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Identification and Evaluating of some Alkaloids compounds from *Zingiber officinals* (L.) as Coagulant Agent

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Abstract :
The present study was conducted in biotechnology laboratories and animal house of the Department of biology in Faculty of Science / University of Kufa for the period from October 2017 until March 2018 to evaluate the biological activity of aqueous and ethanol alcohol alkaloids extracts of *Zingiber officinals* plant on Blood coagulant Agent used adult male Albino rats .This study was orally administration of different concentrations of Ginger alkaloids extract on Blood coagulant biochemistry during 21days. Medicinal plant is collected from Al-Najaf shops , rhizome parts was separated after cleaning from dust and dry at the shape and grounding by Blender. The active compounds were studied using ethanol alcohol and water in extraction methods . Phytochemical analysis used thin Layer Chromatography (TLC) technique of extracts .Ginger extract were divided into three concentration (1,5& 10) mg/kg (B.W) with treatment groups in 6 replicates. At the end of the experiment bleeding & clotting time, RBC,WPC, Platelets, were measured. 21 healthy male Albino rats aging (6-8 month) were divided randomly into 3 groups with control group by 3 male animals in each concentration, as in the below:
Group 1: Healthy or control groups, Group 2: healthy rats were given daily dosage orally by concentrations of 1,5and 10 mg/kg. (B.W) once daily for 21 days respectively of aqueous extract. Group 3: healthy rats were given daily dosage orally by concentrations of 1,5and 10 mg/kg. (B.W) once daily for 21 days respectively of ethanolic extract.
After 21 days, blood samples were taken for biochemical analysis.
Thin Layer Chromatography (TLC) technique of both type of alkaloids extracts (Alcoholic and aqueous) investigated presence of several bioactive compounds include the Alkaloids, Phenols , glycoside and flavonoids compounds .However, both type of Ginger extracts containing more active compounds in ethanolic extract . The results of the current study indicated a significant decrease ($p<0.05$) in the bleeding & clotting time for both type of extracts of sample compared with the control group. Also, The current study investigated that a significant increase ($p <0.05$) in the level of each of WBC , RBC and platelets for both type of extracts compared with the control group. Also, alcoholic and aqueous extract of Ginger caused non significant effect ($p<0.05$) in all Clotting factors for both two extracts in (1,and 5)mg/kg (B.W) but caused significant effects with 10 mg/kg (B.W) compared with the control group. Therefore, this study recommended that alcoholic and aqueous extract of Ginger has increased dietary intake of for both type of extracts which may be beneficial for blood coagulation.

INTRODUCTION

The relationship between medicinal plants and human have grown and increase with increases number of plants that used as medicines .The growth of knowledge to treat diseases continues to accelerate by increasing number of drugs originated from plant (1). Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, disease,and herbiv orous mammals include phenolic , flavonoids , alkaloids , glycoside and tannin very important to stress adaption besides the important for the plant itself, antimicrobial and anti-inflammatory plants phytochemicals used as source of potential and powerful drugs .(2) .The World Health Organization (WHO) reported that about 70-80% of the world populations, depend on natural products in their primary health care (3). The fact that medicinal plants is a widespread result from that they are healthier and safer than synthetic medicine (4).During the last twenty years, the haemostasis system was a subject of intense interest through field phytochemicals importance; reviews are available that describe these theoretical studies of blood coagulation and platelet-dependent (5). The aim of the study: In the present study we have studied the effect of ethanolic and aqueous extract of ginger plant on the blood biochemistry parameters in through 21 days of treatment.

Significance of study

The significance of this current study is to document information on the phytochemicals profile from Ginger plant as a nutraceuticals and pharmacological material. So far, there is few data in literature on the bioactive components isolated from Ginger plant as properties of haemostasis . Therefore, knowledge is needed to facilitate effective popularization of the Ginger in Iraqi an increasing evidence that for medicinally important ; offering great hope for the development of new drugs that have effect on Blood Coagulant properties which afflict humanity today (6).

MATERIALS AND METHODS

Ethanolic and water rhizomes extraction: The rhizomes plants were extracted with two type of solvent , distilled water , ethanol alcohol. Take 20 grams of finely powdered material of sample and 200 ml of distilled water were refluxed in flask 500ml with magnetic stirrer plate for 24 hour at room temperature , then filtered by multi- layer of muslin cloth then by filter paper type Whattman, No (1) and dried in oven (40-30)°C. After that the extract was kept in refrigerator until it was used. (7). The same procedures of ethanolic extraction methods of ginger rhizomes used according to methods by (8).
Three concentration of extract sample used in this study were prepared by dissolved **(2)g** of extracted plant material in (30)ml of normal saline to prepare a stock solution, which is used to prepare concentrations (1,5,10)mg \kg (B.W) of both aqueous and alcoholic extract of Ginger.

Preliminary Qualitative Detection:

Thin Layer Chromatographic (TLC):

TLC was used in the identification of isolated alkaloid compounds. The dried plates were activated at 100 °C for 30 minutes in an oven before used and cooled at room temperature. The sample was spotted by capillary tube . The spot was allowed to dry and then the plate was placed in the glass container (Jar) with the solvent previously placed in the bottom of the tank to a depth of 10 mm. The mobile phase solvent of alkaloid was prepared from (25%NH4OH:Water:Acetone) ratio (3:7:90). This solvent allowed compound to rise by capillary flow to the top of the plate. However, the mobile phase reaches to two centimeters from the top of the plate then the plate was removed and allowed to dry. then separated spots sites was identified with the naked eye and then under the ultraviolet rays (UV) at 366 wavelengths. Relative flow (R*f*) was calculated from below equation: (2)

R *f* = distance a compound moves / distance solvent front

Experimental Animals design

Twenty one adult albino male rates and age between (8-10) weeks were purchased at animal house of university of kufa / College of Science, were kept in the in plastic cages at temperature (23- 28)°C, 50% of humidity and lighting (11 hours of light and 13 hours of darkness) , and these have received the same diet. The animals were allowed free access to pellets and water. The experimental animals (21 rats) were divided to 3 groups by 3 male animals in each concentration and, as in the below:

Group (1): were received food and water and were considered as control.

Group (2): Animals were received orally with aqueous alkaloid extract of *Z.officinals* at (1,5 and 10) mg/kg (B.W) concentration daily for 21 days respectively with each concentration.

Group (3) : Animals were received orally with alkaloid ethanolic extract of *Z. officinals* at (1,5 and 10)mg/kg (B.W) concentration daily for 21 days respectively with each concentration. All groups of rats were kept under same conditions and have received the same diet.

Blood biochemistry

Bleeding Time Measurement

Rats tail was cut with a scalpel 1-2 cm proximal from the end of treatments and bleeding time was calculated from the time of starting of bleeding till bleeding stopped. Spots were made with the bleeding tail on a blotting paper every 15 seconds till bleeding stopped and bleeding time was calculated accordingly. Or the time taken between the appearances of blood to the cessation of bleeding is taken as the bleeding time expressed in minutes (9).

Clotting Time Measurement

Blood was drawn into a capillary tube. The time of appearance of the drop of the blood on the cut tail was noted. The capillary glass tube is then kept between the palms of both hands for 30 second to keep it at body temperature. After 30 second the tube was taken out . the capillary tube was broken at regular intervals of 10 seconds, until a thread of clotted blood appears between the two pieces of capillary glass tube. The time interval between the appearance of the drop of the blood and the thread of the blood clot was the clotting time of the blood sample of the mouse expressed in minutes (9).

Statistical analysis

The experiments were conducted and analyzed as factorial experiments with three replications using a completely Randomized Design (CRD) with two or three factor tested by Least Significant difference (L.S.D) at probability of 1% ($P \leq 0.05$) (10).

RESULTS AND DISSECTIONS

Qualitative phytochemical Tests

TLC profile for Alkaloids

Solvent systems used for the separation of the following phytochemical compounds alkaloids, were a mixture of **(25% NH4OH:Water:Acetone) ratio (3:7:90)v/v/v .** And seen under Uv- light at 366 wavelength to show deferent natural compounds (6). Data in (Table 1) indicate that presence of 4 different spots were recorded with *R*f values that (0.29,0.55,0.79,0.91) at Weak white, , Weak purple , Light pink and Dark orange) color spots in ethanolic extract of Ginger plant respectively. Aqueous extract of Ginger recorded(6) spots with *R*f value of (0.22, 0.32,0.55,0.61 and 0.82) at Blue to green, Dark blue, Weak blue, White to blue and Light brown color spots respectively. The highest number of alkaloids compounds was detected in aqueous extract of Ginger recorded (6) compounds. The *R*f values of both type of extracts in this study , showing the same spots as previously described under the same conditions. The slight differences between these extracts about chemicals contents may be due to the different of cultivation conditions , pH and temperature solvent .The influence of pH on the degradation rate of some phytochemical**. (**11).

Table (1) Alkaloids compounds detection in aqueous and ethanolic extract of Ginger plant .

Type of extract	No. of spot	R*f*	Color in UV light
Zingier			
Ethanol	1	0.29	Weak white
	2	0.55	Weak purple
	3	0.79	Light pink
	4	0.91	Dark orange
Water	1	0.22	Blue to green
	2	0.32	Dark blue
	3	0.55	Weak blue
	4	0.61	White to blue
	5	0.88	Light brown

Figure (1) : TLC detection in water and ethanolic extract of Ginger plant.A:(ethanolic extract), B(aqueous).

Effects of ethanolic & Aqueous extracts of *Z. officinals* on bleeding & Clotting time in experimental rats after 21 days.

This study was carried out to determine the possibility of Zingier rhizomes alkaloid extracts on the hemostatic mechanism, with understand the primary affects on bleeding time. Bleeding time evaluates the platelet responses with hemostasis. (12).Three concentrations (1, 5, 10)mg/kg (B.W) of Ginger crude alkaloid extract for(7,14 and 21) days continuously. Data in the table (2) showed that all concentrations (1,5 and 10) mg/kg (B.W) of ethanolic & aqueous crude alkaloid extract of Ginger plant decreased the bleeding time to (30 ± 0.8; 35 ± 0.8 ;24 ±1.6) minute for ethanolic extracts and (40 ± 3.6 ; 38 ± 1.6 ; 40 ±4.1) for aqueous extracts respectively and compared with control rats treatment (48 ± 0.19) minute after (21) days of treatments .

However, Data in the same table showed that all concentration (1,5 and 10) mg/kg (B.W) of ethanolic & aqueous crude alkaloid extract of Ginger also decreased the Clotting time to (24±2.4 ; 20±1.6 ; 10±1.6) minute for ethanolic extracts and (35 ±0.8; 30 ± 4.1 ; 29 ± 0.8) minute for aqueous extracts respectively compared with control rats treatment ((10±0.60) minute after (21) days of treatments.

Ethanolic extracts concentration of oral administration at (21 days) of Ginger plant extracts has significant effect ($p<0.05$) on reducing bleeding time and Clotting time in compared with the control group. Oral administration of 1mg/kg (B.W) of Ginger ethanolic extract show that significantly reduced ($p<0.05$) of bleeding & Clotting time in compared with control groups . All extract concentrations has effect on bleeding & Clotting time but the 10mg/kg (B.W) concentration at 21 days is more effect and recorded significantly decreased ($p<0.05$) of bleeding time& Clotting time . On the other hand it was found that ethanolic extract of Ginger plant in 5mg/kg (B.W) concentrations did show non significantly effect ($p<0.05$) on bleeding & Clotting time in compare with other concentration. Also, bleeding time & Clotting time effected with ethanolic extracts was less than that for normal bleeding time by 3-4 min in blood samples of all the subjects tested. Bleeding & Clotting time determination is an importance laboratory test, carried out to diagnose abnormality in blood clotting time .

Decrease in normal bleeding time signifies effects on coagulant properties. As ethanolic & aqueous alkaloids extracts of the Ginger plant reduces the bleeding time uniformly in the blood samples of all the experimental rats , it can be suggested that ethanolic & aqueous alkaloids extracts of Ginger plant is the same possesses hemostatic activity. (10).

The result show in figure (2A) that the aqueous extract of Ginger have significantly increased ($p<0.05$) of bleeding time more effect on than ethanolic extract,. Also, show that the aqueous extract of Ginger that have significantly increased ($p<0.05$) of clotting time more effect on than ethanolic extract , figure(3B).

Table 2: Effects of Ethanolic & Aqueous alkaloids extracts of *Z.officinals* on blood character in experimental rats after 21 days.

Blood character mean (± SD)	Conc. of **cruds** alkaloid extract mg/kg b.w				LSD
	Controls	1mg/kg B.W	5mg/kg B.W	10mg/kg B.W	
Ethanolic extracts					
Bleeding time	48±0.19	30±0.8 **ň**	35±08 **Ş**	24±1.6 **Ş**	6.6
Clotting time	40± 5	24±2.4 **ň**	20±1.6 **Ş**	10±1.6 **Ş**	4.6
Platelets count	330±49.7	335±40	464±115 **Ş**	467±143 **ň**	55.4
RBC	4.9±0.6	5.8±0.8	5.8±1.3	5.7±1.3	non
WBC	4.5±0.8	4.9±0.8	5.5±1.7 **ň**	6.1±.1 **Ş**	1.4
Aqueous extracts					
Bleeding time	48±0.19	40±0.4s	38±1.6 **ň**	40±4.1 **ň**	6
Clotting time		35±0.8	30±4.1 **Ş**	29±0.8 **ň**	2.2
Platelets count	330±49.7	535±137	380±120 **Ş**	414±152 **Ş**	33.7
RBC	4.9±0.6	5.6±0.9	5.7±0.7 **Ş**	6±1.4 **Ş**	0.8
WBC	4.5±0.8	7.1±1	7.5±1.8 **Ş**	7.9±1.5 **Ş**	2.7
(Ş) $p < 0.05$ is statistically significant when compared to control group, while (ň) = not significant when compared to the control group.					

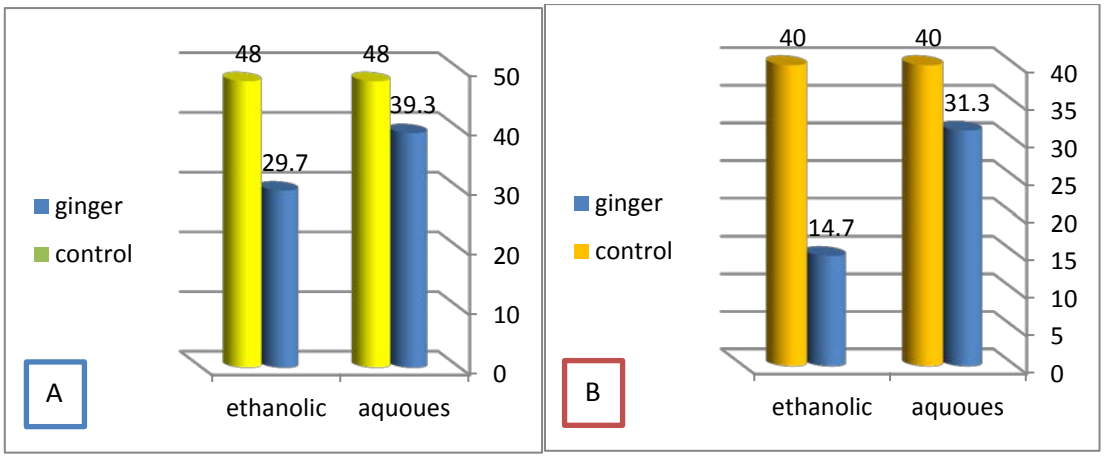

Figure (2) : Comparative effect of ethanolic and aqueous extracts of ginger plant on ,A: bleeding time , B: Clotting time .

The current study agree with the study by (10) shown that, plant constituents such as carbohydrates, alkaloids, phenol, and flavonoids compounds , in ethanolic extracts is an important metabolite is useful for the haemostatic activity which arrest bleeding from damaged or injured vessels by precipitating proteins and thus to form vascular plugs. Some factors affected bleeding time including vasoconstrictive effect of blood vessels, the formation of hemostatic plug and platelet activity. In general, anticoagulants and aspirin have been reported to increase BT in animals and humans while coagulants have opposite effect.(8).

Results agree with study of (13) indicted that , both types of alkaloids extract from Ginger plant decreases the bleeding time while increases the platelets count, Red blood cells (RBC), White blood cells (WBC), at significant difference of (P<0.05). Also, these differences in bleeding time might be due to plant part used , the periods of alkaloids extracts administration and the cruds concentration.

Indeed, a recent study suggests that an decreased of bleeding time with all types of extracts be used as a therapeutic target in the treatment of coagulating agent . Many secondary metabolite supply from exogenous sources(extract) is confirmed with the resultant feedback response leading to decreased of bleeding time is reflected on the stimulation activation of some coagulating factors. Some studies reported that bioactive compounds in Ginger plant extract may increase of the platelets accounts and activation of coagulating factors .Recent study demonstrated that the bioactive compounds present in Ginger plant extract, increase of an intracellular enzyme activates that stimulate number of bloods coagulating factors production or regulated .

Effects of Ethanolic& Aqueous extracts of *Z. officinals* Platelets, RBC& WBC count in experimental rats after 21 days.

The result in the table(2) show that all concentration (1,5, and 10) mg/kg (B.W) of ethanolic and aqueous curd alkaloid extract of Ginger plant increased in (WBC) numbers to (4.9±0.8 ;5.5±1.7; 6±1.1) L for ethanolic extract and (7.1±1; 7.5±1.8; 7.9±1.5) L for aqueous extract in compare with control group after (21) day of treatment.

However , the result in the same table show the present significant increased in (RBC)at all concentration (1,5, and 10)mg/kg (B.W) of ethanolic and aqueous alkaloid extract to (5.7 ± 1.3 , 5.8 ± 0.8, 5.8 ± 0.8)L for ethanolic extract and (5.6 ± 0.9 ; 5.7 ± 0.7 ; 6 ± 1.4)L for aqueous extract of Ginger plant in compare with control group (4.9 ± 0.6)L after (21) day of treatment . Oral administration of 1mg/kg (B.W) of Ginger plant ethanolic extract show that non significantly increased (p<0.05) of (WBC) numbers in compare with the control groups . All the concentration has effect on the (WBC) numbers but the (10) mg/kg (B.W) concentration in (21) day has more effect and recorded significantly increased (p<0.05) . while , the ethanolic extract of the Ginger at 5mg/kg (B.W) have non significantly effect (p<0.05) on WBC numbers compare with the normal white blood cell in control treatment . The result show that the aqueous extract of Ginger plant that have significantly increased (p<0.05) on WBC numbers more effect than ethanolic extract figure (3C).Also, the aqueous & ethanolic extract of Ginger plant that have the same significantly increased (p<0.05) on RBC numbers, figure (3D).

Study by (1) show the both plant extracts has increased the level of immunity by stimulate the immune system and due to the fact that Ginger has the potential to stimulate cellular immunity. While , the result of the RBC numbers after (21) day of oral administration of ethanolic concentration of both the Ginger has non significantly effect (p<0.05) , but the aqueous extracts have significantly increased (p<0.05)in all concentration , the concentration of 10mg/kg (B.W) is the most effect on the RBC numbers compare with the control rat and no significantly (p<0.05) between the ethanolic and aqueous extraction . Ginger alkaloids extracts stimulate the synthesis (erythropoiesis) and the concentration of erythrocytes and help in RBC membrane stabilization by binding to proteins and carbohydrates which are components of RBC membrane and therefore may prevent breakdown of RBC membrane and antagonize the anemic effect of alloxan. (7). This result agree with (7) and (16).

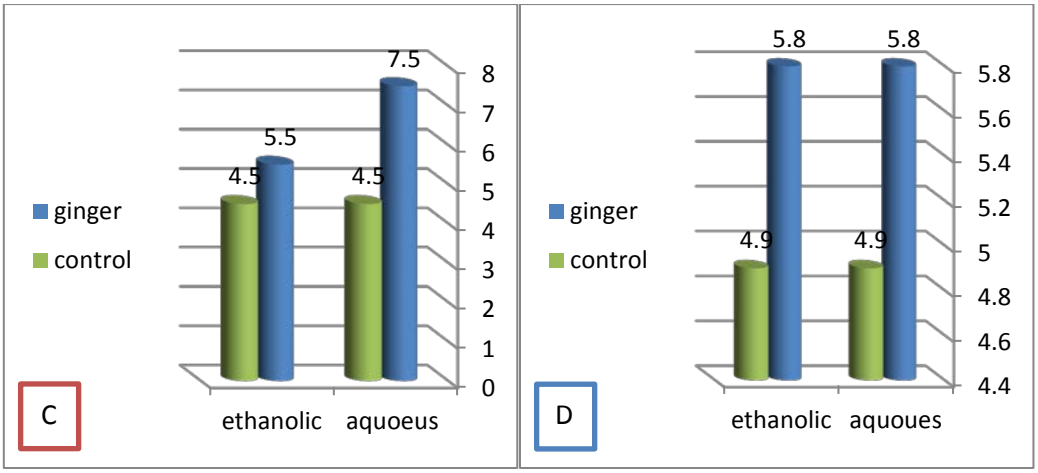

Figure (3): comparative effect of ethanolic & aqueous extracts on , A: WBC , B: RBC.

Data in the table(2) show all concentration (1,5,and 10) mg/kg (B.W) of ethanolic and aqueous alkaloids crud extract of Ginger plant increased in platelets numbers to (335 ± 40 ; 464 ± 115 ; 467 ± 143)for ethanolic extract and (380 ± 120 ; 414 ± 152 ; 535 ± 137) for aqueous extract in compare with control groups (330 ± 49.7) for (21) day of administration . However, The data show that the ethanolic alkaloids extract of the Ginger plant extracts have significantly ($p<0.05$) increased of platelets numbers. The concentration (1 and 5) mg/kg (B.W) of alkaloids extracts show non significant ($p<0.05$) increased compare with the control groups. All the concentration has effect increased of platelets numbers but the most effect of concentration is 10mg/kg (B.W) is more effect at 21 day recorded significantly increased in compare with the control groups . Among the three concentration the most effect is 10 mg/kg (B.W) at 21 day recorded significantly ($p<0.05$) increased in platelets numbers. While , the aqueous extract of the Ginger plant have significantly ($p<0.05$) increased in platelets but there is non significantly increased ($p<0.05$) of platelets in (1,5)mg/kg (B.W) concentration. The 10mg/kg (B.W) at 21 day recorded significantly increased ($p<0.05$) the platelets with both types of extracts . Between the aqueous and ethanolic extracts there is non significantly ($p<0.05$) increased .

CONCLUSIONS

1. The rhizomes of *Z. officinals* vary in their constituent of phytochemicals including,: phenols , alkaloids, flavonoids, , glycosides, tannins.
2. In both treatment (Alkaloid ethanolic and aqueous extracts) are effect on bleeding time , clotting time, RBC,WBC and Platelets with the increased of concentration .
3. The Alkaloids aqueous extraction is more effect than ethanolic extracts on blood parameters .

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