In Vitro Anti-Inflammatory Activities of Red Gemor (Nothaphoebe cf Umbelliflora)

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***Abstract*—Gemor (*Nothaphoebe cf umbelliflora)* is a tree species that found naturally in swampy forest of Sumatra and Kalimantan, Indonesia. The anti-inflammation activity of *red gemor* plant parts have not been investigated, therefore many study should be performed. Thus our study aimed to investigate the anti-inflammation effect of different parts of *red gemor*. Phytochemical analysis of diffrent parts of *red gemor* extracts revealed the presence of various biochemical compounds such as alkaloids, flavonoids, phenolic compounds, triterpenoids and steroid. The anti-inflammatory activities was determined by inhibition protein denaturation method. Result of this study revealed there is inhibitory action on protein denaturation. The percentage inhibition varied from 20,154 to 71,667 for highest concentration to the lowest concentration. The IC50 was found to be 60 for twig, 47,8 for bark, and 116,2 for leaves of *red gemor*. To determined which one of the phytochemical constituent in diffrent parts of *red gemor* are most influence in anti-inflammation activity, we used linear correlation between IC 50 with alkaloid and flavonid content in different parts of *red gemor.* The result suggest that flavonoid are the most influence to protein denaturation (R^2 = 0,9927). The results of the present study suggest that the diffrent parts of *red gemor* have anti-inflammation activity. The anti-inflammation activity of different parts of *red gemor* due to the phytochemical constituents content in different parts of *red gemor*, such as flavonoid and alkaloid.**

***Index Terms*—flavonoid, alkaloid, anti-inflammation activity, *nothaphoebe cf umbelliflora*.**

I. Introduction

Plants are source of antioxidants that are beneficial to health because it contains bioactive compund, and others that can scavenging the free radical. Free radicals are atoms or molecules containing unpaired electrons therefore unstable molecule (Jurnal Tanjung). Numerous physiological and biochemical processes in the human body may produce oxygencentered free radicals and other reactive oxygen species (ROS) as byproducts. Overproduction of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans [1].

It is commonly accepted that, in a situation of oxidative stress, reactive oxygen species, such as superoxide (•O2), hydroxyl (•OH) and peroxyl (•OOH, ROO•) radicals are generated. The ROS play an important role related to the degenerative or pathological processes of various serious diseases inflammation (S.S. Sakat, 2010). Inflammation is the reaction of living tissues to injury, infection or irritation [1].

Inflammation is our body's natural reaction to invasion by an infectious agent, burn, toxin or physical, chemical or traumatic damage. One purpose of inflammation is to protect the site of an injury [2]. The mechanism of inflammation injury is attributed, in part, to release of ROS from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating release of cytokines such as interleukin-1, tumor necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophils and macrophages [3]. Moreover, these reactive species are involved in the biosynthesis of prostaglandins and cyclooxygenase in the cycloxygenase- and lipoxygenase-mediated conversion of arachidonic acid into proinflammatory intermediates [4].

The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary [5]. Since ROS, NO production, related enzymes, proinflammatory cytokines might cause inflammatory damage, many studies about inflammation focused to find materials which selective modulate these free radicals and inflammatory mediators from traditional plant-derived medicines [6].

Mueller et al. screened 30 extracts from a wide range of plant families for their anti-inflammatory activities in macrophage cells [7]. *O. corniculata* is widely used in ethnomedicine for the treatment of inflammatory and related disorders [8]. Chilli pepper extract was shown to have the strongest anti-inflammatory activity along with allspice, apple, basil, bay leaves, black pepper and liquorice [7].

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South Kalimantan of Indonesian, as a tropical district, shows on amazing diversity of plants species. Some of them have been long used as traditional medicines [9]. For example is *red gemor , red gemor* is a vernacular or trade name of *gemor*-bark-producing tree species (or *gemor* tree), which belongs to genus *Nothaphoebe* of the family *Lauraceae*. The species has been commonly identified as belonging to the genus *Alseodaphne* in the same family [10], [11]. Locally they are called as *gemor, menuk* (Kutai, Dayak Tunjung) or *tempuloh* (Dayak Bahau), which cover *Nothaphoebe coriacea* and *N. umbelliflora*. According to Sosef et al., *Nothaphoebe coriacea* occurs in Peninsular Malaysia, Singapore and Indonesia (Sumatra and Kalimantan); while *Nothaphoebe umbelliflora* occurs in Indo-China, Thailand, Peninsular Malaysia, Singapore, Indonesia (Sumatra, Java, Kalimantan) and Papua New Guinea. In Indonesia, the two species are found naturally in swampy forests of Sumatra and Kalimantan [12].

The bark of *gemor* is used as material for insecticide, hio (a stick used for budha's ritual) and glue [12]. *Gemor* tree was potentially have medicinal benefits of the parts of the tree are either leaves, twig, bark. Phenol compound, 4-(3-hydroxy-1-propenyl) – 2 – methoxy-(CAS) coniferyl alcohol in *Gemor* bark can be used as an inhibitor of viral activity, namely, anti-influenza, antiviral and anti-herpes. Furthermore *gemor* contain phytochemical components in bark, twig, and leaves suc ah alkaloid, steroid, flavonoid, saponin, triterpenoid, tanin, and phenolic compounds [13].

The anti-inflammation activity of *red gemor* plant parts have not been investigated, therefore many study should be performed. Thus our study aimed to investigate the anti-inflammation effect of different parts of *red gemor*.

II. Material and Methods

A. Collection and Extraction of Plant Materials

The fresh bark, twig and leaves of *red gemor* was collected from Tumbang Nusa, Center Kalimantan, Indonesia. Before use, it was ensured that the bark, twig and leaves were free from contamination, sand and no microbial growth. The bark, twig and leaves were shade dried and was made into coarse powder using commercial blender. The powdered material was macerated with aquadest (1:1 weight/volume) at room temperature for 4 days with occasional shaking, followed by remaceration for 3 days. After filtration, the filtrate was evaporated at 30^{O}C under reduced pressure in a rotary evaporator. The dry extract was kept in a refrigator till use. Preeliminary phytochemical qualitative (flavonoid, alkaloid, steroid, triterpenoid, and phenolic compund) and quantitative analysis revealed the presence of flavonoid and alkaloid .

B. Qualitative Analysis of Phytochemical Constituents

Phytochemical screening of the crude aqueous extract of *gemor* was carried out using standard phytochemical procedure : [14]-[16]

- *Flavonoid:* A few chop of 1% NH_3 solution is added to the aqueous extract of each plant sample in a test tube. A yellow coloration is observed if flavonoids compound are present.
- *Alkaloid:* A 2 ml of test solution are taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity formed indicates the presence of alkaloids.
- *Steroid:* A 2 ml of test solution and minimum quantity of chloroform are added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Formation of purple color changes into green color that indicates the presence of steroids.
- *Triterpenoid:* 5ml of aqueous extract of each plant sample is mixed with 2ml of $CHCl_3$ in a test tube 3ml of concentrated H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if triterpenoids constituent is present.
- *Phenolic Compounds:* The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

C. Quantitative Analysis of Phytochemical Constituents

- *Alkaloid Content:* alkaloid determination using Harborne (1973) method: 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a waterbath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [17]. The alakaloid content was calculated as percentage
- *Flavonoid Content:* Flavonoid determination by the method of Bohm and Kocipai- Abyazan (1994). 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [17]. The flavonoid content was calculated as percentage

D. In Vitro Anti-Inflammation Activity

Test solution (0,5 ml) consist of 0,45 ml of Bovine Serum Albumin (BSA) (5% w/v aqueous solution) and 0,05 ml of test solution (250 μg/L).

Test control solution (0,5 mL) consists of 0,45 ml of BSA (5% w/v aqueous solution) and 0,05 ml of distilled water.

Product control solution (0,5 ml) consists of 0,45 ml of distilled water and 0,05 ml of test solution (250 μg/ml).

Standard solution (0,5 ml) consists of 045 ml of BSA (5% w/v aqueous solution) and 0,05 ml diclofenac sodium (250 μg/ml).

All the above solution were adjusted to pH 6,3 using 1 N hydrochloric acid. The samples were incubated at 37^{O}C for 20 min and the temperature was increased to keep the samples at 57^{O}C for 3 min. After cooling 2,5 ml of phosphate buffer saline was added to the above solutions. The absorbance was measured using UV visible spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated as, [2]

$$\% \text{ Inhibition} = \frac{100 - 0.D \text{ test solution} - 0.D \text{ of product solution}b}{0,D \text{ of test control}} \quad (1)$$

The control represents 100% protein denaturation. The results were compared with diclofenac sodium (250μg/ml).

E. Statictical Analysis

For the anti-inflammation activity, Inhibitory Concentration 50% (IC_{50}) was measured. The IC_{50} was calculated from the linear curve using microsoft excell 2007 and obtained by plotting the percentage of inhibition versus the concentrations.

For determined which of the phytochemical constituents are most influence on anti-inflammation activity, linear correlation was used between the phytochemical concentration and IC 50 of different parts of *gemor*.

III. Results and Discussion

A. Phytochemical Screening

Herbal medicines make an enormous contribution to primary health care and have shown great potential in modern phytomedicine against numerous ailments and the complex diseases and ailments of the modern world. There will always be risks when appropriate regulations do not mandle the appropriate formulation of the remedies or when self medication fosters abuse [18]

Traditional plant based medicines still exert a great deal of importance to people living in developing countries and also lead to discovery of new drug candidates. Majority of human population worldwide is getting affected by the inflammation related disorders. It is believed that current analgesia inducing drugs such as opiates and NSAIDS are not useful in all cases, because of their side effects like gastrointestinal irritation, liver dysfunction and many others [18].

Therapeutic agents suitable for the treatment of chronic inflammatory diseases are highly desirable, which has resulted in an increased interest in complementary and alternative medicines [18]. In recent years, the search for phytochemicals possessing antioxidant, antimicrobial and anti inflammatory properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases [19].

Preeliminary qualitative phytochemical analysis results are showed in Table I.

Table I shows *red gemor* twig, leaves and stem barks contain the phytocemichal constituents with flavonoid is the highest and followed by phenolic, alkaloid, steroid and triterpenoid.

TABLE I. Qualitative Phytochemical Analysis of Different Parts of Red and White Nothaphoebe cf Umbelliflora

Phytochemical Constituents	*Gemor* Parts		
	Twig	Leaves	Bark
Flavonoid	++	+++	++
Alkaloid	+	++	+
Steroid	+	+	+
Triterpenoid	+	+	+
Phenolic	+	+++	++

Preelimnary quantitative phytochemical analysis are showed in Fig. 1 and Fig. 2. The alkaloid content was found highest in leaves and followed by twig and bark. Flavonoid concentration was found highest inleaves and followed by twig and bark.

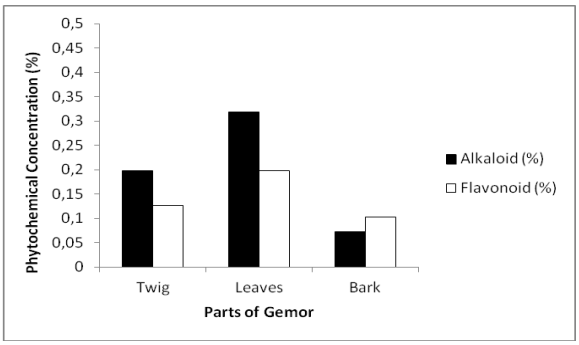

Figure 1. Alkaloid and flavonoid concentration of different part of *Nothaphoebe cf umbelliflora*

Results of our findings confirmed the use of *Gemor* as traditional medicine because the presence of phytochemical constituent in the parts of *gemor*.

The first phytochemical contituent is flavonoid. Flavonoids are a group of polyphenolic compounds ubiquitously found in fruits and vegetables. They have multiple biological activities, including antioxidative, vasodilatory, anticarcinogenic, anti inflammatory, antibacterial, immune–stimulating, anti-allergic, antiviral and estrogenic effects, as well as being inhibitors of phospholipase A2, cycloxygenase, lipoxygenase, glutathione reductase and xanthine oxidase. The detection of high quantity of flavonoids in the leaves of *red gemor* attaches more nutritional and medicinal value to the plant [20].

Another constituent is phenolic compounds like flavonoids act as anti-inflammatroy and antimicrobial agents. For this reason, the presence of phenolic compounds in the different parts of *red gemor* indicates that this plant could be used as anti-inflammmatory and antimicrobial agent [20].

Furthermore, metabolite constituents of diffrent parts of *red gemor* detected are alkaloids. The leaves contained

more alkaloids. Alkaloids are plant bases which exhibit certain physiological properties when used in herbal medicine. A lot of them have anti-malaria and antimicrobial activities. An example is quinine and its derivatives. The presence of tannins in plant has been associated with ulcer management, wound healing, control of bleeding and burns in herbal medicine [20].

B. *Anti-Inflammation Activity*

Anti-inflammation activity of all extracts of *red gemor* was measured by protein denaturation inhibition method. The results are showed in Fig. 3.

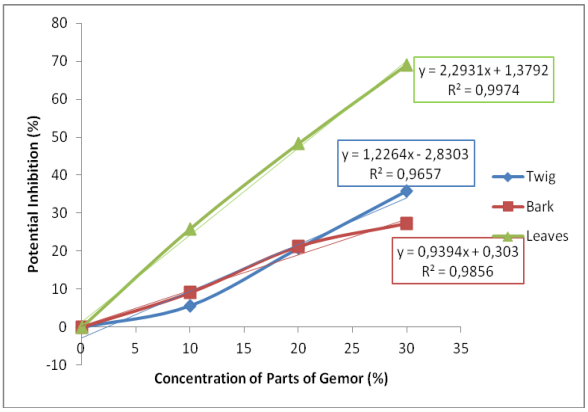

Figure 2. Anti-Inflammation Activity of Different Parts of *Nothaphoebe cf umbelliflora*

TABLE II. EFFECT OF DIFFERENT PARTS OF *RED GEMOR* ON PROTEIN DENATURATION

Sample	Concentration (%)	% Inhibition (%)	IC 50
Twig	0 10 20 30	0,000 20,154 38,154 39,368	60
Bark	0 10 20 30	0,000 23,333 49,444 71,667	47,8
Leaves	0 10 20 30	0,000 33,047 48,182 55,387	116,2
Natrium Diclofenac	0 10 20 30	0,000 70,16 77,22 85,65	11,87

Result of this study revealed there is inhibitory action on protein denaturation. The percentage inhibition showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 20,154 to 71,667 for highest concentration to the lowest concentration. The concentration required for 50% inhibition (IC50) was found to be 60 for twig, 47,8 for bark, and 116,2 for leaves of *red gemor* (Table II). Meanwhile, sodium diclofenac as the control showed the maximum inhibition at 85,65 with IC 50 11,87.

Denaturation of proteins is a well documented cause of inflammation. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins in vivo. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development [21]. Moreover it was already proved that Conventional NSAID's like phenylbutazone and indomethazine does not act only by the inhibition of endogenous prostaglandins production by blocking COX enzyme but also by prevention of denaturation of proteins [22].

As part of the investigation on the mechanism of the anti-inflammation activity, ability of different solvent diffrent parts of plant extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition 71,667 % was observed from bark of *red gemor* followed by leaves 55,387% and twig 39,368 % (Table II). All the solvent inhibited the albumin denaturation. The bark of *red gemor* has the percentage of inhibition near to the control.

Protein denaturation is inhibited by several agents, which would be used for the anti inflammatory drug development. Several reports have been reported that flavonoids and polyphenols present in the extract might be responsible for the major pharmacological activities such as anti- inflammatory, anti cancer, cardio protective and analgesic activity [23]. In the present study, the protein denaturation inhibition activity of different parts of *gemor* may be attributed due to its flavonoid and alkaloid content (Fig. 3).

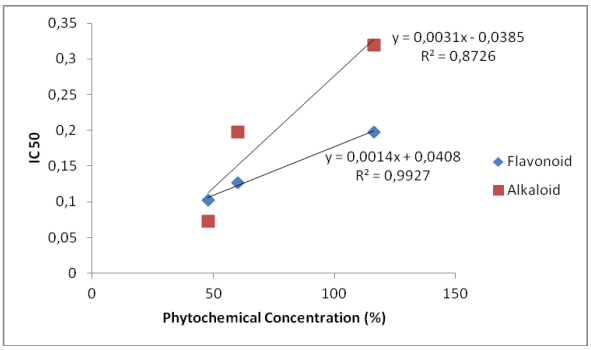

Figure 3. Correlation between flavonoid and alkaloid content and IC 50 of Different Parts of *Nothaphoebe cf umbelliflora*

To determined which one of the phytochemical constituent in diffrent parts of *red gemor* are most influence in anti-inflammation activity, we used linear correlation between IC 50 with alkaloid and flavonid content in different parts of *red gemor*. The result suggest (Fig. 3) that flavonoid are the most influence to protein denaturation (R^2 = 0,9927).

The therapeutic applications of flavonoids on inflammation have previously been reported [24]. Favonoids has long been utilized in Chinese medicine by applying crude plant extracts. Many investi gations have shown that a variety of flavonoid molecules exhibit anti-inflammatory activity both, in vitro and in various animal models of inflammation [25].

Flavonoids are a polyphenols subclass which are widely distributed in the plant kingdom, and are characterized by two or more aromatics rings, each bearing at least one aromatic hydroxyl and connected with a heterocyclic pyran [25]. More than 4000 varieties

of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruit, and leaves. Flavonoids occur as aglycones, glycosides and methylated derivatives [26].

Until 50 years ago, information on the working mechanisms of flavonoids was scarce. However, it has been widely known for centuries that derivatives of plant origin possess a broad spectrum of biological activity. They have been shown to exert antimicrobial, antiviral, antiatherosclerosis, cardioprotective, antiulcerogenic, cytotoxic, antineoplastic, mutagenic, antidiabetic, anti-inflammatory, antioxidant, anti-aging, antihepatotoxic, antihypertensive, hypolipidaemic and antiplatelet activities [26].

A number of flavonoids are reported to possess antiinflammatory activity in vitro and in vivo. Although not fully understood, several mechanisms of action are proposed to explain in vivo anti-inflammatory action. The important mechanism for anti-inflammatory activity is inhibition of eicosanoid generating enzymes including phospholipase A2, cyclooxygenases and lipoxygenases, thereby reducing the concentrations of prostanoids and leukotrienes. Other mechanisms include inhibition of histamine release, phosphodiesterase, protein kinases and activation of transcriptase [26].

Other constituent in different parts of *red gemor* might be has anti-inflammation activity are alkaloid. Alkaloids are most common in fl owering plants, and usually in the Papaveraceae (poppies), Papilonaceae (lupins), Ranunculaceae (aconites), and Solanaceae (tobacco and potatoes). They are also found in lower plants, insects, marine organisms, microorganisms and animals. The pharmacological studies in alkaloids have been largely concerned with the effect of alkaloids on physiological processes other than inflammation Isoquinoline, indole and diterpene alkaloids were the most studied about their activities on infl ammation. Aconitine and others alkaloids were screened for anti-infl ammatory activity. They were effective on different assays including carrageenin-induced paw on edema, adjuvant-induced arthritis and acetic acid induced vascular permeability tests [27].

IV. Conclusion

In the present study, the anti-inflammation activity of diffrent parts of *gemor* was evaluated. The results of the present study suggest that the diffrent parts of *gemor* have anti-inflammation activity. The anti-inflammation activity of different parts of *gemor* due to the phytochemical constituents content in different parts of *gemor*, such as flavonoid and alkaloid.

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