*Jurnal Kejuruteraan dan Sains Kesihatan*
*Journal of Engineering and Health Sciences*
*Jilid 1 2017: 57-74*

# SCREENING OF HAEMOSTATIC ACTIVITY OF *NIGELLA SATIVA* SEED EXTRACT

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## ABSTRACT

The present study was performed with the aim to determine the haemostatic effect of the seed extract of Nigella sativa. The anticoagulant or procoagulant activities of the methanol extract from seeds of Nigella sativa Linn was studied using in vitro coagulation assays which included prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT). Extraction of the seeds resulted in two separate compounds of the extract. The first compound was greasy termed the oil compound while the other compound was viscous and dark called the crude compound. The tests were carried out on fresh frozen plasma (FFP) with different concentrations of the oil and crude compounds (20%, 40%, 60%, 80% and 100%). The coagulation time recorded for both compounds toward the PT, aPTT and TT assays showed transient prolongation of the clot formation. Statistical analysis using the one-way variance (ANOVA) showed statistically significant difference for both compounds, as opposed to the control, since their p-values were less than 0.05. Thus, both compounds of the Nigella sativa seed extract appear to possess anticoagulant activity. In addition, the coagulation time recorded by the oil compound was shown to be higher in PT and TT assays compared to the crude extract. Hence, the oil compound may have a more potent effect in inhibiting the coagulation activity within the extrinsic and common pathways. Meanwhile, from the results of the aPTT tests, the crude compound managed to delay the coagulation time longer than the oil compound. Thus, the crude compound may have more effect towards the coagulation activity of the intrinsic pathway.

**Key words:** *Nigella sativa Linn seed, haemostatic activity, anticoagulant.*

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# SARINGAN AKTIVITI HEMOSTATIK DARIPADA EKSTRAK BIJI *NIGELLA SATIVA*

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## ABSTRACT

Kajian ini dilaksanakan dengan tujuan untuk menentukan kesan hemostatik yang terdapat di dalam ekstrak biji Nigella sativa. Kajian aktiviti antipembekuan atau propembekuan darah oleh ekstrak metanol biji Nigella sativa dijalankan menggunakan ujian pembekuan darah secara in vitro seperti ujian masa protrombin (PT), ujian asas separa pengaktifan tromboplastin (aPTT) dan ujian masa trombin (TT). Pengekstrakkan biji Nigella sativa menghasilkan dua pecahan sebatian. Pecahan pertama adalah sebatian berminyak yang dipanggil sebatian minyak manakala sebatian kedua adalah likat dan hitam dipanggil sebatian mentah. Ujian ini diaplikasikan terhadap plasma beku segar (FFP) dengan kepekatan sebatian minyak dan sebatian mentah yang berbeza (20%, 40%, 60%, 80% dan 100%). Keputusan yang dicatatkan oleh kedua-dua sebatian terhadap ujian PT, aPTT dan TT menunjukkan masa pembekuan yang lebih panjang dalam pembentukan bekuan. Analisis statistik menggunakan "ANNOVA" menunjukkan kedua-dua sebatian mencatatkan nilai p kurang daripada 0.05 jika dibandingkan dengan ujian kawalan yang secara statistiknya amat signifikan. Secara amnya, kedua-dua sebatian ekstrak biji Nigella sativa kelihatan mempunyai aktiviti antipembekuan. Disamping itu, masa pembekuan yang direkodkan oleh sebatian minyak menunjukkan ujian PT dan TT lebih tinggi jika dibandingkan dengan sebatian mentah. Oleh itu, sebatian minyak lebih mempunyai potensi untuk menghalang aktiviti pembekuan darah dalam laluan luaran dan laluan umum. Manakala, daripada keputusan ujian aPTT , sebatian mentah berjaya untuk melambatkan masa pembekuan lebih lama dari sebatian minyak. Oleh itu, sebatian mentah mempunyai kesan lebih kepada aktiviti pembekuan darah dalam laluan dalaman.

**Kata kunci** : *biji Nigella sativa, aktiviti hemostatik, antipembekuan*

## 1.0 INTRODUCTION

*Nigella sativa* Linn seed belongs to the botanical family of Ranunculaceae (Saad, 1975). It is commonly known as black cumin or black seed. In Malaysia, it is commonly known as *Habbatus sawdah*. *Nigella sativa* is a plant that grows to 20-90 cm tall with finely divided leaves. The flowers are white, yellow, pink, pale blue or pale purple with five to ten petals. The plant has fruit which looks like capsule and consists of several united follicles. Each follicle contains numerous seeds (Sharma et al., 2009). *Nigella sativa* is an annual plant that is traditionally used in the Indian subcontinent (Nadkarni, 1976), Arabian countries (Sayed, 1980), and Europe (Lautenbacher, 1997). The herb is widely used for culinary and medicinal purposes. It acts as medication to cure diseases and illnesses such as hypertension, diabetes, infection, inflammation, headache, eczema, fever, and influenza (Ali & Blunden, 2003). Among Islamic believers, *Habbatus sawdah* is the medicine for every type of diseases except death and ageing (Al-Bukhari, 1983). The seeds of *Habbatus sawdah* contain fixed oils, proteins, alkaloids, saponin and essential oil (Lautenbucher, 1997). The component of fixed oil is unsaturated fatty acid. The major components in essential oil are thymoquinone (2-isopropyl-5-methyl-benzoquinone), ρ-cymene, carvacrol, t-anethole, 4-terpineol and longifoline (El-Dakhakhny, 1963). The most active component is thymoquinone. The effects of the *Nigella sativa* seed extract or its active compound (thymoquinone) have been discovered since 1960 (El-Tahir & Bakeet, 2006). The study showed that the extract has an antioxidant effects, antibacterial, anti-hypertension, anti-inflammatory, antiulcer, anti-ischemic and hypoglycemia agents (Haq et al., 1995; Zuridah et al., 2008; Al-Gaby, 1998; Zaoui et al., 2002; Al-Hader et al., 1993; Akhtar et al., 1996; Hosseinzadeh et al., 2006). Therefore, the present study is design to investigate the presence of anticoagulant activities in the methanol extract of *Nigella sativa* Linn seeds by using *in vitro* laboratory test of blood coagulation parameters and its phytochemical compounds.

## 2.0 MATERIALS AND METHODS

Nigella sativa seeds
The seeds were purchased from a local herbal in Kuantan, Pahang.

### 2.1 Extraction method

A modification of reflux extraction and wetting procedure by H. Zuridah et al. (2008) was used. Six hundred gram of *Nigella sativa* seeds were ground into powder form and soak in 1500 mL of methanol (HmbG Chemical, Germany).

The extracts were incubated for seven days at room temperature (18-25˚C) with at least 5 times vibration per day. Then, the extracts were filtered using Whatman filter paper and evaporated using rotary distillation apparatus. The extracts were finally kept at 4˚C until further testing.

## 2.2 Phytochemical Screening of *Nigella sativa* Linn seeds

The extracts were tested with froth test, xanhoproteic test and alkaline reagent test. The tests were done to screen the presence of saponins, proteins abd flavonoids.

## 2.3 Concentration of extracts

The extraction of the seeds using this method yielded an oleoresin compound and a yellow to greenish oil which were kept in sterile petri dishes and stored at 4˚C for further use. In preparation of the stock solution, the extracted compounds were dissolved in dimethyl sulfoxide (DMSO). Then, the stock solution was diluted with normal saline (0.9% NaCl) to obtain different concentrations of the extracts ranging from 20%, 40%, 60%, and 80%.

## 2.4 Coagulation test

The coagulation assay comprises of the thrombin time (TT), prothrombin time (PT), and activated partial thromboplastin time (APTT) tests. Tests were conducted as described by the manufacturer (Diagnostic STAGO Company, France). The Fresh frozen plasma (FFP) blood group O positive was used in the study. Before used, the FFP was thawed by putting it into a water bath at 37˚C. To assure its viability, the FFP was tested by the PT, TT and aPTT tests.

## 2.5 Statistical Analysis

Data collected in this study was subjected to the one-way analysis of variance (ANOVA) followed by post-hoc Dunnett's multiple comparison using Predictive Analytics Software Statistic (PASW) version 18 for determination of significance difference in mean. P-value of less than 0.05 ($p<0.05$) was considered as significant.

## 3.0 RESULTS

When the seeds were subjected to methanol extraction, the resulting compound was separated into two layers which consisted of an oil and crude bilayer. The finding of an oil product from the extraction was supported by a chemical

investigation on the *Nigella sativa* Linn seeds performed by Greenish (1880). In report, he mentioned that the seeds contain 37% oil and 4.1% ash (calcium salts).

Phytochemical screen show that the both compounds contain similar constituents except for crude that lacks of saponins (Table 1). A rapid evaluation for anti- or pro-coagulant activities, consisting of prothrombin time (PT), thrombin time (TT) and activated partial thromboplastin time (aPTT), was used as screening methods. The pure and diluted (20%, 40%, 60% and 80%) oil and crude components of the methanol extract of the *Nigella sativa* Linn seeds were added to FFP sample at a 1:4 ratio before being applied to blood coagulation assays. As for control, normal saline was added to the FFP sample. The anticoagulant activity of the extract can be detected by the prolongation of the coagulation time taken for the respective screening tests. While reduction in the coagulation time may be due to procoagulant activity of the seed extract.

The PT, aPTT and TT tests shows prolongation in oil compounds and there are significant difference since the p-value was less than 0.05 ($p<0.05$) when compared to control. Meanwhile, the post-hoc Dunnett's multiple comparison showed that results from all concentrations of oil compound of *Nigella sativa* Linn seed were significant (Table 2, Table 3 and Table 4).

The results for PT, aPTT and TT tests also shows transient prolongation of crude compounds and there are significant difference since the p-value was les than 0.05 ($p<0.05$) when compared to control. Meanwhile, the post-hoc Dunnett's multiple comparison showed that results from all concentrations of oil compound of *Nigella sativa* Linn seed were significant (Table 5, Table 6 and Table 7).

Table 1: The phytochemical test of oil and crude compounds of Habbatus sawdah seeds

| **Phytochemical compound** | **Oil compound** | **Crude compound** |
|---|---|---|
| Saponins | + | - |
| Proteins | + | + |
| Flavonoids | + | + |

### 3.1 Haemostatic effect of the oil compound of Habbatus sawdah seeds extract

Table 2: Comparing the mean coagulation time for PT from different concentrations of the oil compound of *Nigella sativa* to that of control using analysis of one-way variance (ANOVA).

| **Variables** | **N** | **Mean Coagulation Time (SD)** | **F-stats (df)** | ***P*-value** |
|---|---|---|---|---|
| **Concentration of Extract (%)** | | | | |
| **20%** | 3 | 20.00 (1.00) | 619.32 (5;12) | < 0.001* |
| **40%** | 3 | 23.67 (3.22) | | |
| **60%** | 3 | 33.00 (2.00) | | |
| **80%** | 3 | 63.33 (1.53) | | |
| **100%** | 3 | 100.00 (3.61) | | |

*The mean difference is significant at the 0.05 level

Table 3: Comparing the mean coagulation time for aPTT from different concentrations of the oil compound of *Nigella sativa* L to that of control using one-way analysis of variance (ANOVA)

| **Variables** | **N** | **Mean Coagulation Time (SD)** | **F-stats (df)** | ***P*-value** |
|---|---|---|---|---|
| **Concentration of Extract (%)** | | | | |
| **20%** | 3 | 38.33 (1.53) | 1173.198 (5;12) | < 0.001* |
| **40%** | 3 | 66.33 (1.16) | | |
| **60%** | 3 | 93.33 (3.06) | | |
| **80%** | 3 | 117.33 (1.16) | | |
| **100%** | 3 | 163.67 (4.93) | | |

*The mean difference is significant at the 0.05 level

Table 4: Comparing mean coagulation time for TT from different concentration of *Nigella sativa* L oil compound to that of control using one-way analysis of variance (ANOVA)

| **Variables** | **N** | **Mean Coagulation Time (SD)** | **F-stats (df)** | ***P*-value** |
|---|---|---|---|---|
| **Extracts concentration (%)** | | | | |
| **20%** | 3 | 44.33 (2.08) | 3231.647 (5;12) | < 0.001* |
| **40%** | 3 | 75.67 (1.53) | | |
| **60%** | 3 | 108.67 (2.52) | | |
| **80%** | 3 | 133.67 (1.53) | | |
| **100%** | 3 | 177.00 (2.00) | | |

*The mean difference is significant at the 0.05 level

### 3.2 Haemostatic Effect of the Crude Compound of the Habbatus sawdah seeds

Table 5: Compared mean coagulation time by different concentration of *Nigella sativa* L. crude compound with mean coagulation time of control using one-way analysis of variance (ANOVA) for PT test.

| **Variables** | **N** | **Mean Coagulation Time (SD)** | **F-stats (df)** | ***P*-value** |
|---|---|---|---|---|
| **Extracts concentration (%)** | | | | |
| **20%** | 3 | 19.67 (3.51) | 36.21 (5;12) | < 0.001* |
| **40%** | 3 | 26.67 (1.16) | | |
| **60%** | 3 | 25.00 (4.36) | | |
| **80%** | 3 | 36.00 (2.00) | | |
| **100%** | 3 | 41.00 (2.65) | | |

*The mean difference is significant at the 0.05 level

Table 6: Compared mean coagulation time of different concentration of *Nigella sativa* L. crude compound with mean coagulation time of control using one-way analysis of variance (ANOVA) for aPTT test.

| **Variables** | **N** | **Mean Coagulation Time (SD)** | **F-stats (df)** | ***P*-value** |
|---|---|---|---|---|
| **Extracts concentration (%)** | | | | |
| **20%** | 3 | 53.00 (2.65) | 5333.733 (5;12) | < 0.001* |
| **40%** | 3 | 98.33 (1.53) | | |
| **60%** | 3 | 141.00 (2.00) | | |
| **80%** | 3 | 196.33 (1.53) | | |
| **100%** | 3 | 240.00 (2.00) | | |

*The mean difference is significant at the 0.05 level

Table 7: Compared mean coagulation time of different concentration of *Nigella sativa* L. crude compound with mean coagulation time of control using one-way analysis of variance (ANNOVA) for TT test.

| **Variables** | **N** | **Mean Coagulation Time (SD)** | **F-stats (df)** | ***P*-value** |
|---|---|---|---|---|
| **Extracts concentration (%)** | | | | |
| **20%** | 3 | 45.33 (3.06) | 1149.725 (5;12) | < 0.001* |
| **40%** | 3 | 71.67 (2.31) | | |
| **60%** | 3 | 93.67 (1.53) | | |
| **80%** | 3 | 117.33 (2.08) | | |
| **100%** | 3 | 145.33 (3.51) | | |

*The mean difference is significant at the 0.05 level

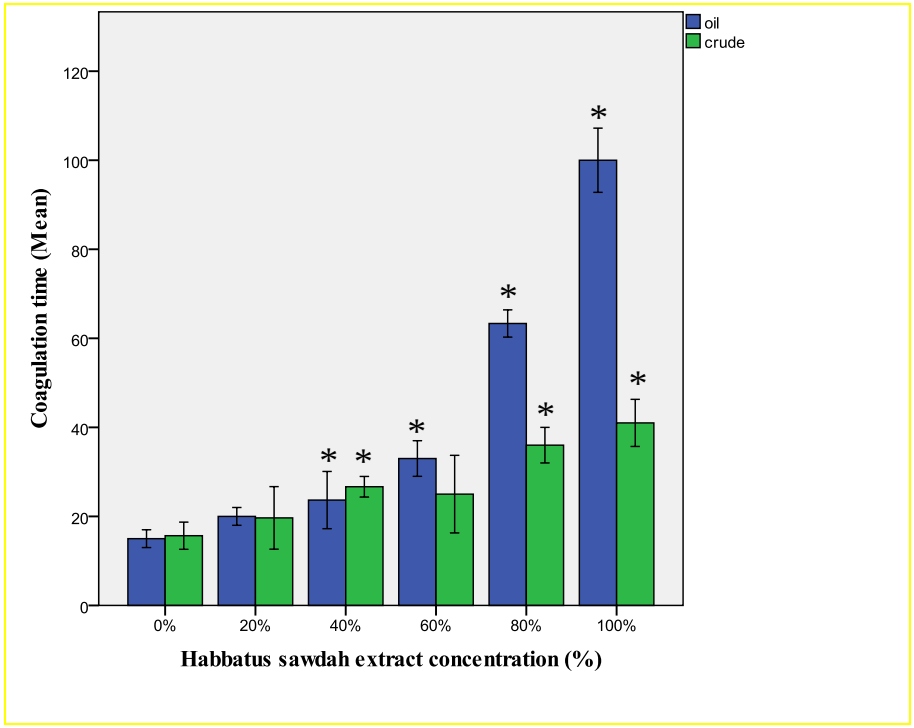


Figure 1: Prothrombin time (PT) for control (0%) and different concentration of *Nigella sativa* L. oil and crude compound. The bars represent the mean ± SD of triplicate reading. The * indices the p-value less than 0.05 which was significant compared to the control (0%) by post-hoc Dunnett's multiple comparison (2-sided).

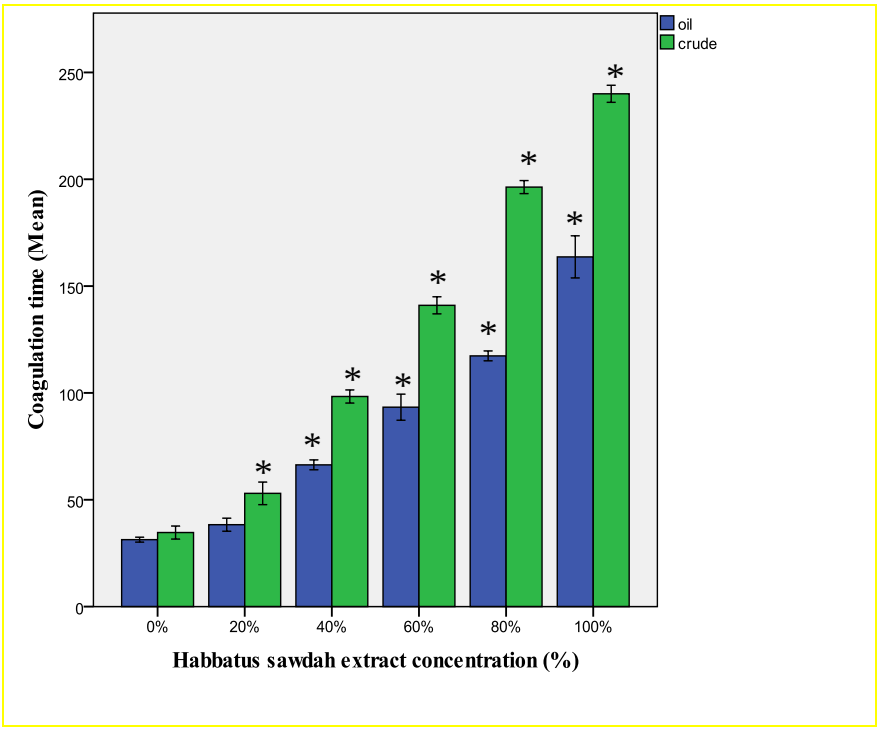


Figure 2: Activated partial thromboplastin time (aPTT) for control (0%) and different concentration of *Nigella sativa* L. oil and crude compound. The bars represent the mean ± SD of triplicate reading. The * indices the p-value less than 0.05 which was significant compared to the control (0%) by post-hoc Dunnett's multiple comparison (2-sided).

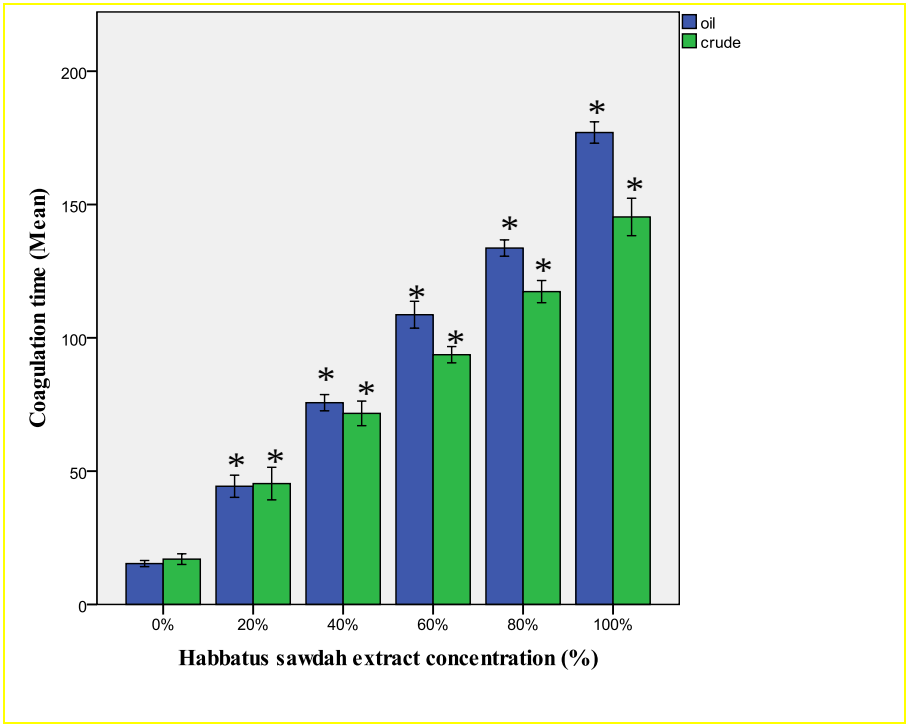


Figure 3: Thrombin time (TT) for control (0%) and different concentration of *Nigella sativa* L. oil compound. The bars represent the mean ± SD of triplicate reading. The * indices the p-value less than 0.05 which was significant compared to the control (0%) by post-hoc Dunnett's multiple comparison (2-sided).

## 4.0 DISCUSSION

*Nigella sativa* Linn or Black cumin is an herbs belonging to the family Ranunculaceae. In our country, this herb is known as Habbatus sawdah. The most special part of the herb is its' seed. A lot of work has been done on the *Nigella sativa* seed by researchers from Egypt (Awad and Binder,2003; Mahfouz et al., 1962), Sudan in Africa and extending to Saudi Arabia (Al-Jishi and Abuo Huzoifa, 2003; Al-Ghamdi, 2001), India (Nadkarni, 1976) and Pakistan (Anwar-ul et al., 2004) in Asia.

The medicinal properties from the seeds of *Nigella sativa* Linn have been written in the Holy Quran. It was stated in the Al-Quran that the Habbatus sawdah is a universal healer for all diseases except ageing or death. In the ancient time, it was the most famous herbs known for its wide range of healing capabilities.

Traditionally, the seeds have been used to treat headache, cough, abdominal pain, diarrhea, asthma, rheumatism and others disease (Hala Gali-Muhtasib et al., 2006; El-Tahir et al., 1993).

However, there have been very few reports on the effect of this seed on haemostasis. To date, the only study was reported in 2003. In their work, Al-Jishi and co-workers have done the research about the influence of *Nigella sativa* seed on haemostatic function in rats (Al-Jishi & Abou Huzoifa, 2003). From their study, they managed to show that the prothrombin time (PT) was transiently prolonged but in contrast, the thrombin time (TT) and activated partial thromboplastin time (aPTT) were reduced as compared to the controls (Al-Jishi and Abuo Hozaifa, 2003). Hence, the seed was suggested to possess variable effect towards the coagulation process in the rats.

Therefore, in the present study, a similar study was conducted to test for possible anticoagulant or procoagulant activity of the *Nigella sativa* Linn methanol seed extract. However, an *in vitro* approach was adopted whereby extracts were mixed with fresh frozen plasma (FFP) before analysis was performed.

The PT was performed to determine the coagulation process in the extrinsic pathway of haemostatic process. The test will determine the functionality of factors I, II, V, VII and X (Preissner, 2004; van Ryn et al., 2010). The results from the PT assay carried out on all of the concentration of the oil compound of *Nigella sativa* Linn seeds showed transient prolonged coagulation time as compared to the control. The results obtained were analyzed by one-way variance (ANOVA) followed by post-hoc Dunnett's multiple comparison. ANOVA showed that the results obtained were significant.

As for the crude compound of *Nigella sativa* seed obtained from the methanol extraction, similar dilution and PT assay were performed. And as predicted, all of the FFP which were mixed with the crude compound appeared to show prolongation in the coagulation time as compared to the control. For statistical purposes, the one way variance (ANOVA) was used to determine the p-value which was essential to classify the differences in coagulation times recorded between the extracts as significant or not. Statistical analysis performed demonstrated that results obtained from the extract were significant since the p-value was less than 0.05 ($p<0.05$) except 20% and 60% for crude compound.

Therefore, the present part of the study appeared to suggest that both oil and crude compound of the *Nigella sativa* Linn seeds possess anticoagulant properties which can inhibit the coagulation process in the extrinsic pathway. The results obtained further support the study of Al-Jishi and Abuo Huzoifa (2003) which found that the prolongation of PT test in the blood of rats after the treatment with the whole crushed *Nigella* sativa seed.

In the clot formation process, the conversion of fibrinogen to fibrin is enhanced by thrombin bypassing all other clotting factors. The functional thrombin could be assayed by performing thrombin time (TT) test. When the oil compound of the *Nigella sativa* extract was tested for their effect on the thrombin time, the results showed that for all concentrations the average coagulation time were highly prolonged compared to the control. In the TT screening tests, the coagulation time appeared to increase as the concentration of the extract was increased. Subsequently, the result obtained were analyzed by one-way variance (ANOVA) followed by post-hoc Dunnett's multiple comparison which showed that results from all concentrations of oil compound *Nigella sativa* Linn seeds were significant.

Determination of the influence of the crude compound on haemostasis using the TT assay also resulted in significant extension of the coagulation time. The coagulation time was shown to increase accordingly as the concentration of extract used was increased. The concentrations of each dilution showed greater prolongation in the coagulation time of TT as compared to that of controls. Thus, in this study, the oil and crude compounds of *Nigella sativa* Linn seed extract were tested using an *in vitro* approach. Results obtained showed a statistical significant transient prolongation of the clotting time. Prolongation of TT result was in contrast to the result from the study by Al-Jishi and Abuo Hozaifa (2003). For the aPTT tests, all concentrations of the oil and crude compound of *Nigella sativa* Linn seed extract also had successfully showed prolongation in their coagulation time when compared to the control. The concentration of each dilution showed extension in the time required for clot formation. Statistical analysis showed that the results obtained were significant.

The prolongation of aPTT indicates that the *Nigella sativa* Linn seeds possess anticoagulant properties which can inhibit the coagulation factors activities of intrinsic pathway such as factor VIII, IX, XI, XII, X, V, prothrombin (II) and fibrinogen.

## 4.1 Interpretation of Oil and Crude Compound for PT, TT and aPTT Result

When taken together, results from the PT, APTT and TT assays for both the oil and crude seed extract of *Nigella sativa* Linn generally showed that the seeds possess anticoagulant properties which can prolong the time required for the FFP to form clot. In other words, the *Nigella sativa* Linn seed extract has the potential to alter the rate of conversion of soluble fibrinogen into insoluble fibrin and also in the polymerization of the fibrin forming visible fibrin clot.

The prolongation of the PT, TT and aPTT test can be due to the presence of coumarine like substance in the both oil and crude compound. Similar hypothesis has been suggested by Al Jishi and Abuo Hozaifa (2003). The presence of coumarine-like-substance will block the carboxylation of several

glutamate residues in prothrombin and factors VII, IX and X as well as the endogenous anticoagulant protein C and S (Deloughery, 2004). The blockade would result in formation of incomplete coagulation factor molecules which are biologically inactive.

The protein carboxylation reaction requires the reduction of vitamin K to reactivate it. The coumarine would prevent this reductive metabolism of the inactive vitamin K (Deloughery, 2004). Hence, the coagulation factors that depend to the vitamin K in coagulation pathway to form the clot will be inhibited. Factor II, VII, IX and X are vitamin K dependent, which mean the prolongation in the coagulation time of PT, TT and aPTT test are due to the presence of coumarine like substance in the oil and crude compound.

As such, disturbance to the activities of factors IX and X explained the prolongation in the aPTT test. As for the extended clotting time for PT and TT, that could be due to reduced activities of factors VII and II, respectively. All of the factors are vitamin K dependent factor.

However, results obtained from this study are not in accordance to reports by previous researchers. In their work, Ghoneim et al. (1982) suggested that Nigella *sativa* Linn induced a reduction in the aPTT and TT test. The present study is not concomitant to the previous study due to difference method of administration and extraction of *Nigella sativa* Linn seeds. Ghoneim et al. (1982) reported an enhancement of coagulation after parenteral administration of *Nigella sativa* petroleum extract in rats while Al-Jishi and Abuo Hozaifa (2003) reported a haemostatic effect after oral administration of *Nigella sativa* powdered seeds also in rats.

### 4.2 Comparison of Anticoagulant Activity between Oil and Crude Compound

The results from the PT assay for the oil and crude seed extracts of *Nigella sativa* Linn showed that both compounds possess anticoagulant properties which can inhibit the activity of coagulation factors such as factor VII, X, V, prothrombin (II) and fibrinogen in the extrinsic coagulation pathway. However, the anticoagulant potential of the oil compound was shown to be more prominent in prolongation of the PT coagulation time as opposed to the oil compound.

When comparing the haemostatic effect of the two compounds on the PT and TT assay, it shown that the *Nigella sativa* Linn seeds oil extract was significantly prolong the coagulation time. Different to the pattern demonstrate by the PT assay, the crude extract was more prominent in extending the coagulation time for APTT.

The difference between the coagulation results of oil and crude compounds can be related to the difference in the phytochemical components for each compound. The screening assay performed in this study managed to showthat both compound contained saponin, protein and flavonoids.

Unfortunately, the full screening test could not be done due to the absence of the Girard's and the Dragendroff's reagents. Previous study that has been done by Mahfouz and El-Dakhakhny (1960) had reported on the isolation of 'nigellone' from the oil extract. In another study conducted by Houghton et al. (1995), it was shown that the main constituent of the volatile oil is thymoquinone which is a sub compound of 'nigellone'. Thymoquinone was among the component of interest as it has been linked many medicinal properties (Mahfouz et al., 1965; El-Dakhakhny, 1963; Houghton et al., 1995). The ability of the oil compound to delay coagulation in the PT and TT tests may be due to the presence of thymoquinone or other compositions like alkaloid, saponin and flavonoids. These elements may be more in the oil compound compared to the crude compound. Therefore, further tests should be performed to assess the presence of thymoquinone and other compositions and also to quantify the amount of each compound in the oil and crude extracts.

Meanwhile, the crude compound exhibited a more potent effect on the aPTT. This may be due to the different constituents in the crude compound. Anticoagulant effect exits in plant may be because the plant contains polysaccharides that have been linked to the production of unfraction heparin drug which is usually extracted from animals. At present, the polysaccharide was also found in plants such as marine alga (Mayer et al., 2007). It is likely that the crude compound may possess more fiber of polysaccharides that may affect the mechanism of the intrinsic pathway.

## 5.0 CONCLUSION

The results of the present study show that *Nigella sativa* seems to have transient anticoagulant effect. Since both compounds have the anticoagulant activity towards haemostatic activity, there is potential to use the *Nigella sativa* seed as one of the anticoagulant agents. Besides, it can fulfill the characteristic of an ideal anticoagulant agent. Bounameaux (2009) stated in his article that the ideal anticoagulant must inhibit both free and clot-bound activated coagulation factors, nonspecific plasma protein binding, little interaction with food and other drugs and no routine monitoring of coagulation or platelet count required (Bounameaux, 2009). Further studies are need to determine the exact effect of *Nigella sativa* on the clotting factors by assaying their levels, on the recently identified markers of haemostatic activation and on the other natural anticoagulants.

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