Journal of Advances in Medical and Pharmaceutical Sciences
4(4): 1-5, 2015, Article no.JAMPS.20550
ISSN: 2394-1111

SCIENCEDOMAIN *international*
www.sciencedomain.org

Assessment of Thrombolytic Activity of Selected Species from Lamiaceae Family Growing Naturally in Kurdistan Region\Iraq Using *In-vitro* Model

Lana Yousif Mutalib[1*]

[1]*Department of Pharmacognosy, Pharmacy College\Hawler Medical University, Iraq.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAMPS/2015/20550
Editor(s):
(1) Jinyong Peng, College of Pharmacy, Dalian Medical University, Dalian, China.
Reviewers:
(1) Mohamed Lotfy Ashour, Ain Shams University, Egypt.
(2) Dorota Wojnicz, Wroclaw Medical University, Poland.
(3) Ayona Jayadev, All Saints' College, Trivandrum, Kerala, India.
Complete Peer review History: http://sciencedomain.org/review-history/11696

Original Research Article

Received 31st July 2015
Accepted 16th September 2015
Published 6th October 2015

ABSTRACT

Aims: The present study was aimed to assess most commonly used medicinal plant belong to Lamiaceae family for their thrombolytic activity.
Place and Duration of the Study: Pharmacy College\ Hawler Medical University, June, 2015.
Methodology: Dried leaf part ethanolic extracts of selected species have been evaluated for their thrombolytic activity using fresh human blood specimen at concentrations of 200, 400, 600, 800 and 1000 µg\ml, streptokinase drug and distilled water were incorporated in the study as control positive and negative, respectively.
Results: Showed significant thrombolytic activity of leaf extracts at all tested concentrations in comparison with control negative distilled water ($P < 0.01$), highest clot lysis activity were recorded for *Salvia officinalis* leaf extract (68.43±1.457%) and minimum activity were exhibited by *Rosmarinus officinalis* leaf extract (49.27±3.256%). *Salvia officinalis* and *Thymus vulgaris* expressed significant thrombolytic activity in comparison with control positive streptokinase ($P < 0.05$) at highest tested dose concentration (1000 µg\ml).
Conclusion: Selected species from Lamiaceae plant family can open a venue for production of thrombolytic drugs derived from natural source with greatest activity and lowest adverse effects.

**Corresponding author: E-mail: lanayousif82@yahoo.com;*

Keywords: Thrombolytic drugs; Salvia officinalis; Thymus vulgaris; Rosmarinus officinalis.

1. INTRODUCTION

World Health Organization (WHO) have estimated that more than 80% of world population in developing countries depend on herbal remedies for maintaining their health [1]. The nature is considered as repository of medicinal agents from ancient times, since herbal remedies are believed to be safe as they are derived from natural source [2]. Blood clot (thrombus) causes extensive diseases of cardiovascular system. Thrombolytic agents such as urokinase (UK), streptokinase (SK), tissue plasminogen activator (t-PA) are used to dissolve the formed clot, but they are accompanying by series side effects like haemorrhage and anaphylactic reactions [3-6]. Outstanding attempts were continued for discovering natural derived drugs form either animal or plant source with thrombolytic or antithrombotic activity [7-10].

Lamiaceae plant family is one of the most important plant family including about 400 geneses, in which about 40% of them are aromatic plants [11]. Lamiaceae plant families known for their volatile oil contents, also they contain many other phytochemicals responsible for their therapeutical activities such as flavonoids and polyphenolic acids. The most commonly used species of this family are: *Salvia officinalis*, *Rosmarinus officinalis* and *Thymus vulgaris*. These plants are chiefly used as antimicrobial, antioxidant. In digestive system aliments they are applied as spasmolytic and carminative. Different species of Lamiaceae contain varianous amounts of polyphenolic acid, which in turn affect the therapeutic activities of each species [12].

The aim of present study was to assess the thrombolytic activity of selected species from Lamiaceae family which are most commonly used by the local community for variant purposes either culinary or medicinal.

2. MATERIALS AND METHODS

2.1 Plant Collection

Leaf part of *Salvia officinalis, Rosmarinus officinalis* and *Thymus vulgaris* were collected in different places of Erbil city, Kurdistan Region\Iraq have been identified by Department of Pharmacognosy, College of Pharmacy\ Hawler Medical University. Leaves were dried in shade and kept in closed air tight container under 21-23℃.

2.2 Plant Material Extraction

Powdered plant leaves were extracted separately using ethanol (75%) according to method described by Alupuli et al. [13] using ultrasonic assisted extractor machine. The extracts were concentrated and dried under vacuum. The dried extracts were reconstituted using distilled water forming 1 mg\ml concentration used as stock solution, extracts were prepared in a serial dilution concentrations of 200, 400, 600, 800 and 1000 µg/mL using distilled water, the solutions were kept overnight. The insoluble material were removed by filtration using 0.22- µm syringe filter, the clear solution have been assessed for thrombolytic activity [14,15].

2.3 Streptokinase (SK) Solution Preparation

Commercially available lyophilized Streptokinase vials of 1500000 I.U (Abbott), was reconstituted using distilled water, mixed properly. This suspension was used as a standard stock solution from which 100 µl (30,000 I.U) was used for *in-vitro* thrombolytic assay [14,15].

2.4 Blood Specimen

Ten ml of fresh blood sample were drawn from healthy human volunteers (n=5) without a history of taking anti-inflammatory and contraceptive for a minimum duration 7-10 days. A volume of (0.5 mL) of blood sample was transferred to ten pre-weighed properly labelled eppendorf tubes in a aseptic condition [14,15].

2.5 Thrombolytic Assay

Thrombolytic assay were carried out according to described method by Daginawala et al. [14] and Sweta et al. [15]. Each labelled filled eppendorf tube was allowed to stand at 37℃ for 45 min for clot formation. After clot formation the serum was withdrawn without disturbing the formed clot using syringe and the tubes were reweighed. The clot weight was estimated from the following equation:

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

Aliquot of 100 µl of each concentration of leaves extracts separately have been added to each tube, and incubated at 37℃ for 90 min. After incubation, released fluid from the clot lysis were removed using syringe without disturbing the clot and the tubes were weighed again. Positive control (Streptokinase drug, SK) and negative control (distilled water) were included in the study. The deviation in clot weight were expressed as percentage of clot lysis according to the following equation:

% clot lysis = (weight of released clot \ weight of clot) x 100

2.6 Statistical Analysis

All data were estimated from triplicate procedure work, mean values ± standard deviation were determined. Comparison between control and tested samples were performed using two way ANOVA method using graph pad prism 6 considering ($P < 0.05$) statistically significant.

3. RESULTS AND DISCUSSION

In-vitro thrombolytic activity assessment for all extracts showed dose dependent manner, highest activity where expressed at greatest concentration of 1000 µg\mL for all tested plants (Table 1) and (Fig. 1). The significant thrombolytic activities were exhibited by the leaf extracts in comparison with control negative ($P<0.001$) (Table 2). A significant mild to moderate activity were expressed by the leaf extracts of *S. officinalis* and *T. vulgaris* in comparison to the positive control after incubation for 90 min at 37℃ at (1000 µg\mL) concentration ($P < 0.05$). In general all tested concentrations were showed activity ranging from (19.53±0.784%) for *R. officinalis* at (200 µg\mL) to the highest activity (68.43±1.457%) for *S. officinalis* at (1000 µg\mL). The thrombolytic activity of leaf extracts can be arranged as (*S. officinalis* > *T. vulgaris* > *R. officinalis*). From literatures the rosmarinic acid, is a characteristic phytochemical constituent of Lamiaceae family plant which is responsible for thrombolytic activity of the plants [15]. It was reported that the quantities of rosmarinic acid in tested plants were as follow: In *S. officinalis* (39.3±0.5 mg\g) was highest followed by *T. vulgaris* (23.5±0.5 mg\g) and *R. officinalis* (7.2±0.1 mg\g) at third rank [16], which was explained the demonstrated activity expressed by the tested plants.

Table 1. Thrombolytic activity of *Salvia officinalis, Thymus vulgaris* and *Rosmarinus officinalis*

Concentration (µg\mL)	% Clot lysis (mean ± SD) (*n=3*)		
	Salvia officinalis	***Thymus vulgaris***	***Rosmarinus officinalis***
200	30.74±0.505	24.30±1.000	19.53±0.784
400	50.49±0.83	54.57±0.814	30.17±0.660
600	58.49±1.020	53.37±1.102	33.20±1.105
800	65.20±1.000	60.33±0.902	42.07±1.145
1000	68.43±1.457	61.67±0.569	49.27±3.256

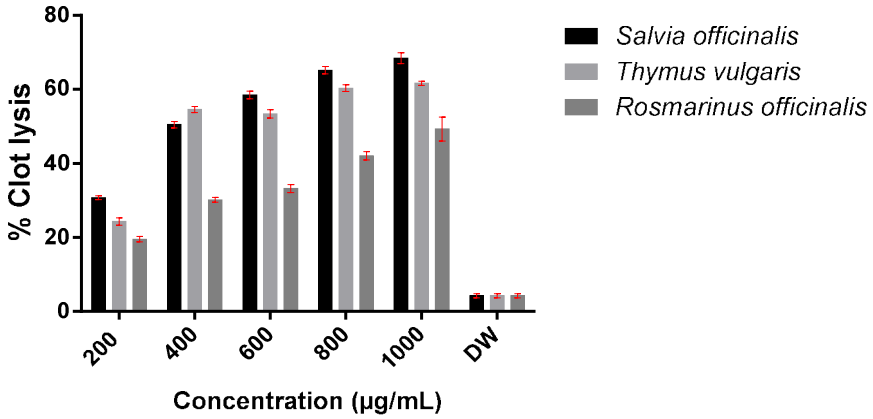

Fig. 1. Concentration gradient clot lysis % of *Salvia officinalis*, *Thymus vulgaris* and *Rosmarinus officinalis* along with control positive streptokinase (SK) and control negative (DW)

Table 2. Thrombolytic activity of negative and positive control

Control	% Clot lysis (mean ± SD) (*n=3*)
Distilled water (negative control)	72.16±1.945
Streptokinase SK (positive control)	4.25±0.526

4. CONCLUSION

In a continuous venue for developing drugs from natural source for protection of cardiovascular system with maximum efficacy and lower side effects, it is concluded that the tested selected species from Lamiaceae plant family can play an important role in developing thrombolytic drugs derived from nature specially *Salvia officinalis* leaf.

CONSENT

Author declared that written informed consent was taken from patients for publication of this study.

ETHICAL APPROVAL

Author declares that all experiments were examined under approval of Pharmacy College ethics committee.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Mulat Y. The role of cultural practices in trees management practices among the Qemant community Chilga Woreda (district), Ethiopia. IJRD. 2013;7:177–80.
2. Gesler WM. Therapeutic landscapes: Medical issues in light of the new cultural geography. Soc Sci Med. 1992;34:735-46.
3. Sai SY, Mamata P, Divya GC, Beena DB. Evaluation of *in vitro* thrombolytic activity of phytochemicals in Bacopa monnieri Linn. J Pharm Res. 2012;5(1):100-1.
4. Apurba SA, Fariha AC, Farjana K, Jamaluddin ATM, Atiqul HPAP. Phytochemical screening and *In vitro* evaluation of pharmacological activities of *Aphanamixis polystachya* (Wall) parker fruit extracts. Trop J Pharm Res. 2013;12(1):111-6.
5. Mucklow JC. Thrombolytic treatment – Streptokinase is more economical than alteplase. Brit Med J. 1995;311:1506.
6. Rouf SA, Moo-Young M, Chisti Y. Tissue-type plasminogen activator: Characteristics, applications and production technology. Biotechnol Adv. 1996;14:239-26.
7. Rajapakse N, Jung WK, Mendis E, Moon SH, Kim SK. A novel anticoagulant purified from fish protein hydrolysate inhibits factor XIIa and platelet aggregation. Life Sci. 2005;76:2607-19.
8. Md. Al Amin S, Abu Bakar S, Nawshad Hossian AKM, Md. Kowser M, Mohammad AK, Mohammad AR. Evaluation of thrombolytic activity of four Bangladeshi medicinal plants, as a possible renewable source for thrombolytic compounds. J Pharm Nutr Sci. 2011;1:4-8.
9. Nargis SC, Badrul AM, Tanbirul HASM, Ronok Z, Ehsanul HM, Ekramul HM. *In vitro* free radical scavenging and thrombolytic activities of Bangladeshi aquatic plant *Aponogeton undulatus* Roxb. GJP. 2011;5(1):27-32.
10. Irfan NK, Md. Razibul H, Md. MR, Adnan M, Md. Mominul IS, Sourav H. Thrombolytic potential of *Ocimum sanctum* L., *Curcuma longa* L., *Azadirachta indica* L. and *Anacardium occidentale* L. JBCP. 2011;2(3):125-27.
11. Stănescu U, Hăncianu M, Miron A, Aprotosoaie C. Medicinal plants from A to Z; monographs of drugs with therapeutical uses. Iaşi: Gr. T. Popa Publishing House. 2004;2. (Romanian)
12. Sharma O. Plant taxonomy. New Delhi: Tata McGraw-Hill Publishing; 1993.
13. Alupuli A, Calinescu I, Lavric V. Ultrasonic vs. microwave extraction intensification of active principles from medicinal plants. AIDIC Conference Series. 2009;9:1-8.
14. Daginawala HF, Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM. Development of an *In vitro* model to study clot lysis activity of thrombolytic drugs. Thromb J. 2006;4:14.
15. Sweta P, Rajpal SK, Jayant YD, Hemant JP, Girdhar MT, Hatim FD. Effect of *Fagonia arabica* (Dhamasa) on *in vitro*

thrombolysis. BMC Complement Altern Med. 2007;7:36.
16. Maryam S, Homa H, Soodabeh S, Ahmad RG, Morteza PH. Comparative study of rosmarinic acid content in some plants of Labiatae family. Pharmacogn Mag. 2012; 8(29):37–41.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11696