absorbance value obtained from the measurement results using a UV-Vis spectrophotometer. Percent inhibition or stability is a measure to observe the ability of a sample in stabilizing red blood cells through the absorption ratio between the absorbance of the test solution and the negative control absorbance. The percentage value of volatile oil stability that approaches or exceeds positive control considered an excellent substance, thus because it has the same or more anti-inflammatory activity than the positive control.

Anti-inflammatory activity may be identified from the decrease in the absorbance of hemoglobin. The smaller the absorption detected in the mixture of test solution means the red blood cell membrane undergoes lysis. The positive control used was aspirin, with a percent inhibition value of 72.26%. Aspirin is used as a positive control since aspirin is an anti-inflammatory drug that can prevent the release of inflammatory mediators, one of which is inactivating cyclooxygenase (COX) in prostaglandin synthesis (Ouellet

et al., 2001). A study conducted by (Juvekar et al. 2009) showed that aspirin with a concentration of 100 µg / mL provides stability to the red blood cell membrane by 72.56%.

CONCLUSION

The ethanol extract derived from the hook of *U. tomentosa* contains better phytochemicals than methanol extract and contributes a higher impact on *Salmonella thypi* compared to *Staphylococcus aureus.* The higher concentration applied will increase the antibacterial activity. The hook extract of *U. tomentosa* (Wild. Ex Schult) DC originates Borneo, Southeast Asia, contributes to the better impact against RBC stability at level ≥ 50 ppm compared to 100 ppm aspirin with EC_{50} was 5.21 ppm.

Acknowledgment

This work fully supported by Tanjungpura University.

REFERENCES

1. Akbar, E., Malik, A., (2002). Antimicrobial Triterpenes from Debregeasia Salicifolia. Natural Product Letters 16, 339–344. https://doi.org/10.1080/10575630290033088
2. Aquino, R., De Feo, V., De Simone, F., Pizza, C., Cirino, G., (1991). Plant metabolites. New compounds and anti-inflammatory activity of Uncaria tomentosa. J. Nat. Prod. 54, 453–459. https://doi.org/10.1021/np50074a016
3. Batiha, G.E.-S., Magdy Beshbishy, A., Wasef, L., Elewa, Y.H.A., Abd El-Hack, M.E., Taha, A.E., Al-Sagheer, A.A., Devkota, H.P., Tufarelli, V., (2020). Uncaria tomentosa (Willd. ex Schult.) DC.: A Review on Chemical Constituents and Biological Activities. Applied Sciences 10, 2668. https://doi.org/10.3390/app10082668
4. de Paula, L.C.L., Fonseca, F., Perazzo, F., Cruz, F.M., Cubero, D., Trufelli, D.C., Martins, S.P. dos S., Santi, P.X., da Silva, E.A., del Giglio, A., (2014). Uncaria tomentosa (Cat's Claw) Improves Quality of Life in Patients with Advanced Solid Tumors. The Journal of Alternative and Complementary Medicine 21, 22–30. https://doi.org/10.1089/acm.2014.0127
5. Deharo, E., Baelmans, R., Gimenez, A., Quenevo, C., Bourdy, G., (2004). In vitro immunomodulatory activity of plants used by the Tacana ethnic group in Bolivia. Phytomedicine 11, 516–522. https://doi.org/10.1016/j.phymed.2003.07.007
6. Gillespie, R.J., Popelier, P.L.A., (2001). Chemical bonding and molecular geometry: from Lewis to electron densities, Topics in inorganic chemistry. Oxford University Press, New York.
7. Gupta, V., Chauhan, S., Prakash, A., Mathur, A., (2013). Evaluation of in vitro and invivo anti-inflammatory activities of Parthenium camphora. Recent Research in Science and Technology 5.
8. Halliwell, B., Whiteman, M., (2004). Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? British Journal of Pharmacology 142, 231–255. https://doi.org/10.1038/sj.bjp.0705776
9. Harborne, A.J., (1998). Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. Springer Science & Business Media.
10. Heitzman, M.E., Neto, C.C., Winiarz, E., Vaisberg, A.J., Hammond, G.B., (2005). Ethnobotany, phytochemistry and pharmacology of Uncaria (Rubiaceae). Phytochemistry 66, 5–29. https://doi.org/10.1016/j.phytochem.2004.10.022
11. Juvekar, A., Sakat, S., Wankhede, S., Juvekar, M., Gambhire, M., (2009). Evaluation of antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. Planta Med 75, s-0029-1234983. https://doi.org/10.1055/s-0029-1234983
12. Kloucek, P., Polesny, Z., Svobodova, B., Vlkova, E., Kokoska, L., (2005). Antibacterial screening of some Peruvian medicinal plants used in Callería District. Journal of Ethnopharmacology 99, 309–312. https://doi.org/10.1016/j.jep.2005.01.062
13. Kośmider, A., Czepielewska, E., Kuraś, M., Gulewicz, K., Pietrzak, W., Nowak, R., Nowicka, G., (2017). Uncaria tomentosa Leaves Decoction Modulates Differently ROS Production in Cancer and Normal Cells, and Effects Cisplatin Cytotoxicity. Molecules 22. https://doi.org/10.3390/molecules22040620
14. Kumar, B.S., Kunwar, A., Singh, B.G., Ahmad, A., Priyadarsini, K.I., (2011). Anti-hemolytic and Peroxyl Radical Scavenging Activity of Organoselenium Compounds: An In Vitro Study. Biol Trace Elem Res 140, 127–138.https://doi.org/10.1007/s12011-010-8692-3
15. Leelaprakash, G., Dass, S.M., (2011). In vitro anti-inflammatory activity of methanol extract of Enicostemma axillare. International Journal of Drug development and Research 3.
16. Masriani, Fadly, D., Bohari, B., (2020). α-Glucosidase Inhibitory Activity of Ethanol Extract Obtained from Dillenia suffruticosa and Pycnarrhena cauliflora. Journal of Global Pharma Technology 12, 881–887.
17. Montoro, P., Carbone, V., de Dioz Zuniga Quiroz, J., De Simone, F., Pizza, C., (2004). Identification and quantification of components in extracts ofUncaria tomentosa by HPLC-ES/MS. Phytochem. Anal. 15, 55–64. https://doi.org/10.1002/pca.740
18. Mujahid, A., Akiba, Y., Toyomizu, M., (2007). Acute Heat Stress Induces Oxidative Stress and Decreases Adaptation in Young White Leghorn Cockerels by Downregulation of Avian Uncoupling Protein. Poultry Science 86, 364–371. https://doi.org/10.1093/ps/86.2.364
19. Navarro Hoyos, M., Sánchez-Patán, F., Murillo Masis, R., Martín-Álvarez, P.J., Zamora Ramirez, W., Monagas, M.J., Bartolomé, B., (2015). Phenolic Assesment of Uncaria tomentosa L. (Cat's Claw): Leaves, Stem, Bark and Wood Extracts. Molecules 20, 22703–22717. https://doi.org/10.3390/molecules201219875
20. Olchowik, E., Lotkowski, K., Mavlyanov, S., Abdullajanova, N., Ionov, M., Bryszewska, M., Zamaraeva, M., (2012). Stabilization of erythrocytes against oxidative and hypotonic stress by tannins isolated from sumac leaves (Rhus typhina L.) and grape seeds (Vitis vinifera L.). Cellular and Molecular Biology Letters 17. https://doi.org/10.2478/s11658-012-0014-7
21. Orafidiya, L.O., Oyedele, A.O., Shittu, A.O., Elujoba, A.A., (2001). The formulation of an effective topical antibacterial product containing Ocimum gratissimum leaf essential oil. Int J Pharm 224, 177–183.https://doi.org/10.1016/s03785173(01)00764-5

22. Ouellet, M., Riendeau, D., Percival, M.D., (2001). A high level of cyclooxygenase-2 inhibitor selectivity is associated with a reduced interference of platelet cyclooxygenase-1 inactivation by aspirin. Proceedings of the National Academy of Sciences 98, 14583–14588. https://doi.org/10.1073/pnas.251543298
23. Oyedapo, O., Akinpelu, B., Akinwunmi, K., Adeyinka, M., Sipeolu, F., (2010). Red blood cell membrane stabilizing potentials of extracts of Lantana camara and its fractions. International Journal of Plant Physiology and Biochemistry 2, 46–51.
24. Parekh, J., Chanda, S., (2007). In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk J Biol 31, 53–58.
25. Saleem, T.M., Azeem, A., Dilip, C., Sankar, C., Prasanth, N., Duraisami, R., (2011). Anti–inflammatory activity of the leaf extacts of Gendarussa vulgaris Nees. Asian Pacific Journal of Tropical Biomedicine 1, 147–149. https://doi.org/10.1016/S2221-1691(11)60014-2
26. Sandoval, M., Okuhama, N.N., Zhang, X.-J., Condezo, L.A., Lao, J., Angeles, F.M., Musah, R.A., Bobrowski, P., Miller, M.J.S., (2002). Anti-inflammatory and antioxidant activities of cat's claw (Uncaria tomentosa and Uncaria guianensis) are independent of their alkaloid content. Phytomedicine 9, 325–337. https://doi.org/10.1078/0944-7113-00117
27. Shailesh, G., Seema, K., Dwivedi, S., (2011). In-Vitro anti-inflammatory activity of Sarcostemma acidum Wight. and Arn. Indian herb by Human red blood cell membrane stabilization method. International Journal of Pharmacy Teaching & Practices 2, 1–5.
28. Somkuwar, D.O., Kamble, V.A., (2013). Phytochemical Screening of Ethanolic Extracts of Stem, Leaves, Flower and Seed Kernel of Mangifera Indica L. Pharmacognosy 4, 383–389.
29. Turner, I.M., (2018). Notes on the genus Uncaria (Rubiaceae) in Singapore. GBS 70, 9–12. https://doi.org/10.26492/gbs70(1).2018-02
30. Valerio, L.G., Gonzales, G.F., 2005. Toxicological Aspects of the South American Herbs Cat's Claw (Uncaria tomentosa) and Maca (Lepidium meyenii): A Critical Synopsis. Toxicological Reviews 24, 11–35. https://doi.org/10.2165/00139709-200524010-00002
31. Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., Gritsanapan, W., (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Industrial Crops and Products 44, 566–571. https://doi.org/10.1016/j.indcrop.2012.09.021
32. White, G., Bourbonnais-Spear, N., Garner, F., (2011). Antibacterial constituents from Uncaria tomentosa. Phytopharmacology 1, 16–19.
33. Zhang, Q., Zhao, J.J., Xu, J., Feng, F., Qu, W., (2015). Medicinal uses, phytochemistry and pharmacology of the genus Uncaria. Journal of Ethnopharmacology 173, 48–80. https://doi.org/10.1016/j.jep.2015.06.011