# IN VITRO THROMBOLYTIC / FIBRINOLYTIC EFFECTS OF RUE AQUEOUS DISTILLED EXTRACT

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## Abstract

Ruta graveolens showed several biological and pharmacological actions. In vivo, Its effect on the circulating blood is secondary rather than primary. The aim if this work was to study its effect on human blood clot, in vitro experimental model. This work was done in Department of Chemistry, College of Science, Baghdad University in Baghdad, Iraq. Aqueous distilled extract of *Rue* was prepared by simple distillation and scanned by UV-Visible spectrophotometer. The ability of this extract to generate peroxynitrite was investigated in vitro experimental mode. The effect of 100 μL of 1% *Rue* extract was tested on human blood clot prepared from five healthy volunteers. The extract was added either before the clot is formed or after it. UV-visible spectra of aqueous *Rue* extract showed the presence of single peak at 195nm.*Rue* extract enable to generate peroxynitrite in a concentration dependent manner (r = 0.98). It is significantly reduced the formed clot size by 12.534 ± 5.674% while the simultaneous administration of blood and aqueous extract resulted in reduction of clot size by 4.772 ± 2.207%. *Rue* aqueous distilled extract has thrombolytic and fibrinolytic effect by the evidence of clot lysis. This effect may be related to donation of nitric oxide.

## Introduction

The hemostatic system helps to maintain the integrity of the circulatory system after severe vascular injury, whether traumatic or surgical in origin. Part of these events, in any patient, is stimulation of clot breakdown (fibrinolysis), which may become pathology-ical (hyperfibrinolysis) in some [(1)].

The common name of *Ruta graveolens* L. is *Rue*. All parts of the plant contain the active principles although they are mostly encountered in leaves especially before blooming. The most frequent international use of Rue has been for induction of abortion. Methyl-nonyl-ketones, one of Rue active substances, induced uterine contractions and pelvic congestion leading to uterine hemorrhage and possibly abortion in pregnancy[(2)].

Several substances were isolated from *Rue* including rutin (glycoside), frucocoumarins, alkaloids (quinolones), tannin and essential oils. Extracts of *Rue* proved experimentally to have several beneficial pharmacological actions including antibacterial [(3-5)], antifungal [(6)], antihelmintic, antiparasitic[(7)], antitumor [(8)], anti-inflammatory [(9)], and antiandrogenic [(10)]. Also it showed several toxic effects including dermal photosensitization[(11,12)], cytotoxicity [(5)], antifertility [(13)], mutagenicity [(14)] and abortifacient effect [(15)].

Its effect on blood, due to its content of coumarin derivatives, is secondary rather than primary. As a result of its toxic effect on the liver, it caused coagulation disorders.

Recently, there is an evidence that peroxynitrite, a molecule formed from nitric oxide and superoxide, decreased the activity of tissue plasminogen activator, i.e., antifibrinolysis [(16)].

The aim of this study is to investigate the distilled aqueous extract of *Rue* leaves on human blood clot, in vitro experimental model, in an attempt to show whether such extract has fibrinolytic activity in relation to the peroxynitrite.

## Experimental

This study was conducted at Department of Chemistry, College of Science, Baghdad University in Baghdad, Iraq. After obtaining permission from the local ethics committee

and informed consent, five healthy male volunteers were allocated randomly from the college students.

**Materials**

All the chemicals used in this work were of analar grade. Commercially available *Ruta graveolens* L. leaves was obtained from the north of Iraq.

**Herbal preparation**

Aqueous extract of *Ruta graveolens* L. leaves was prepared by simple distillation. In brief one gram o dried *Rue* leaves in 100 mL distilled water (1%) was heated, the vapors separated and recondensed them to obtain a clear extract liquid that was more concentrated in the more volatile components.

**UV-visible spectra**

UV-visible spectra was recorded on a Aquarius (Cecil series with scanning ability) spectrophotometer (France). The spectra of distilled aqueous *Rue* extract (1%) was measured from 150-900nm at room temperature with a 10 mm path length quartz cell with a scan rate of 600 nm/min.

**Determination of peroxynitrite**

Peroxyntrite levels in the distilled aqueous extract of *Rue* was determined according to the method described by Beckman et al (1992) [17] cited by VanUffelen et al (1998)[18]. Peroxynitrite mediated nitration of phenol, resulting in nitrophenol formation, formed the basis of the peroxynitrte assay.

In brief, serial dilutions of 1% distilled aqueous *Rue* extract were placed in a glass test tubes and then 5 mM phenol in 50 mM sodium phosphate buffer to a final volume of 2 mL were added, mixed well and incubated for 2 hours at 37 Cº. Then the reaction was stopped by addition 15 µL of 0.1M sodium hydroxide, mixed and immediately recorded the absorbance of the samples at 412 nm.

**Clot lysis**

Venous blood samples (3 mL each) were drawn from five healthy human male volunteers. 500 µL of blood was transferred to each of four previously weighed Eppendorff tubes for each subject. In the first series, the transferred 500 µL allowed to form clots at 37 Cº for 45 minutes [19]. After clot formation, serum was completely removed and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube- weight of the tube alone). To each Eppendorrf containing pre-weighed clot, 100 µL of distilled aqueous *Rue* extract or 100 µL distilled water as a negative control were added. All the tubes were then incubated at 37 Cº for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot stabilization. The obtained difference in weight was expressed as percentage of stabled or lysed clot. In the second series of experiments, simultaneous addition of 500 uL blood and 100 uL either distilled water or distilled aqueous *Rue* extract , incubated at 37 Cº for 45 minutes. The obtained clot weight was determined as above.

**Statistical analysis**

Data are expressed as mean ± SE of number of experiments (n=5). The significance ($p < 0.05$) between % clot weight changes induced by distilled water or *Rue* extract was tested by paired and unpaired one tailed "t" test.

**Results**

UV-visible spectra of aqueous *Rue* extract showed the presence of one peak at 195nm with optic density 0.141 Fig.(1). Fig.(2) showed that *Rue* extract had the ability of generation peroxynitrite in a concentration dependent manner (r = 0.98). One hundred microliters of *Rue* extract, the volume that was used in clot lysis experiments, generated 15.9 uM of peroxynitrite.

Distilled water did not show any significant lytic effect on the formed clot. The clot weight is decreased by 0.229 ± 0.447 %. Aqueous extract of *Rue* was significantly ($p$=0.0495) showed thrombolytic effect. It reduced the clot size by 12.534 ± 5.674%. The difference between distilled water and aqueous extract of *Rue* reached to the level of significant ($p$ = 0.05) Fig.(3). The simultaneous administration of blood and aqueous extract resulted in reduction of clot size by 4.772 ± 2.207% as compared with the effect of distilled water. Such effect was approximated the level of significant ($p$ = 0.06) Fig.(3).

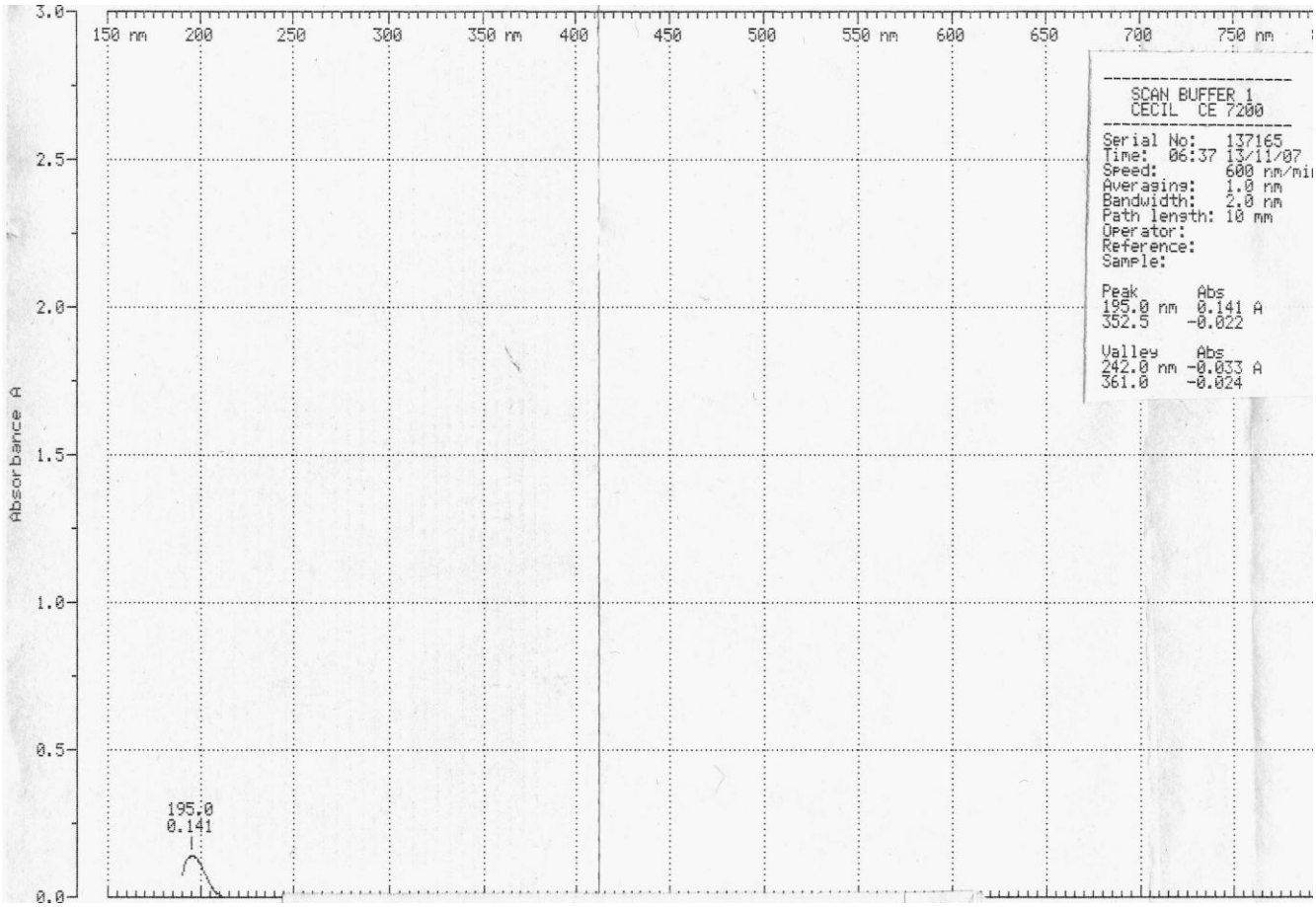


**Fig.(1) :UV-Visible spectra of aqueous distilled extract of Rue.**

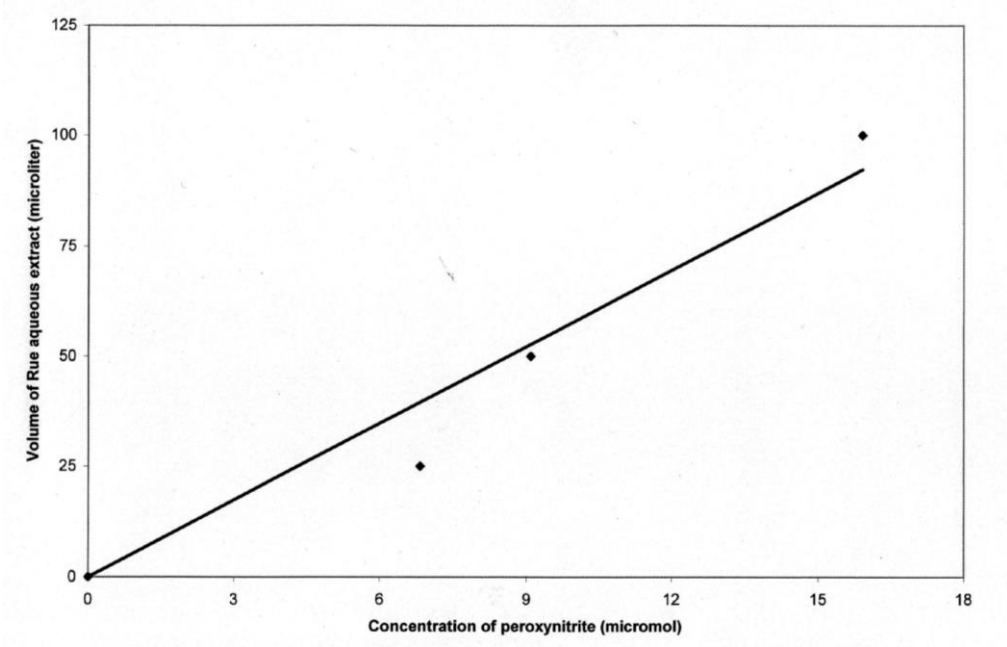


**Fig.(2) : The relationship between the volume of aqueous Rue extract and the formation of peroxynitrite.**

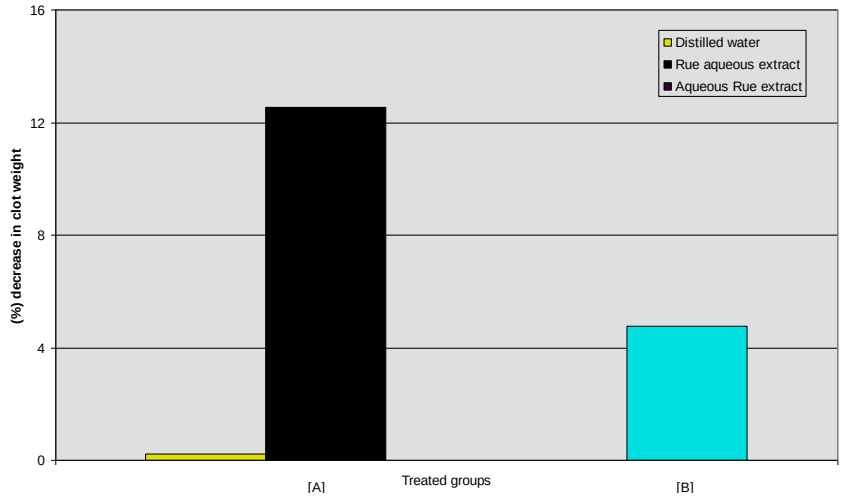


**Fig. (3) : The effect of distilled aqueous extract of Rue on the blood clot. (A) Rue extract was added after the clot formation, (B) Rue extract was simultaneously added to fresh blood.**

## Discussion

The present results clearly show that aqueous extract of *Rue* has thrombolytic and/or fibrinolytic effect (s) as it reduces the clot weight. Its effect seems to be solely related to the active substance demonstrated by UV-visible spectra.

Although the detected active substance generates peroxynitrite but its effect on the clot seems to be not related to peroxynitritite i.e. exerts direct effect. Its fibrinolytic and/or thrombolytic effect(s) is reported here for the first time. Ethanolic extract of *Ruta chalepenis* had significant inhibitory effect on the number of circulating red cells in mice [(20)].

Few herbal medicines exert thrombolytic or fibrinolytic effects like Fangonia Arabica (Dhamasa) [(21)], Artmisiae folium (Gaiyoh) [(22)], Hemidesmus indicus [(23)], and garlic [(24)].

Some plants/extracts products exert their throbolytic or fibrinolytic effects via their content of certain fibrinolytic proteases enzymes like Pleurotus ostreatus [(25)], Spirodel polyrhiza [(26)] and others. The thrombolytic /fibrinolytic effect(s) of *Rue* in this work is not related to the effect of proteases enzymes because the aqueous distilled extract was used not crude solvent extract.

The interesting finding in this study is the concentration dependent formation of peroxynitrite by *Rue* aqueous distilled extract. This means that *Rue* extract behaves as nitric oxide donor, since peroxynitrite is the result of non enzymatic reaction of nitric oxide and superoxide anion in solution. Therefore, the effect of *Rue* extract on clot lysis may be related to the vital role of nitric oxide on the blood platelets [(27)].

In conclusion, *Rue* aqueous distilled extract shows thrombolytic and fibrinolytic effect by the evidence of clot lysis. Further study is recommended to identify the chemical structure of its active ingredient and to elucidate the exact mechanism of action. Also it is recommended to study the *Rue* extract as nitric oxide donor.

## References

[1] Lawson JH and Murphy MP (2004). Challenes for providing effective hemostasis in surgery and trauma. Semin Hematol, 41, 55-56.

[2] Jonglard J (1977). Intoxication d'origine vegetale. Encylopedie Medico Chirurg-icale, 16065 A10.

[3] Ojala T., Remes S., Haasuu P., Vuorela H., Hiltunen R., Haahtela K., Vuorela P (2000). Antimicrobial activity of some coumarin containg herbal plants growing in Finland. J Ethnopharmacol, 73(1-2), 299-305.

[4] Alzoreky NS., Nakahara K (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int J Food Microbiol, 80, 223-230.

[5] Ivanova A., Mikhova B., Najdenski H., Tsvetkova I., kostova I (2005). Antim-

icrobial and cytotoxic activity of Ruta graveolens. Fitoterapia, 76(3-4), 344-347.

[6] Oliva A., meepagala KM., Wedge DE., Harries D., Hale AL., Aliotta G., Duke SO (2003). Natural fungicides from Ruta graveolens L. leaves including a new quinolone alkaloid. J Agric Food Chem, 51, 890-896.

[7] Guarrera PM (1999). Traditional antihel-mintic, antiparasitic and repellent uses of plants in central Italy. J Ethnopharmacol, 68(1-3), 183-192.

[8] Preethi KC., Kuttan G., Kuttan R (2006). Antitumour activity of Ruta graveolens extract. Asian Pac J Cancer Prev , 7, 439-443.

[9] Atta AH and Alkofahi A (1998). Anti-noceceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts, 60, 117-124.

[10] Khouri NA and El-Akawi Z (2005) Antiandrogenic activity of Ruta graveolens L. in male Albino rats with emphasis on sexual and aggressive behavior. Neuro Endocrinol Lett, 26, 823-829.

[11] Hale AL., Meepagala KM., Oliva A., Alliotta G., Duke SO (2004). Phytotoxins from the leaves of Ruta graveolens.J Agric Food Chem, 52, 3345-3349.

[12] Furniss D and Adams T (2007). Herbs of grace; an unusual cause of phytodermatitis mimicking burn injury. J burn Care Res, 28,:767-769.

[13] Kong YC., Lau CP., Wat KH., Ng KH., But PP., Cheng KF., Waterman PG (1989). Antiinfertility principle of Ruta graveolens. Planta Med, 55, 176-178.

[14] Paulini H., Eilert U., Schimmer O (1987). Mutagenic compounds in an extract from ruta herba (Ruta graveolens L.). I. Mutagenicity is partially caused by fluorquinoline alkaloids. Mutagenesis, 2, 271-273.

[15] Conway GA and Slocumb JC (1979). Plants used as abortifacients and emmenagogues by Spanish New Mexicans. J Ethnopharmacol, 1, 241-261.

[16] Nielsen VG., Crow JP., Zhou F., Parks DA (2004). Peroxynitrite inactivate tissue plasminogen actiator. Anaesth Analg , 98, 1312-1317.

[17] Beckman JS., Ischiropoulos H, Zhu L., Vander Woerd M., Smith C., Chen J et al (1992). Kinetics of superoxide dismutase-and iron-catalyzed nitration of phenolics by peroxynitrite. Arch Biochem Biophys, 298, 438-445.

[18] VanUffelen BE, Van Der Zee J., DeKoster BM, Vanstereninck J., Elferink JGR (1998). Intracellular but not extracellular conversion of nitroxyl anion into nitric oxide leads to stimulation of human neutrophil migration. Biochem J, 330, 719-722.

[19] Prasad S., Kashyap RS., Deopujari JY., Purohit HJ., Taori GM., Daginawala HF (2006). Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thromb J, 4, 14.

[20] Shah AH., Qureshi S., Aqeel AM (1991). Toxicity studies in mice of ethanol extracts of Foeniculum vulgare fruit and Ruta chalpensis aerial parts. J Ethnopharmacol, 34(2-3), 167-172.

[21] Prasad S., Kashyap RS., Deopujari JY., Purohit HJ., Taori GM., Daginawala HF (2007). Effect of Fagonia Arabica (Dhamasa) in vitro thrombolysis. BMC Complement Altern Med, 7, 36.

[22] Niwa M., Yuasa K., Kondo S., Sakuragawa N (1985). Studies of Waken-Yakus (traditional herbal drugs): especially on the effects of Gaiyoh (Artemisiae folium) on blood coagulation. Thromb Res , 38, 671-672.

[23] Mary NK., Achuthan CR., Babu BH., Padikala J (2003). In vitro antioxidant and antithrombotic activity of Hemidesmus indicus (L.) R.B. J Ethnopharmacol , 87 (2-3), 187-191.

[24] Bordia A., Verma SK., Srivastava KC (1998). Effect of garlic (Allium sativum) on blood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease. Prostaglandins Leukot Essent Fatty acids, 58, 257-263.

[25] Choi HS., Shin HH (1998). Purification and characterization of a fibrinolytic protease in Pleurotus ostreatus. Mycologia, 90, 647-679.

[26] Choi HS., Sa YS (2001). Fibrinolytic and antithrombotic protease from Spirodela

polyrhiza. Biosci Biotechnol Biochem, 65, 781-786.
[27] Karmohapatra SK., Kahn NN., Sinha AK (2007). The thrombolytic effect of asoirin in animal model. J Thromb Thrombolysis, 24, 123-129.

**الخلاصة**

تمتلك عشبة السذاب العديد من الفعالية البايولوجية والدوائية. ويبدو ان تأثيرها في جهاز الدوران داخل الجسم الحي ثانويا وليس أوليا أي بصورة مباشرة.

تهدف هذه الدراسة الى معرفة عملها في خثرة الدم البشري خارج الجسم الحي. تم تحضير المستخلص المائي المعقم للعشبة بوساطة عملية التقطير البسيط واجراء مسح طيفي للمحلول بوساطة المطياف الضوئي المرئي وفوق الآشعة البنفسجية. كما وتم التحري على قابلية المستخلص في توليد بيروكسي نتريت في أنموذج خارج الجسم الحي. وتم اختبار فعالية المستخلص(100 مايكرولتر من 1%) في خثرة الدم البشري المحضرة من خمسة متطوعين أصحاء ، حيث أضيف المستخلص قبل او بعد تكون الخثرة.

أظهرت نتائج المسح الطيفي وجود ذروة مفردة عند طول موجي 195 نانومتر. كما واستطاع المستخلص من توليد بيروكسي نتريت بتراكيز تزايدت بتزايد حجوم المستخلص المستخدم. عمل المستخلص على تقليص حجم الخثرة بدلالة نوعية متميزة بمقدار 12.534 ± 5.674% عند اضافة المستخلص بعد تكوين الخثرة بينما عمل على تقليص تكوين الخثرة بمقدار 4.772 ±2.207% عند اضافته قبل تكوين الخثرة.

يستنتج من ذلك ان للمستخلص صفة تحلل الخثرة وتحلل الفابرين بدلالة حصول تحلل الخثرة. ومن المحتمل ان يرد هذا التأثير الى قابلية المستخلص على وهب اوكسيد النتريك.