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The Anti-inflammatory, Anti-pyretic and Wound Healing Activities of *Cocos nucifera* (MATAG Types) Fresh Juice and Kernel Extract in Experimental Animals

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Abstract: The present study was carried out to evaluate the potential of *C. nucifera* as antipyretic, anti-inflammatory and wound healing agents. The fresh juice of *C. nucifera* (FJCN) was directly used while its aqueous kernel extract (AKCN) was obtained after 72 h soaking of 1:2 (w/v) fresh kernel in 2:1 (v/v) chloroform:methanol. The extracts, in the concentrations/strengths of 10, 50 and 100%, were used in the anti-pyretic and anti-inflammatory studies while those in the concentration of 100% were used only in the wound healing study. The fresh juice and aqueous kernel extract of *C. nucifera* exhibited significant ($p<0.05$) anti-inflammatory and antipyretic activities and promote wound healing with the latter producing a more effective effects in all assays used. This finding has scientifically supported the folkloric used of *C. nucifera* in the treatment of inflammation, pyrexia and wound.

Key words: *Cocos nucifera*, anti-inflammatory, antipyretic, wound healing

Introduction

Cocos nucifera (*C. nucifera*), known in Malay as *'kelapa'*, belongs to the family of *Palmae* in *Araecaeae* order and it is originated from Malaysia, Polynesia and Southern Asia and is now prolific in South America, India and the Pacific Island (Ahmad and Raji, 1993). The juice and kernel (meat) of *C. nucifera* have been used in Malays folk medicine for number of ailments such as to relieve fever, stomach upset, headaches, diarrhea and dysentery (Ahmad and Raji, 1993; Musa, 1998). The juice has adequate natural minerals and high quality proteins, which are valuable for growth and repair of the body (Pehowich *et al*., 2000). Medical research has discovered that the coconut oil obtained from the kernel consists of monolauric and lauric acid, which helps the immune system in a beneficial manner. The monolauric acids were use by the body to produce high levels of anti-microbial activity (Mid-American Marketing Corp., 2004). Lauric acid was the basic of monolaurin and was part of the chemical constituents of sodium lauryl sulfate that has been discovered to promotes health and used in adjunct treatment of viral diseases. It also found to be of the active chemicals in controlling Human Immunodeficiency Virus (HIV) disease (Dayrit, 2004). Recent study by Alanis *et al*. (2005)

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has demonstrated that the *C. nucifera* aqueous and methanol extract possess antibacterial properties against the *Escerichia coli*, *Shigella flexneri*, *Shigella sonnei* and *Salmonella* spp. with the latter being more active than the former.

Although *C. nucifera* has been use for a long time as herbal medicine in Malaysia, no pharmacological studies have been reported on the anti-inflammatory, antipyretic and wound healing activities of this plant. Although there is one report on *C. nucifera* antinociceptive and antioxidant activities (Alviano *et al*., 2004), the sample used was different from the one used in the present study. The present study was carried out to elucidate the potential anti-inflammatory, antipyretic and wound healing activities of the aqueous extracts of *C. nucifera* juice and kernel in animal models.

Materials and Methods

Plant Materials

The fruits of *Cocos nucifera* (Palmae) were collected from Pusat Pembangunan Komoditi, Teluk Bharu Hutan Melintang, Perak. It was identified to be from MATAG types (a hybrid combination of Malayan Dwarf and the Tagnanan Tall coconut tree). Two materials from the fruits (juice and kernel) were tested for its anti-inflammatory, antipyretic and wound healing activity.

Preparation of the Juice of C. nucifera

The fresh juices of *C. nucifera* (FJCN) used in the study was obtained prior to the experiments. This is to retain most of its organoleptics and nutritional characteristics. Three concentrations were being tested: 100% concentration/strength (pure juice) which was taken directly from the fruit and, 10 and 50% concentrations/strengths of FJCN that was prepared by diluting the pure juice in distilled water (DH_2O) with the ratio of 1:1 (w:v).

Preparation of Extract of the Kernel of C. nucifera

The aqueous kernel extract of *C. nucifera* kernel (AKCN) were obtained using the chloroform: methanol (CM) solution mixture (2:1) (v:v) as described by Zakaria (2001) but with slight modifications. Briefly, the kernel were macerated with CM in a ratio of 1:2 (w:v) for overnight (24 h). After 24 h, it was filtered and the supernatant were collected and left for another 30 min to finish the process. Two layers can be seen; the aqueous supernatant on the upper layer and the CM supernatant on the lower layer. The aqueous supernatant also called as the stock solutions were collected and evaporated at 70°C to remove the excess methanol residue. The product from the evaporation considered as the 100% concentrations/strengths of the AKCN.

Chemicals

The following chemicals were used: acetylsalicylic acid (ASA) (Bayer co. Ltd., Malaysia), chloroform, methanol and ethyl acetate (AR grade, Merck KGaA, Germany), brewer's yeast (AR grade, Sigma Chem. Co., St. Louis, USA), sodium chloride and formaldehyde (AR grade, Carlo Erba, Germany), acriflavine (Bayer Co. Ltd., Malaysia). All solutions were prepared immediately prior to the experiments.

Animals

All animals used in this study were obtained from the Animal House, Faculty of Veterinary, Universiti Putra Malaysia (UPM). The *Sprague dawley* rats with mean weight of 162 g were used in

all of the experiments except for the wound healing test, in which *Balb-C* mice with weight ranging from 25-30 g were used. The animal were housed in cages under laboratory standard conditions in a temperature (22°C)-controlled room, with free access to food and water.

Anti-inflammatory Activity

The anti-inflammation study was carried out according to the method described by Chakraborty *et al*. (2004) with slight modifications. The *Sprague dawley* rats were treated with intraperitoneal (i.p) injection of DH_2O, ASA (200 mg kg^{-1}), FJCN (10, 50 and 100% concentration) and AKCN, 30 min prior to 5% formalin sub plantar administration on the right hind paw of the rats. The paw thickness resulting from edema caused by formalin was measured in all groups for 6 h at every 30 min intervals.

Antipyretic Activity

The antipyretic activity of the FJCN and AKCN was measured by slightly modifying the method described by Reanmongkol *et al*. (2002). *Sprague dawley* rats were fasted overnight with water and food before the experiment. The rectal temperature of the rat was measured using digital thermometer (Temp Teller model OST; China) before treatment (BT) and 0, 1, 2, 3, 4, 5, 6 and 7 h after treatment (i.p.) with DH_2O, ASA (200 mg kg^{-1}), FJCN (10, 50 and 100% concentrations) and AKCN (10, 50 and 100% concentrations). The brewer's yeast (2 g kg^{-1}) (BY) which act as the pyrexia inducer were given i.p after 30 min of the test solutions administration.

Wound Healing Activity

The wound healing activity of JFCN and AKCN was carried out according to the method described by Reezal (2003). A single surgical incision of about 2 cm was inflicted at the dorsal surface of the *Balb-C* mice. The incision was treated daily by applying topically with acriflavine, JFCN (100%) and AKCN (100%) for a period of nine days. Three mice from each group groups were randomly selected and sacrificed after 24 h of treatment as well as on the 3rd, 6th and 9th day for the collection of the skin from the wound site for histological study. The skins collected were kept in 10% formalin to ensure it is well preserved for the histological procedure. Evaluation of the wound healing activity was based on the designate values developed by Speroni *et al*. (2002) but modified by Reezal (2003). The evaluation for wound healing process was made by observation of the cicatrizing activity, indicated by the formation of the granulation tissue and the conversion to fibrous mass called scar and the anti-inflammatory activity of both extracts on the skin sample. The length, colour and inflammatory aspects of the area around the wound were also examined. The inflammatory response, which is the normal acute reaction of the tissue after any injury was also closely observed.

Statistical Analysis

The results are presented as Mean±Standard Error of Mean (SEM). The Analysis of Variance (ANOVA) test was used to analyze and compare the data, with $p<0.05$ as the limit of significance.

Results

Effects of C. nucifera on Anti-inflammatory Response in Rats

As can be seen from the Fig. 1 the formalin-induced inflammatory edema was significantly ($p<0.05$) suppressed by 20 mg kg^{-1} ASA throughout the experiment, starting 30 min after the latter

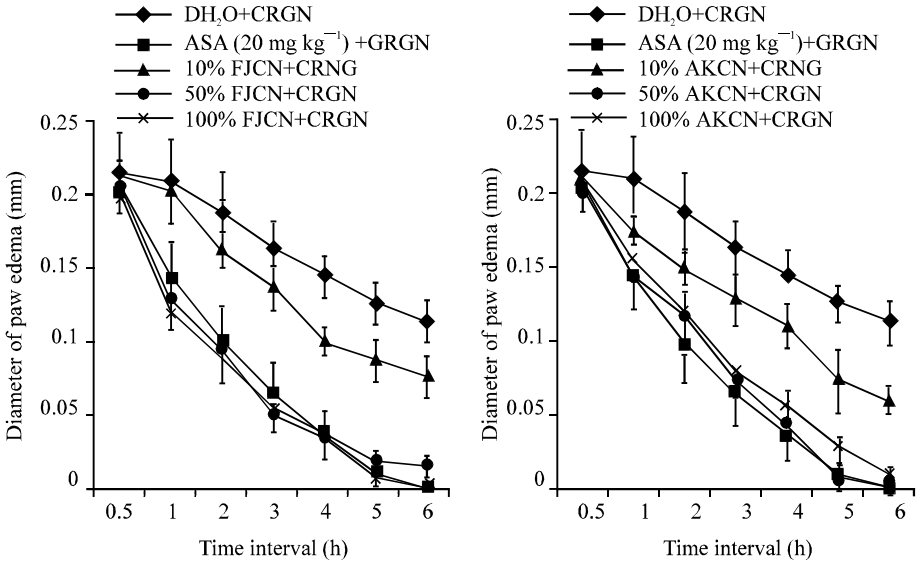


Fig. 1: The anti-inflammatory profiles of different concentrations of fresh juice (panel a) and aqueous kernel extract (panel b) of *C. nucifera*

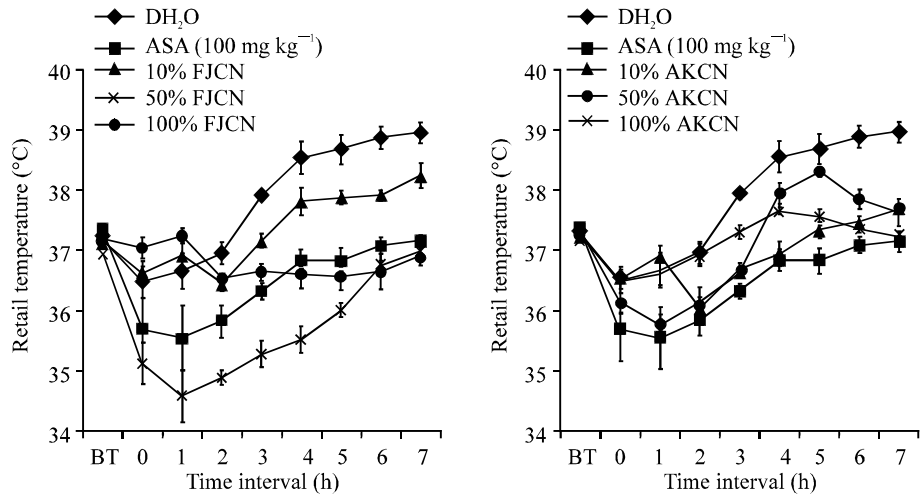
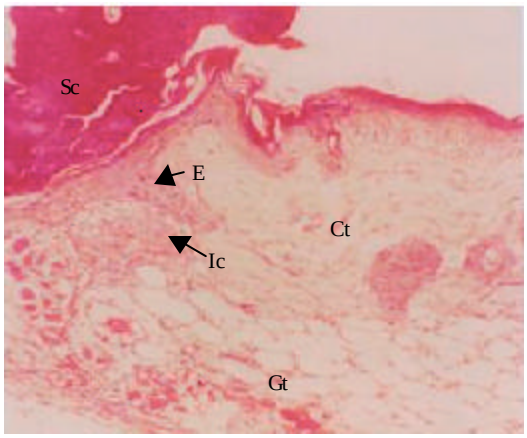


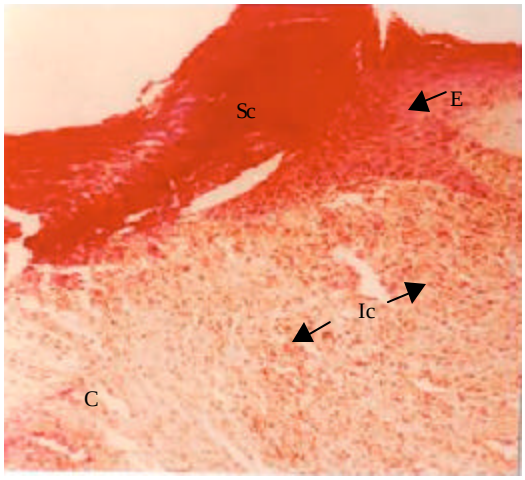
Fig. 2: The anti-pyretic profiles of different concentrations of fresh juice (panel a) and aqueous kernel extract (panel b) of *C. nucifera*

administration. Concomitantly, both extracts were also found to exhibit significant ($p<0.05$) anti-inflammatory activity with the FJCN producing a more effective effect. In addition, both extracts at the concentration of 100% produced total recovery from inflammatory edema as can be seen after 300 min of their administration.



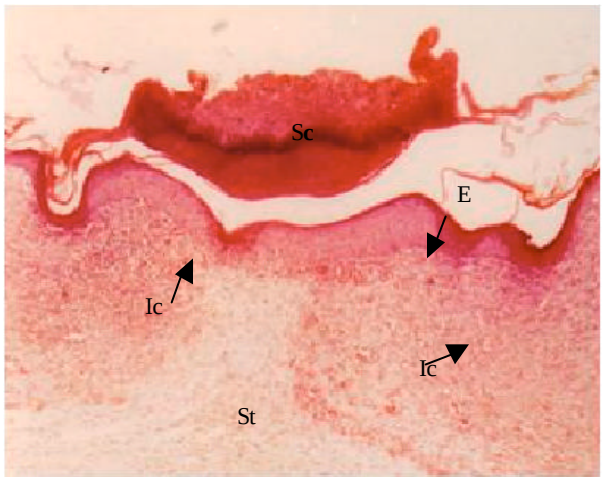
Ct: Connective tissue; E: Epithelium; Ic: Inflammation cells;
Gt: Granulation tissue; Sc: Scab

Fig. 3(a): The AKCN-treated mice, at day 3 of the experiments, showed progressive wound healing activity with visible scab formation and inflammation cells. Well developed granulation and counective tissues were present as the healing process takes place. Proliferative re-epithelialisation were observed under the formed scab



C: Cicatrisation; E: Epithelium; Ic: Inflammation cells; Sc: Scab

Fig. 3(b): Advanced skin reactions were observed on the AKCN-treated mice, at day 6 of the experiments, with re-epithelialisation was more proliferate on treated mice. Inflammation cells can be seen in abundance at the wound site together with visible cicatrisation which indicates advance healing process. Bit and pieces of the scab appeared to be sloughing off the skin



E: Epithelium; Ic: Inflammation cells; Sc: Scab; St: Scar tissue

Fig. 3(c): Well-developed organization of scar tissue and new thick lining of epithelium cells were observed at the end of the experiment. Inflammation cells have been reduced as the dermis layer starts to shape. The scab appeared to be separated from the skin surface signifying a comprehensive healing process

Effects of C. nucifera on Yeast-induced Fever in Rats

The antipyretic effects of FJCN and AKCN assessed using the BY-induced assay were shown in panel a and b of Fig. 2, respectively. The BY-induced pyrexia was observed after 240 min of its administration and continues to increase until the end of observation. Interestingly, the ASA was found to significantly ($p<0.05$) suppress the rat's body temperature for the first 3 h during which the BY did not yet produce its pyretic effect and this hypothermic inducing activity was still observed during the next 5 h when BY start to exhibit its pyretic effect. As can be seen from the graph, between 4 h to 8 h where pyretic activity was clearly observed, the FJCN extracts was found to be more effective in reducing fever in rat when compared to its counterpart (AKCN). In addition, both extracts, in the concentration of 50%, were also found to cause hypothermia in rats during the first 3 h after BY administration. On the other hand, the 100% concentration FJCN did not show any activity during the same range of time while the AKCN also exhibit significant ($p<0.05$) hypothermic activity.

Effects of C. nucifera on Wound Healing Activity in Mice

For the skin sections of the untreated group (negative control), severe necrosis and hemorrhages on the surface of wound were observed on the 3rd day of the experiment. Large number of infiltrating neuthrophils can be seen below the necrotic area and the growth of granulation tissue observed. The scab also appeared at this period of study with the non-proliferative thickening of epithelium cells on the edge of the wound area. On the 6th day of the experiment, severe necrosis and hemorrhages with slow growth of granulation tissue can still be seen on the surface of wound. A large number of neutrophils can be seen infilltrating the wound area. At this duration of study, the epithelium cells have thickened due to epithelial regeneration and can be seen under the scab. On the 9th day of treatment, the presence of large number of infiltrating macrophages and moderate amount of granulation tissue indicated delayed and incomplete wound healing.

For the acriflavine-treated group, the healing process started to develope after 3rd day of treatment as evidence from moderate necrosis and hemorrhages on the surface of wound. Furthermore, the presence of infiltrating neutrophils was found to be accompanied by a small number of infiltrating macrophages below the necrotic area and the growth of granulation tissue was observed at this period of study. The formation of scab and thickening of the epithelium cells can also be seen at this time of study. These lesions indicated good growth of healing tissue. On the 6th day of the experiment, the healing process has developed extensively as evidence from little necrosis and hemorrhages on the surface of wound. Furthermore, a large number of infiltrating macrophages below the necrotic area and extensive growth of the granulation tissue was also observed. On the 9th day of the experiment, extensive deposition of collagen fibers, elongated fibrocytes and few capillaries can be clearly observed. There was complete absence of hair follicles in healed area and keratinization was also noticed on completely grown epidermis suggesting complete healing of wounds.

For the group treated with FJCN, the sections of skin wound on the 3rd day of treatment revealed a more progressive healing indicated by the extensive formation of granulation tissue with the migration of infiammation cells to the wound site. The thickening of epithelium cells was visible at the edge of the wound that has already closed because of the scab formation. On the 6th day of treatment, the granulation tissue began to mature and appeared to form a fibrous counective tissue with the presence of monocytes observed. The inflammation cells showed less redness as the wound healing progressed. The scab has started to slough off from the skin as the epithelium cells cover almost half of the wound area. On the 9th day of the experiment, the sections of skin wound have demonstrated a more progressive healing characterized by extensive proliferation of fibrous counective tissue, which lead to the formation of fibrous mass or scar and the presence of a small number of monocytes. The number of inflammation cells began to decrease and the scab almost sloughed off. Keratinization was also observed on completely grown epidermis indicating complete healing of wounds.

For the group treated with AKCN, the sections of skin wound result showed that the granulation tissue was yet to form. However, the epithelium cells on the edge of the wound were found to thicken and the migration of inflammation cells like neutrophils at the wound area was visible. Furthermore, the AKCN treated group also appeared with closed wound and formation of the scab (Fig. 3a). On the 6th day of the experiment, the section of skin wound treated with AKCN exhibited the formation of granulation tissue at the wound area (Fig. 3b). The epithelial regeneration has developed under the scab, which lined up by the epithelium cells to form a new layer of epidermis. The inflammation cells like macrophages and monocytes can be seen accumulated at the wound area. After 9th day of treatment, the skin sections treated with AKCN revealed the formation of extensive fibrous mass, elongated fibrocytes and deposition of collagen fibers while the infiamed cells appeared less red than before (Fig. 3c). At this time, the scab of the wound has sloughed off from the skin and a new layer of epidermis formed and completely covering the healed area. Keratinization on the completely grown epidermis was also noticed indicating complete healing of wounds.

Discussion

C. mucifera is one of the most valuable plants to man and was used as a primary source of food, drinks and even medication. It has been used as a traditional medicine by many cultures (Cano and Volpato, 2004; Alanis *et al*., 2005), including in Asian countries, for hundreds of years to treat a wide range of illnesses (Ahmad and Raji, 1993; Musa, 1998). For example, the fruit of *C. mucifera* contains the coconut water (juice) and coconut meat (kernel) was very famous in the Malays folklore medicine

for its ability to relieve fever (Yahaya, 2005). In addition, Alleyne *et al*. (2005) have also reported on the *C. nucifera* juice extract ability to control hypertension in a clinical trial carried out at the University of the West Indies, St Augustine, Trinidad and Tobago, West Indies. Previous studies have also demonstrated that the aqueous extract of husk fiber of *C. nucifera* possessed peripherally- and centrally-mediated antinociceptive and antioxidant (Alviano *et al*., 2004), antimicrobial and antiviral (Esquenazi *et al*., 2002) and leishmanicidal (Mendonca-Filho *et al*., 2004) activities.

The present study has revealed the potential use of *C. nucifera* as anti-inflammatory, antipyretic and wound healing agents. The demonstrated antipyretic and wound healing activities of *C. nucifera* have confirm the Malays' traditional used of *C. nucifera* in the treatment of high fever (Yahaya, 2005) and Ayurvedic writings that accounts the efficacy of *C. nucifera* in the cure of wounds (Dayrit, 2004). Injury to tissues, especially those that lead to wound, will trigger the release of prostaglandins that is known to involve in pain sensation, inflammation and wound healing (Katzung, 1995). Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It depends upon the reparative abilities of the tissue, type and extent of the damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen is the major component of extra cellular tissue, which gives support and strength and is composed of amino acid (Hydroxyproline).

In addition, the ability of *C. nucifera* to produce anti-inflammatory activity by reducing the diameter of edema induced by carrageenan can be link to its observed antipyretic activity since both mechanisms of pyrexia and inflammation also involved the formation of prostaglandin. It is believe that the observed antipyretic and anti-inflammatory activities of the extract are probably due to the inhibition of prostaglandin release or blocking of the enzyme, cyclo-oxygenase, that is responsible for prostaglandin production and similar mediators involved in those mechanisms (Di Rosa *et al*., 1971; Spector, 1962). Further extensive research to screen for their inhibitory effect on cyclooxygenase, lipoxygenase and elastase activity in intact lenkocytes and platelets (Preito *et al*., 2003) will be carried out in the near future to determine the actual mode of action for both extracts of *C. nucifera*.

Furthermore, *C. nucifera* have also been reported to contain high amount of lauric acid, followed by myristic, caprylic, palmitic and capric acids as its major source of fatty acids (Mid-American Marketing Corp., 2004) with the juice itself was low in fatty acids content (Pehowich *et al*., 2000). Although we used the aqueous extract of the *C. nucifera* kernel throughout the study, the possibility of extracting lipid-based compounds or fatty acids into AKCN should not be ruled out based on our previous study with *Channa striatus* (Zakaria *et al*., 2005). The presence of methanol in AKCN, indicated by the failure of AKCN to freeze at -20°C, has lead to the latter evaporation and only after this process did the AKCN freeze at the said temperature. It is generally known that methanol is an intermediate solvent that has a capability to extract aqueous- as well as lipid-based compounds. The presence of those lipid-based compounds/fatty acids in AKCN was also thought to contribute to the AKCN observed activity. According to Crocker *et al*. (2001) fatty acids might take part in the attenuation of polymorphonuclear lenkocytes (PMN) activity and thus, suppressed inflammatory processes. In addition, Crocker *et al*. (2001) also suggested that a change in membrane fluidity through the proportions of fatty acids incorporated into membrane phospholipids can influence cell function, as membrane fluidity depends on the chain length and saturation of phospholipid fatty acids. The high content of unsaturated and monounsaturated fatty acids will undoubtedly influence membrane

fluidity and will affect many cell functions, including PMN activation. Thus, the presence of various types of fatty acids in *C. nucifera* could be used to explain its observed anti-inflammatory activity.

Although isolation and identification of bioactive compounds are not part of the objective of this study, various types of compounds have been isolated and identified from the *C. nucifera* juice and kernel such as zeatin-O-glucoside and dihydrozeatin-O-glucoside (Ge *et al*., 2004), ortho-topolin (Ge *et al*., 2005a), kinein and kinetin riboside (Ge *et al*., 2005b) and α-Galactosidase (Balasubramaniam *et al*., 1976; Mujer *et al*., 1984). In addition, the endosperm of *C. nucifera* was reported to contain cocosin (Garcia *et al*., 2005) while the virgin coconut oil was found to contain polyphenol components that help in lowering lipid levels in serum and tissues and LDL oxidation by physiological oxidants (Nevin and Rajamohan, 2004). In addition, Mini and Rajamohan (2004) have also reported on hypolipidemic effect of *C. nucifera* kernel protein, which is thought to be due to the present of high content of L-arginine while Mantena *et al*. (2003) have reported on the antioxidant properties of the *C. nucifera* juice that is believed to be attributed to the ascorbic acid, the main constituents of the juice. Two types of protein namely glutelin and prolamin, which were found in coconut milk, were also reported to be present in the juice of *C. nucifera* (Birosel *et al*., 1976). Although various types of compounds have been reported as mentioned above, their potential as anti-inflammatory, anti-pyretic or wound healing agents have not been proven. It seems important to recommend that further studies using isolated constituents instead of whole extract to be carried out in this field since *C. nucifera* have been proven to contain various types of biological compounds and to possess various pharmacological effects. Thus, the result may provide a basis for the isolation of compounds of biological interest from *C. nucifera*. Based on the finding, *C. nucifera* juice and kernel extracts were found to possess anti-inflammatory, antipyretic and wound healing activities.

Acknowledgments

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