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Determination of Antioxidant Compounds and *In-Vitro* Thrombolytic Activity of *Borago Officinalis* Leaves Growing Naturally in Kurdistan Region\Iraq

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ABSTRACT

Phytochemical are compounds derived from natural source play important role in mankind health system with various physiological activities such as antioxidant and thrombolytic activity. *Borago officinalis* leaves have been assessed for quantity of antioxidant compounds comprise in estimation of total phenolic, total flavonoid and total tannin contents, and thrombolytic activity of hydrolacholic leaves extract at different concentration ranges. The hydroalcholic extract of leaves showed low phenolic contents about (26.45 ± 0.126 mcg GAE\g DW) with approximately similar quantity of total flavonoid and total tannin contents about (212 ± 0.278 mcg QE\g DW) and (216 ± 0.521 mcg GAE\g DW), respectively. Leaf extract exhibited a dose dependent manner thrombolytic activity ranges between (8.346± 0.669 - 44.6697±1.1076) considered significant in comparison with control negative distilled water (p value < 0.0001) and moderate activity in comparison with control positive streptokinase. *Borago officinalis* leaf was a good source for antioxidant compounds and open a venue for production of thrombolytic agents from the leaf with less side effects in comparison to the conventional drugs.

Keywords: *Borago officinalis*, thrombolytic activity, total phenolic content, total flavonoid contents, total tannin contents.

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INTRODUCTION

Phytonutrients or phytochemicals are plant derived substances known for their antioxidant activities. Antioxidant phytochemicals comprise a large number of substances such as phenol, flavonoid and tannins, approximately 3000 flavonoids substances have been described and attributed in the antioxidant activities of herbal preparations and products[1,2]. Major complication may be as a consequence of hemostasis failure of the circulatory system like thrombus formation, in turn leadings to acute myocardial or cerebral infarction. Conventional thrombolytic drugs are streptokinase, altepase, urokinase, ainstreplase and tissue plasminogen activator (TPA) which characterized by series adverse effects such as sever anaphylactic shock, lack of specificity, intracranial haemorrhage and bleeding[3,4]. There were a significant limitation of availability of the thrombolytic drugs, in addition to the need of large doses to reach the maximum efficacy of the drugs. Because of the deficiency of availability of thrombolytic agents, attempts were ongoing for discovering of new agents from medicinal plants with less side effects and lower costs[5]. Proven thrombolytic activity were documented for various medicinal plants, some of them have significant therapeutic value in clot lysis activity[6,7,8]. Herbal preparation have been used for treatment of different diseases since ancient times. Herbal preparations and product were preferred by people because they known as to be safe because they are natural[8]. *Borago officinais* L.(Borage), is herbaceous annual plant native to North Africa, Europe and widely distributed in many places of Mediterranean Region belong to Boraginaceae family. It is medicinally important plant attributed by many pharmacological activities such as diuretic, emollient, demulcent, antioxidant and expectorant. The plant also used for culinary purposes as vegetables included in different meal recipes[9,10,11]. *Broago officinalis* plant is widely used by Kurdish community for culinary purposes, locally known as "Zman-ga" have been selected for present study to evaluate the quantity of antioxidant compounds of the leaves which comprised in total phenolic, total flavonoid and total tannin contents, pharmacologically the plant have been assessed for it is thrombolytic activity using *in-vitro* model.

MATERIALS AND METHOD

Plant collection

Leaf part of *Borago officinalis* was collected in mountain places (Shaqlawa) of Erbil city, Kurdistan Region\Iraq during March-April 2015, have been identified by Department of Pharmacognosy, College of Pharmacy \ Hawler Medical University. Plant leaves were dried in shade and kept in closed container under 21-23 ^{o} C.

Determination of antioxidant compounds

Plant materials have been assessed for quantitative antioxidant compounds by estimation of total phenolic, total flavonoid and total tannin contents.

Estimation of total phenolic content

Total phenolic compounds have been estimated according to the Folin-Ciocalteu method with slight modifications[12]. Briefly, a volume of 1ml of extract prepared from (1g) dried leaves was mixed with (9 ml) of distilled water. One ml of Folin-Ciocalteu reagent was added and the mixture stand for 5 minute at room temperature, later (10ml) of (7%) Na_2CO_3 were added. The volume have been completed to (25ml) and incubated for 90 minutes at room temperature with distilled water. The absorbance was read at 750nm using UV visible spectrophotometer. Total phenolic content was estimated from standard equation (y= 0.008x-0.120, r^2=0.983) of calibration curve (figure.1.) obtained from standard concentrations of gallic acid [20, 40, 60, 80 and 100 mcg\ml in distilled water]. The results were expressed as mcg gallic acid equivalent (GAE) \ gram of dry weight (DW).

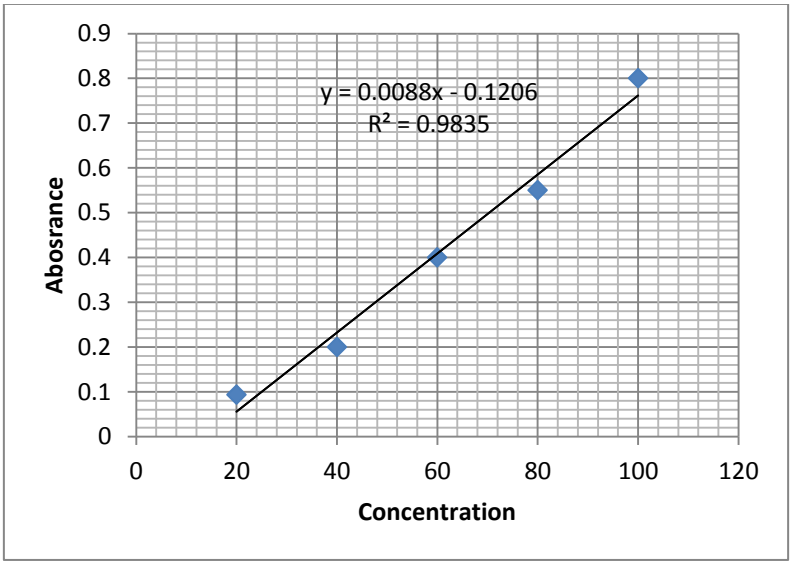

Figure.1: Calibration curve of gallic acid for total phenol estimation

Estimation of total flavonoid content

Total flavonoid content was measured by the aluminium chloride colorimetric method[13]. A volume of (1 ml) of extract prepared from (1g) dried leaves was added to (10 ml) volumetric flask containing (4 ml) of distilled water. Then (0.3 ml) sodium nitrite 5% was added to the flask, stands for 5 min then (0.3 ml) aluminium chloride solution (10%) was also added. At sixth min, (2 ml) sodium hydroxide (1 M) was added and the volume has been adjusted to (10 ml) using distilled water. The solution was vortex and the absorbance was read at 510 nm. The total flavonoid content

was estimated from standard equation (y=0.007x+0.036, r^2= 0.98) of calibration curve (figure.2.) obtained from standard concentrations of querstine [20, 40, 60, 80 and 100 mcg\ml in ethanol (80%)]. The results was expressed as mcg querstine equivalents (QE)\ gram dry weight (DW).

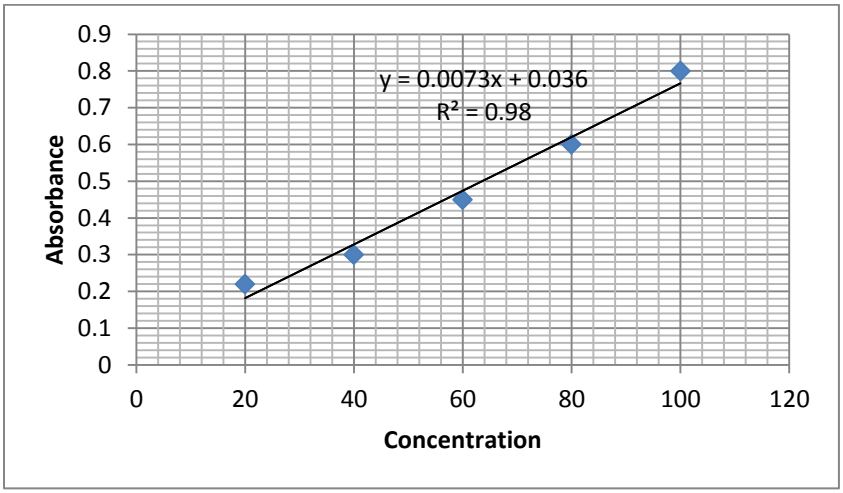

Figure.2: Calibration curve of querstine for total flavonoid estimation

Estimation of total tannin content

Total tannin content have been estimated according to the Folin-Ciocalteu method described by Tamil selvi *et al*, 2012[14] with slight modifications. Aliquot of (0.1 ml) of the plant material extract prepared from (1g) of dried leaves was added to the mixture of (7.5 ml) of distilled water and (0.5 ml) of Folin-Ciocalteu reagent, to the mixture (1ml) of (35%) Na_2CO_3 solution. The volume has been adjusted to (10ml) using distilled water. The mixture was shaken and allowed to stand at for 30 min room temperature and absorbance was read at 725 nm. Total tannin content were measured from standard equation (y=0.001x-0.012, r^2=0.996) of calibration curve (figure.3.) obtained from measuring absorbance of standard concentrations of gallic acid [20, 40, 60, 80, 100of gallic acid\ml prepared in distilled water]. Total tannin content were expressed as mcg gallic acid equivalent (GAE)\ gram of plant dry weight (DW).

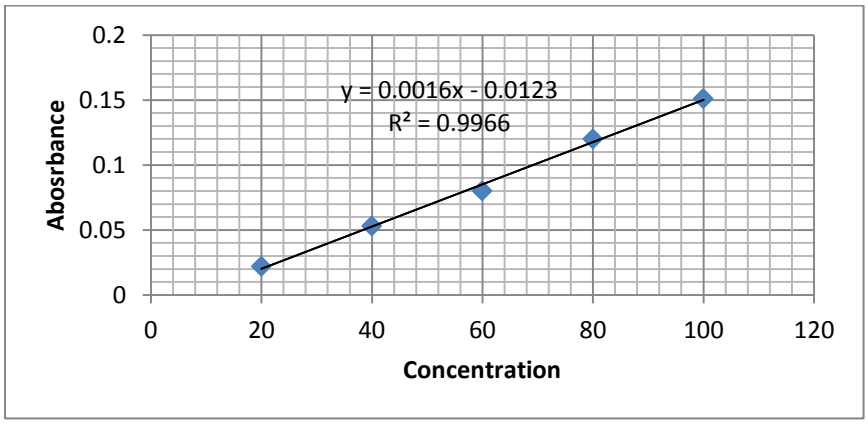

Figure 3: Calibration curve of gallic acid for tannin content estimation

***In-vitro* thrombolytic assay**

Plant extract preparation

Powdered *Borago officinalis* leaf (BO) introduced for hydroalcholic (75%) extraction using ultrasonic assisted extractor according to Alupuli et al, 2009 procedure[15]. The extract was concentrated and dried under vacuum. The dried extract was reconstituted using distilled water at a concentration of 10mg\ml used as stock solution, then a serial dilution extract was prepared with concentrations of [200, 400, 600, 800 and 1000 mcg/ml] using distilled water as diluent, the solutions were kept overnight. The insoluble material were removed through filtration, the clear solution have been assessed for thrombolytic activity[16,17].

Streptokinase (SK) solution preparation

Lyophilized Streptokinase vials of 1500000 I.U commercially available (Abbott), were reconstituted using sterile distilled water, mixed thoroughly. This suspension was used as a standard stock solution from which 100µL (30,000 I.U) was used for *in-vitro* thrombolytic activity assessment[16,17].

Blood sampling

Fresh blood sample (5ml) were drawn from healthy human volunteers (n=5) with a history of not taking anti-inflammatory and contraceptive for 7-10 days duration. (0.5 ml) of blood sample were transferred to ten pre-weighed properly labeled eppendorf tubes in a sterile aseptic condition[16,17].

Thrombolytic assay

Thrombolytic assay were carried out according to a method described by Sweta *et al*, 2007 and Daginawala *et al*, 2006 [16,17]. Each properly labeled filled eppendorf tube were allowed to stand for 45 min at 37 ^{0}C for clot formation. After clot formation the serum (yellow liquid) was withdrawn without disturbing the clot using syringe and the tubes were reweighed. The clot weight was measured from the following equation:

Weight of clot= weight of clot filled tube - weight of empty tube X 100

Aliquot of (0.1ml) of each concentration of leaf extract of *Borago officinalis* have been added to each eppendorf tube, and incubated for 90 min at 37 ^{0}C. After incubation periods the supernatant fluid released from the clot lysis were removed using syringe without disturbing the clot and the tubes were re-weighed. Control positive Streptokinase (SK) drug and control negative distilled water were incorporated in the study. The difference in the weight of clot between two periods of incubation were expressed as percentage of clot lysis according to the following equation:

% clot lysis = weight of released clot \ weight of clot X 100

Statistical analysis

All experiments were carried out in triplicate, the results were expressed as mean ± standard deviation (SD). Comparison between means with controls performed using one tail unpaired t-test method using Graph pad prism 6 program considering p value < 0.001 statistically significant.

RESULTS AND DISCUSSION

Determination of antioxidant compounds

Compounds responsible for antioxidant activity of the plant are phenol, flavonoid and tannin. Phenolic compounds are universal secondary metabolites in plants known for their antioxidant activities[18,19]. *Borago officinalis* leaves have been evaluated for total phenolic of the hydroalcholic extract using Folin--Ciocalteu method showed (26.45 ± 0.126 mcg GAE\g DW) which is lower concentration in comparison to the total phenolic contents recorded by Zaynab et al, 2012[20]. Flavonoids are natural seecondary metabolite known as vitamin P, characterized in different pharmacological activities such as anti-inflammatory, anti-cancer and antiallergic[21]. Total flavonoid exhibited measured by aluminium chloride colorimetric method by the leaf extract was (212 ± 0.278 mcg QE\g DW). Total tannin contents expressed by *Borago officinalis* leaf was (216 ± 0.521 mcg GAE\g DW). Approximately similar amount of total flavonoid and total tannin contents were detected in leaf extract.

In-vitro thrombolytic activity

As a part of screening for cardio-protective agents obtained from plant origin, *Borago officinalis* leaves have been evaluated for thrombolytic activity, which exhibited a dose dependent manner thrombolytic activity (figure.4.) ranges between (8.346± 0.669 - 44.6697±1.1076) (Table.1.) from lower concentration (200mcg\ml) to higher concentration (1000 mcg\ml). *B.officinalis* showed significant activity in comparison to the control negative (P value < 0.0001) in approximately all tested concentrations, The activity ranges between mild to moderate in comparison to control positive streptokinase (30,000 IU). Greater activity was expressed by the highest concentration of the leaf (1000 mcg\ml). There is no any previous studies on the thrombolytic activity of the plant, the plant contains may important phytochemicals which may be responsible for the thrombolytic activity expressed by the plant, such alkaloids, tannins, flavonoids, phenolic acids like rosmarinic acid[22,23,24]. Rosmarinic acid is a phenolic acid with proven thrombolytic activity on rats which cause clot lysis in about 50% followed administration[25].

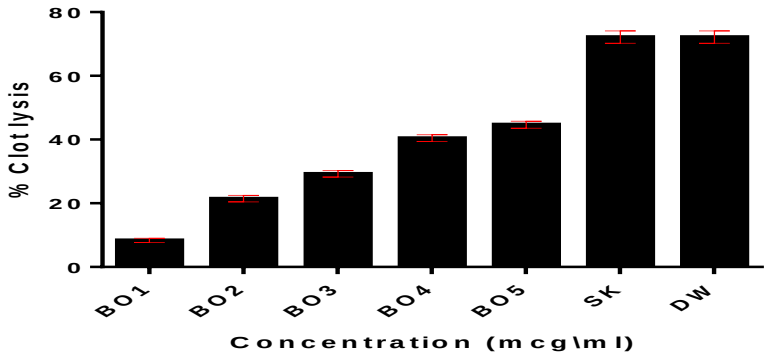

Figure 4: Thrombolytic activity of *Borago officinalis* (BO) leaf at different concentrations in correspondence to control positive streptokinase (SK) and control negative distilled water (DW)

Table 1: Thrombolytic activity of *Borago officinalis* leaf

Tested Herb\Drug*	Clot lysis %(Mean ± SD)**	t-test value	P value in comparison to control negative
BO1	8.346± 0.669	5.42637	< 0.0001
BO2	21.4643±1.050	22.7852	< 0.0001
BO3	29.2363±0.977	33.0695	< 0.0001
BO4	40.443±1.051	47.8987	< 0.0001
BO5	44.6697±1.1076	53.4916	< 0.0001
Streptokinase	72.164 ± 1.945	89.8729	< 0.0001
Distilled water	4.245 ± 0.526	-	-

*Herb concentration, BO1=200 mcg\ml, BO2=400mcg\ml, BO3=600mcg\ml, BO4=800mcg\ml, BO5=1000mcg\ml

** n=3

CONCLUSION

Borago officinalis leaf considered a source of antioxidant compounds with mild to moderate thrombolytic activity, open a new way for production of thrombolytic agents from natural source with lower side effects.

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