

**Antioxidant and anticoagulation activities of flavonoid glycoside group
extracted from ginger**

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Abstract:

The flavonoid group is one of important component of ginger and composed from a large polyphenolic group containing a benzo- γ -pyrone structure that present in tissues of plant with high relatively concentrations of sugar or non sugar (a glycone). Flavonoids have antioxidant activity with polyhydroxylated substitution on A and B rings through they have ability to donate the hydrogen atoms and scavenge the free radicals that formed by lipid and/or by metal ions chelation peroxidation. Substances have highly antioxidant activity also have anticoagulant activity, therefor flavonoid groups also have anticoagulant effects. So flavonoids have many medical application in the treatment of cancer, cardiovascular diseases, age related diseases, reduce inflammation, reduce mutagenesis of human cells as well as reduce blood glucose and lipid. In this study, flavonoid glycoside group was extracted from ginger plant and was measured antioxidant and anticoagulant activities by Lee method (DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging test) and in vitro anticoagulant procedure (prothrombin time test and activated partial thromboplastin time test).

Key words: Flavonoid glycoside, antioxidant activity, anticoagulant activity.

Physiology Classification QP 501-801

Introduction:

Ginger is a famous medicinal plant that widely spread in Asia regions and over the world with a long history of traditional uses as medicinal herbe. It undergoes of Zingiberaceae family[1]. It has widely pharmacological effects [2,3] like relief cold, cough, throat infection [4], nausea, stomach pain, diarrhea, asthma [3], arthritis, joint pain, heart diseases and lung diseases [5].

Ginger is containing many chemical groups include flavonoids, polyphenols, tannins [6], carbohydrates, extractable oleoresins, vitamins, many fats and minerals [7].

Flavonoids are composed from a large polyphenolic group containing a benzo- γ -pyrone structure that present in tissues of plant with high relatively concentrations of sugar or a glycones [8,9]. The family of flavonoid includes flavonols, iso-flavonols, flavones, anthocyanidins, anthocyanins, catechins and proanthocyanidins [10,11]. All flavonoids have 3-ringed structures and they are derived from phenylalanine, aromatic amino acids and tyrosine as shown in figure (1)[12]. The differences between flavonoids structure due to the pattern and the scale of prenylation, hydroxylation, glycosylation and alkalization reactions that change the basic molecule [13].

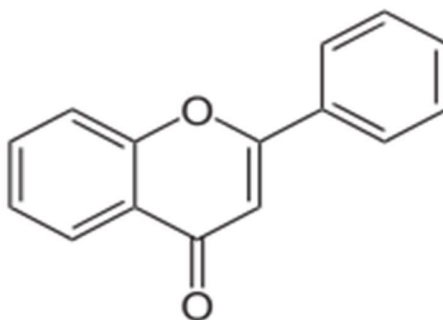


Fig.1: Molecular structure of the flavone backbone (2-phenyl-1,4-benzopyrone) [12]

The flavonoid groups have antioxidant activity with polyhydroxylated substitution on A and B rings through they have ability to donate the hydrogen atoms and scavenge the free radicals that formed by lipid peroxidation [14,15] and/or by metal ions chelation [16,17]. Substances have highly antioxidant activity also have anticoagulant activity[18], therefore flavonoid groups also have anticoagulant effects[19,20,21]. So flavonoids have many medical application in the treatment of viral and bacterial infections, cancer, cardiovascular diseases, age related diseases [22,23,24,25], reduce inflammation [26,27,28], reduce mutagenesis of human cells [29,30,31] as well as reduce blood glucose and lipid [32].

Firstly, The dry ginger rhizomes of ginger plant were collected from herbs market, pulverized by a mechanical grinder and stored in airtight glass containers in dark until extraction.

Extraction of the sample

One gram of ginger powder was weighed and dissolved in 100 ml of ethanol. The mixture was shaken in an electronic shaker for 3 hours at room temperature, then it was centrifuged for 20 minutes at 4000 rpm and filtered by filter paper (whatman no.1) [6].

Extraction of flavonoid glycoside group:

The aim of this study to estimate antioxidant and anticoagulation activities of glycoside flavonid group extracted from ginger.

Materials and methods:

In this study, flavonoid glycoside group was extracted from ginger plant and was measured antioxidant and anticoagulant activities by Lee method (DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging test) [33] and in vitro anticoagulant procedure (prothrombin time test and activated partial thromboplastin time test) [34].

Flavonoid glycoside was extracted from ginger with 50% methanol, then the mixture was acidified by 1.2 M HCl. Ascorbic acid was used to prevent mixture oxidation. At 80 °C, the hydrolysis of the flavonoid glycoside was taken out for 2 hours [35].

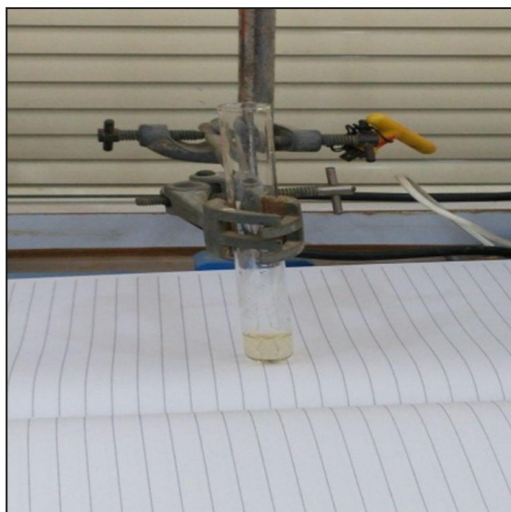
Detection of flavonoids:

Sodium hydroxide test

Five milligrams of the compound was dissolved with water, warmed and filtered. Then aqueous sodium hydroxide (10%) was added to 2 ml of this solution and a yellow color was appeared. After that, dilute hydrochloric acid was added and the color of solution was change

from yellow to colorless that indication for the presence of flavonoid glycoside and the change

of color was appeared in figure 2 [36].



(a)



(b)

Fig.2: detection of flavonoids (a) addition of 10% aqueous sodium hydroxide (yellow color was appeared) (b) addition of dilute hydrochloric acid (yellow color was disappeared)).

**Determination of antioxidant activity of flavonoid glycoside [33]:
DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging test:**

DPPH free radical scavenging by flavonide glycoside was measured by Lee method.

Positive control (stander):

Negative control (blank): 4 ml of ethanol and 1 ml of DPPH

Four different concentrations of flavonide glycoside were prepared

4 ml of flavonoid glycoside (0.05 mg/ml) and 1 ml of DPPH (0.4 mg/ml)

4 ml of flavonoid glycoside (0.1 mg/ml) and 1 ml of DPPH (0.4 mg/ml)

4 ml of flavonoid glycoside (0.15 mg/ml) and 1 ml of DPPH (0.4 mg/ml)

4 ml of ascorbic acid (0.05, 0.1, 0.15, 0.2, 0.25 mg/ml) and 1.0 ml of DPPH (0.4 mg/ml)

4 ml of flavonoid glycoside (0.2 mg/ml) and 1 ml of DPPH (0.4 mg/ml)

4 ml of flavonoid glycoside (0.25 mg/ml) and 1 ml of DPPH (0.4 mg/ml)

Wait for 30 minutes in dark place at room temperature and measured the absorbance by spectrophotometer (520 nm). The ability of flavonide glycoside to scavenge free radical by equation:

$$\text{Control OD} - \text{Sample OD}$$

Radical scavenging activity % = $\frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$

Determination of anticoagulant activity of flavonoid glycoside [34]:

In vitro anticoagulant procedure [34]:

Blood samples were collected from female adult healthy volunteers, don't take medication for at least two weeks and stored in anticoagulant tubes. The plasma was prepared by centrifugation of collected blood (800 g) at room temperature for 10 minutes and then PT and APTT were measured.

Prothrombin Time (PT) test:

PT test was evaluated of The action in extrinsic pathway of coagulation

Procedure: -90 μL of plasma mixed with 10 μL of flavonoid glycoside solutions (0.5 $\mu\text{g}/\mu\text{L}$, 1 $\mu\text{g}/\mu\text{L}$ and 2 $\mu\text{g}/\mu\text{L}$) and incubated for 5 min at 37°C

-Heparin (1IU/ml) was positive control

-Plasma was control (without anticoagulant activity)

-PT assay reagent (200 μL) was pre-warmed for 10 min at 37 °C and added to the samples and recorded the clotting time by coagulometer.

Activated partial thromboplastin time (APTT) test:

APTT test was evaluated of The action in intrinsic pathway of coagulation.

Procedure:

-90 μL of plasma mixed with 10 μL of flavonoid glycoside solutions (0.5 $\mu\text{g}/\mu\text{L}$, 1 $\mu\text{g}/\mu\text{L}$, 1.5 $\mu\text{g}/\mu\text{L}$ and 2 $\mu\text{g}/\mu\text{L}$) and incubated for 5 min at 37°C.

-Heparin (1IU/ml) was positive control

-Plasma was control (without anticoagulant activity)

-APPT reagent was pre-warmed for 2 min at 37 °C and added to the samples and recorded the clotting time by coagulometer.

Analysis:

The data of this study was analysis by SPSS version 15. Where the t-test used for find the differences between antioxidant activities of ascorbic acid and flavonoid glycoside. Regression used to find the relationship between the concentrations of flavonoid glycoside and PT and APTT.

Results:

The antioxidant activity of flavonoid glycoside group was measured by percent of radical (DPPH) scavenging activity. In this study, the absorbance of ascorbic acid was more than absorbance of flavonoid glycoside group and that means the antioxidant activity of flavonoid glycoside group was more than antioxidant activity of ascorbic acid because it was reverse relation between antioxidant activity and absorbance and it was significant difference between absorbance of ascorbic acid and flavonoid glycoside ($P < 0.05$) as shown in table(1) and fig (3).

The anticoagulant activity of flavonoid glycoside group was measured by PT and APTT times. When the concentration of flavonoid glycoside group was increased lead to increase of PT times ($P < 0.05$, $R=0.988$, $R^2=0.976$) as appeared in figure (4) and the higher one at 2.5 mg/ml was 17.3 seconds nearly from heparin (positive control) (20 seconds) as appeared in table (2).

Also APTT times flavonoid glycoside group was raised with concentration ($P < 0.05$, $R=0.9875$,

$R^2=0.975$) as shown in figure (5) and the higher one was 45.2 seconds at 2.5 mg/ml more than

heparin (44.4 seconds) as shown in table (3). that means flavonoid glycoside had anticoagulant activity.

Table 1: absorbance of ascorbic acid and flavonoid glycoside and percent of radical scavenging activity (antioxidant activity) of flavonide glycoside:

No. of samples	Concentrations of ascorbic acid	Absorbance (nm)	Concentrations of flavonoid glycoside	Absorbance (nm)	percent of radical scavenging activity (antioxidant activity) of flavonoid glycoside
1	0.05 mg/ml	768	0.05 mg/ml	108	85.9%
2	0.1 mg/ml	760	0.1 mg/ml	96	87.4%
3	0.15 mg/ml	694	0.15 mg/ml	82	88.2%
4	0.2 mg/ml	535	0.2 mg/ml	77	85.6%
5	0.25 mg/ml	514	0.25 mg/ml	53	89.7%

* $P < 0.05$ (it was significant difference between absorbance of ascorbic acid and flavoniod glycoside)

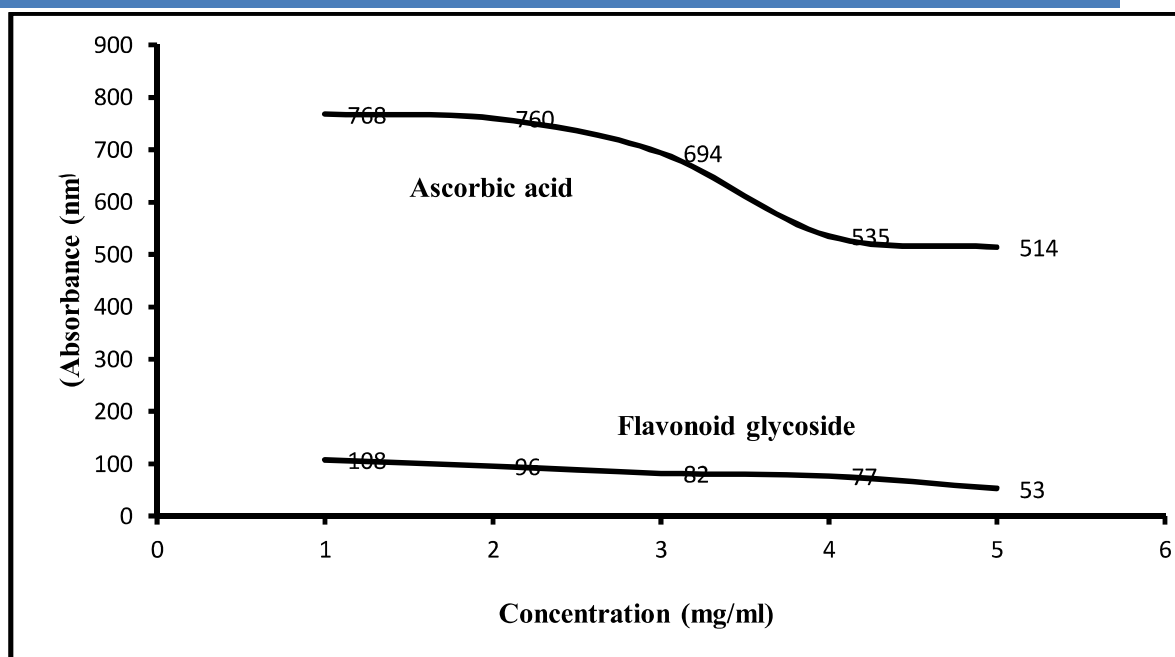


Fig.3: absorbance of ascorbic acid and flavonoid glycoside at different concentrations (1=0.05, 2=0.1, 3=0.15, 4=0.2, 5=0.25)

Table 2: Prothrombin time of flavonoid glycoside compounds at different concentrations

Samples	Prothrombin time (seconds)
Plasma (control)	12.2
Heparin (positive control)	20
Flavonoid glycoside (0.5 mg /ml)	12.6
Flavonoid glycoside (1 mg/ml)	13.7
Flavonoid glycoside (1.5 mg/ml)	15.5
Flavonoid glycoside (2 mg/ml)	16.7
Flavonoid glycoside (2.5 mg/ml)	17.3

*P < 0.05, R = 0.988, R² = 0.976

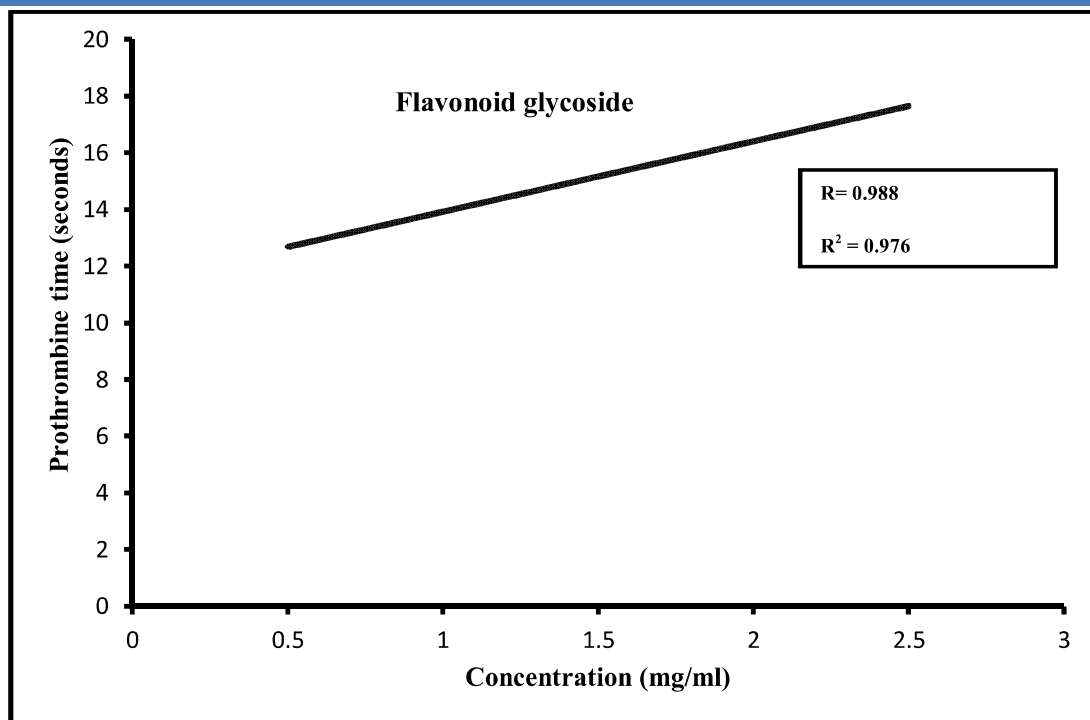


Fig.4: Prothrombin time of flavonoid glycoside at different concentrations (0.5, 1, 1.5, 2, 2.5 mg/ml)

Table 3: Activated partial thromboplastin time of flavonoid glycoside compounds at different concentrations

Samples	Activated partial thromboplastin time (seconds)
Plasma (control)	33.2
Heparin (positive control)	44.4
Flavonoid glycoside (0.5 mg/ml)	32.8
Flavonoid glycoside (1 mg/ml)	34.6
Flavonoid glycoside (1.5 mg/ml)	37.4
Flavonoid glycoside (2 mg/ml)	42.3
Flavonoid glycoside (2.5 mg/ml)	45.2

*P< 0.05, R= 0.9875, R²=0.975

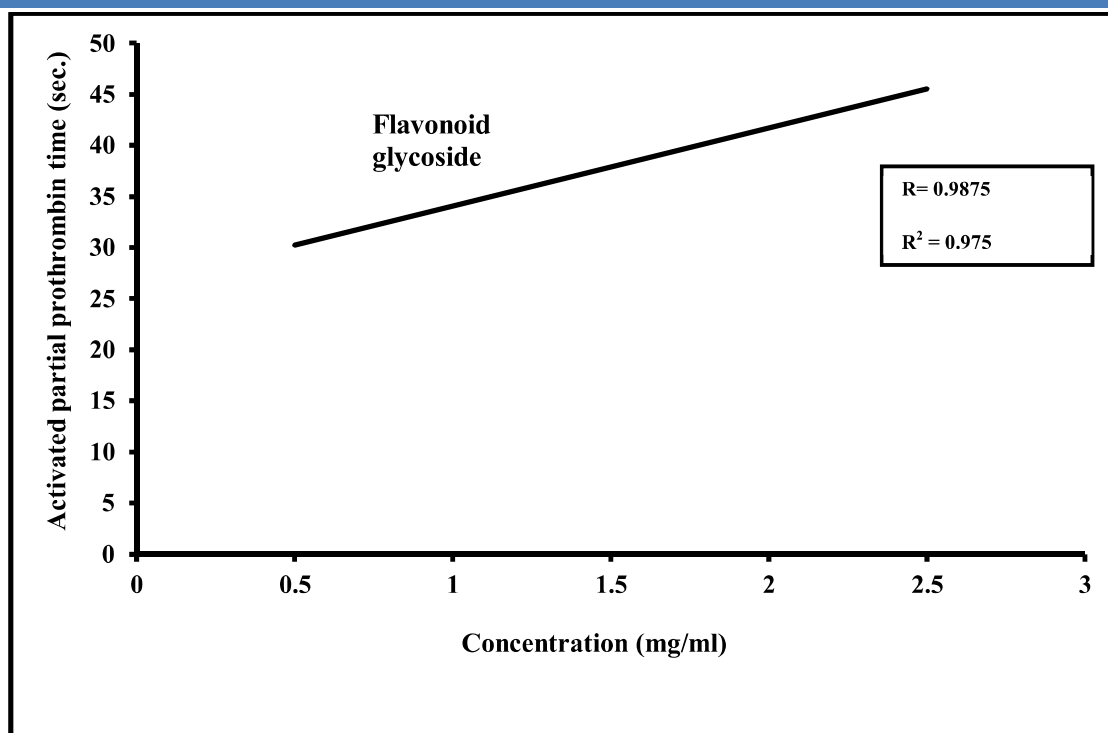


Fig.5: Activated partial thromboplastin time of flavonoid glycoside at different concentrations (0.5, 1, 1.5, 2, 2.5 mg/ml)

Discussion:

After the extraction of pure ginger powder, flavonoid glycoside group was extracted by methanol and 1.2 M HCL at 80°C and then the detection of this group by sodium hydroxide test. Two of important pharmacological effects of flavonoid glycoside was measured (antioxidant and anticoagulant activities) by DPPH radical scavenging test and PT and APTT time tests.

Antioxidant activity of flavonoid glycoside was measured by the ability of flavonoid glycoside to scavenge of free radical DPPH and ascorbic acid was used as a positive control due to its strong antioxidant capacity and that was related to enediol structure which can be oxidized easily to diketones [37].

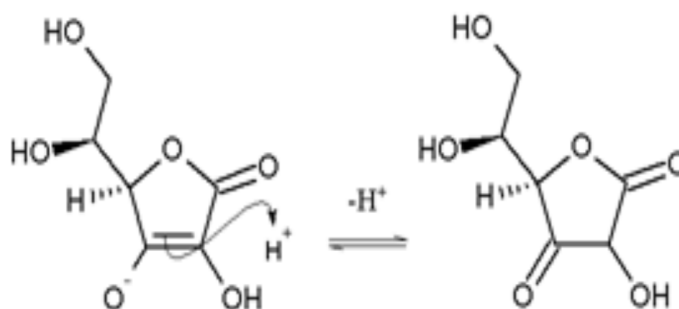


Fig. 6: The mechanism of antioxidant activity of ascorbic acid [39]

$R^2=0.976, 0.975$) and when the concentrations of flavonoid glycoside were increased lead to elevate both PT and APTT times. this result was reflected flavonoid glycoside had anticoagulant activity. Thrombin (factor II) is one of factors that play an important role in coagulation and hemostasis processes [46,47] through many mechanisms include thrombin converts the soluble fibrinogen into the insoluble fibrin clot [48], also thrombin activates platelets and stimulates the other coagulation factors (FV, FVIII, FXI) on the platelet's surface [49] and thrombin stabilizes the clot by activation of factor XIII (transglutaminase) [50,51]. Flavonoid glycoside was consider as a poly phenolic compound and had direct thrombin inhibitor affect by inhibiting thrombin amidolytic activity [47,52], therefore flavonoid compound could reduce cardiovascular risk factors, including blood coagulation, blood pressure, platelet aggregation and plasma lipids [47,53].

Conclusion:

In this study, flavonoid glycoside is one of important content of ginger and had strong antioxidant activity, also had anticoagulant activity. Their activities could be used in many pharmacological applications.

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Flavonoid glycoside was scavenged of free radical DPPH and could be change the color of solution from violet to yellow at 5 minutes that mean this group had antioxidant capacity [6]. This activity was related to either electron donation or hydrogen capture processes [38,39]. The antioxidant capacity of flavonoid glycoside was more than ascorbic acid ($P<0.05$) and this property was increased with concentration (0.05 mg/ml – 108 nm to 0.25 mg/ml – 53 nm). The percent of radical scavenging activity (antioxidant activity) of flavonoid glycoside was high and it was ranged from 85.6 to 89.7% that means flavonoid glycoside had strong antioxidant effect [40,41] .

The other pharmacological effect of flavonoid glycoside was anticoagulant. This effect could be evaluated by measuring PT and APTT times and heparin (sulfated polysaccharide) act as positive control due to its strong anticoagulant property and it has highly negative charge density (3000 to 30000 Da) [42,43] therefore it could bind to the antithrombin III lead to conformational change and activation of it [44]. The activated antithrombin lead to inhibit thrombin and other target proteinases [43,45] .

Prothrombin time of flavonoid glycoside was increased with elevated of concentrations from 0.5 mg/ml=12.6 sec to 2.5 mg/ml=17.3 sec. slightly less than PT of positive control (heparin=20 sec.), while APTT of flavonoid glycoside was increased from 0.5 mg/ml=32.8 sec. to 2.5 mg/ml= 45.2 sec. which was slightly more than positive control (heparin= 44.4 sec.). PT and APTT of flavonoid glycoside at high concentration nearly from PT and APTT of heparin that mean this group had also anticoagulant activity. On other hand, there was significant relationship between the concentrations of flavonoid glycoside and PT and APTT times ($R=0.988, 0.9875$ and

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الخلاصه:

مجموعه الفلافوناييد هي احدى المكونات المهمه في الزنجبيل ويتكون من مجموعته متعدده الفينول كبيره التي تحتوي على بنزو الفايبورين الموجود في انسجه النباتات وهذه المجموعه تحتوي جزء سكري و جزء لاسكري جلايكون. مجموعته الفلافوناييد تمتلك فعاليت مقاومه للتاكسد وذلك لوجود المجاميع متعدده الهيدروكسيل ضمن الحلقات A و B (المكونه للفلافوناييد) من خلال قدرته على وهب ذرات الهيدروجين وازاله الجذور الحره التي تتكون من الدهون او من خلال الارتباط البروكسيد مع ايونات المعادن. ومن المعلوم ان المواد التي تمتلك فعاليت عاليه لمقاومه التاكسد تمتلك ايضا فعاليت مانع للتخثر، لذلك مجموعته الفلافوناييد تمتلك ايضا فعاليت مقاومه التخثر. ولهذا السبب هذه المجموعه لها الكثير من التطبيقات الطبيه في علاج السرطان، الامراض القلبيه، الامراض لها علاقه بالعمر، تقليل الالتهاب، تقليل تغير الجيني لخلايا الانسان وتقليل نسبة السكر والدهون بالدم. في هذه الدراسه استخلصت مجموعته الفلافوناييد جلايكوسايد من الزنجبيل وقمنا بقياس فعاليت مقاومه التاكسد ومانع للتخثر من خلال طريقه (Lee method) و طريقه (In vitro anticoagulant procedure).

الكلمات المفتاحيه: فلافوناييد جلايكوسايد، فعاليت مقاومه التاكسد، فعاليت مانع للتخثر.