



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 3(5): 148-151
Received: 12-11-2014
Accepted: 03-12-2014

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In vitro evaluation of fibrinolytic and antioxidant activities of *Maba buxifolia* (Rottb.) Juss. stem

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Abstract

Studying medicinal plants are more important in recent times to determine their potential source of new drugs. Present study was carried out to investigate fibrinolytic and antioxidant activity of methanol extract of *Maba buxifolia* stem. *In vitro* clot lysis was done by using urokinase as standard, and the total antioxidant activity by the phosphomolybdenum assay method. The efficacy of methanol extract of *Maba buxifolia* stem is based on the percentage of clot lysis, showed highest fibrinolytic and antioxidant activity equivalent to ascorbic acid. The present study revealed the fibrinolytic and antioxidant activity potential of methanol extract of *Maba buxifolia* plant stem to some extent optimistic pointing to the value of further studies in this field.

Keywords: *Maba buxifolia* stem, Methanolic extract, Fibrinolytic activity, Antioxidant activity.

1. Introduction

A wide range of biochemicals can be synthesized by plants through various biosynthetic pathways. They are the potentials of phytochemistry and are stored in different parts of the plant body as primary and secondary metabolites. The application of chemical data to plant systematics has received much attention as this data is useful for solving taxonomic problems. Taxonomic identification and phytochemical characterization of medicinal plants provides authentic means to be used as crude herbs or extracts and pure natural compounds [1]. Advancement in chemical and biological techniques for analysis of herbs from last decade brought transformation of research in pharmacognosy [2]. Evaluation of whole plant or plant parts and their products has always been an important area of research to discover a phytodrug [3]. Herbal medicine is also called as botanical medicine or phytomedicine is currently in demand and its popularity is increasing day by day because of better patient tolerance, compliance, low cost, availability and eco-friendly nature [4].

For the past few years the researchers are focusing on the formulation of ayurvedic herbal medicines on the basis of their traditional uses and its known effectiveness in the treatment of various ailments. Cardiovascular disease caused by a blood clot (thrombus) formation is one among the most severe diseases which are increasing at an alarming rate in the recent years [5]. Thrombolytic agents are used in the management of thrombosis [6]. Thrombolytic agents such as tissue plasminogen activator, Urokinase, Streptokinase [7] etc., are used all over the world for the treatment [8] but their use is associated with hyper risk of haemorrhage [9], anaphylactic reaction and lacks specificity. In the recent past, two decade's efforts have been carried out towards the exploration, discovery and designing and identification of natural products with antiplatelet [10], anticoagulant [11], antithrombotic and thrombolytic activity of the plants [12]. Flavonoids, phenolic acids, tocopherols produced from plants are the main phenolic compounds which are natural antioxidants [13]. The antioxidant activity of flavonoids is due to free radical generations by chelation of metal ions, such as iron and copper and inhibiting enzymes [14]. The leaf juice of *Maba buxifolia* sin. *Diospyros ferrea* is used to strengthen the liver [15]. The literature revealed that these plants contain penta cyclic triterpenes and juglone-based 1,4-naphthoquinones and about 130 species have undergone some phytochemical and/or pharmacological investigations [16].

The present study was designed to evaluate Methanolic extract of *Maba buxifolia* stem for *in vitro* fibrinolytic and antioxidant activities.

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2. Materials and Methods

2.1. Collection of Plant materials

The Stem of *Maba buxifolia* (Rottb.) Juss. of family Ebenaceae, commonly called Utikayalu in Telugu is collected from hill slopes of Tirumala, Andhra Pradesh, India. Taxonomically the plant was authenticated by Mr. A. Ravi Kiran, BSI, Coimbatore, India. A voucher specimen is deposited in Department of Botany, Acharya Nagarjuna University, Guntur and the specimen number is ANU Y9B0R024. The collected stem was shade dried till the moisture content is evaporated and finally pulverized into small pieces.

2.2. Preparation of plant extract

The crude plant extract was prepared by the Soxhlet extraction method. 100 g of plant material was uniformly packed in the thimble and extracted with 300 ml of methanol. The process continued till the solvent in siphon tube became colorless. The extract allows for air drying till the solvent got evaporated.

2.3. *In vitro* fibrinolysis

The Fibrinolytic activity of *Maba buxifolia* stem was done by using urokinase (UK) as a standard reference [17, 18].

2.5. Urokinase solution

5,00,000 IU Urokinase was purchased from Bharat serums and vaccines Ltd (India). To the commercially available lyophilized urokinase vial (5, 00,000 I.U.), distilled water was added and mixed properly. This suspension was used as a stock. Serial dilutions were made to observe the thrombolytic activity using the slightly modified *in vitro* model.

2.6. Blood collection

10 ml. of venous blood was drawn from healthy human volunteers in 20 ml. of centrifuge tube containing anticoagulant sodium citrate without a history of oral contraceptive or anticoagulant therapy and centrifuged at 10,000 rpm for 10 minutes. The plasma is separated in a fresh screw capped tube for further experiments. Preparation of the plasma clot was done according to the method reported by Pharmacopeia (1960) [19].

2.7. Fibrinolytic Method

Experiments for clot lysis and fibrinolytic activity were carried out with slight modification in the procedure [17]. Serial dilutions of urokinase (5,00,000 IU) with distilled water (2, 50,000; 1, 25,000; 62,500 and 31,250 IU dilutions) were taken as a positive control, normal saline as blank and 1mg/ml concentration of metabolic test extract of *Maba buxifolia* was taken for experimental study.

Calculation

% of clot lysis =

$$\frac{\text{Clot weight before lysis} - \text{Clot weight after lysis}}{\text{Clot weight before lysis}} \times 100$$

2.8. Fibrinolytic assay

A sterile 2 ml Eppendorf tube was taken, weighed and later, 0.25 ml of plasma along with 50 μ l calcium chloride (1%) solution. The tube was thoroughly mixed and incubated at 37 $^{\circ}$ C for 45 minutes. After clot formation, the tube was weighed

again to determine the clot weight by subtracting the empty weight of the tube that was taken before the addition of the plasma from the weight of the tube with clot (clot weight = weight of clot containing tube – weight of empty tube). Serial dilutions of Urokinase (2, 50,000; 1, 25,000; 62,500 and 31,250 IU dilutions) or 1 mg/ml test extract were added to the tube containing clot and incubated at 37 $^{\circ}$ C for 90 minutes. After incubation, fluid obtained due to clot lysis was completely removed carefully from the tubes. The tubes were again weighed to observe the difference in weight after clot lysis. The difference obtained in weight, before and after clot lysis was expressed as percentage of clot lysis.

2.9. Phosphomolybdate Assay (Total antioxidant capacity):

The total antioxidant capacity of the extract was evaluated by the phosphomolybdate assay method [20]. It is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acetic condition. 0.3 ml (75 mg) of the extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 $^{\circ}$ C for 90 min. Then, the absorbance was measured at 695 nm using a UV-visible spectrophotometer against a blank (0.3 ml DMSO + 3 ml reagent) after cooling to room temperature. The antioxidant activity was expressed as the number of microgram equivalent of ascorbic acid.

3. Results

Fibrinolytic activity was measured as percent lysis of plasma clot. Urokinase was used as positive control. A standard calibration curve was obtained by plotting different concentrations of urokinase viz. 5, 2.5, 1.25, 0.625 and 0.3125 lac IU against percentage of clot lysis produced (Figure 1). Clot lysis at different concentration of urokinase presented in table-1. The plant extract showed 24.3% of clot lysis showed in table-2.

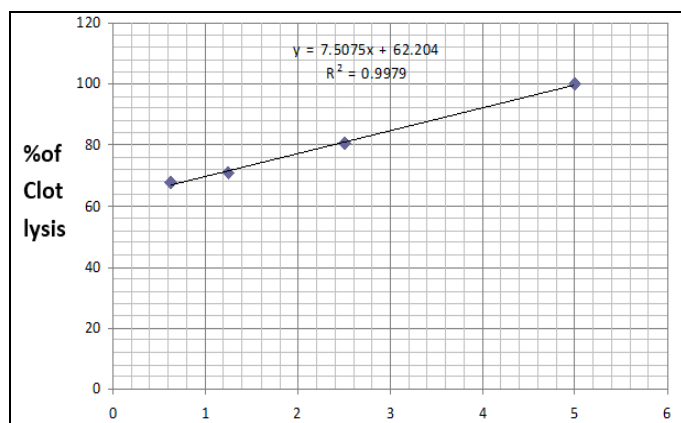


Fig 1: Standard curve for clot lysis (%) at different concentration of Urokinase

The antioxidant activity of each extract at a particular concentration was expressed as the number of micrograms equivalent of ascorbic acid that was calculated by keeping absorbance values of test extracts in the linear regression equation of calibration curve of antioxidant activity of ascorbic acid at known concentrations presented in table 3 and figure-2 demonstrate the absorbance of extracts and Vit 'C' at 20 μ g/ml, 40 μ g/ml, 80 μ g/ml, and 160 μ g/ml respectively.

Table 1: Clot lysis at different concentrations of Urokinase (5,00,000)

S. No.	Urokinase Conc. (IU)	Wt. of empty tube	Wt. of tube with clot	Wt. of tube after clot lysis	(%) of clot lysis
1	5,00,000	1.2102	1.5163	1.2128	100
2	2,50,000	1.2013	1.5152	1.2609	80.6
3	1,25,000	1.2038	1.5183	1.2917	70.9
4	62,500	1.204	1.516	1.3043	67.7
5	31,250	1.2069	1.5031	1.3007	66.6

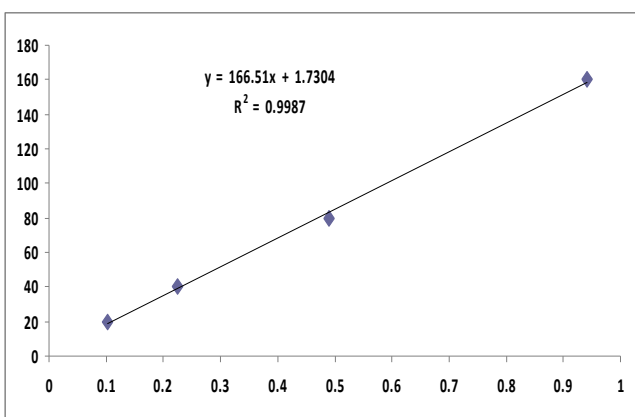
Table 2: Clot lysis at 10 mg/ml concentration of methanol extract of *Maba buxifolia* stem

S. No.	Extract	Weight of empty tube	Weight of tube with clot	Weight of tube after clot lysis	(%) of Clot lysis
1	Methanol Extract of <i>Maba buxifolia</i>	1.1091	1.931	1.0218	24.3

Table 3: Antioxidant activity of vitamin 'C'

Vit 'C' Concentration(µg/ml)	Absorbance (mean of 6 replicates)
20	0.103 ± 0.06
40	0.225 ± 0.04
80	0.49 ± 0.48
160	0.942 ± 0.03

The total antioxidant capacity of the plant extracts expressed as the µg of ascorbic acid activity equivalent present in µg of Methanolic extract of *Maba buxifolia* that was determined by phosphomolybdenum assay. The result of the assay for *Maba buxifolia* stem extracts are presented in the table 4.

**Fig 2:** Antioxidant activity of vitamin 'C' standard curve**Table 4:** Antioxidant activity of methanol extract of *Maba buxifolia* stem expressed as Vitamin 'C' equivalents

Extracts	µg/ml equivalent of vitamin C			
	20 µg/ml	40 µg/ml	80 µg/ml	160 µg/ml
M- Methanol.	18.206 ± 0.25	37.1802 ± 0.56	54.2039 ± 1.0	89.3405 ± 1.67

4. Discussion

The fundamental task of thrombolytic or fibrinolytic therapy is the degradation of fibrin by plasmin, which can be activated by plasminogen [21]. However, there are findings of bacterial contaminants of plants which have plasminogen receptors that bind plasminogen. Few plant extracts and their products having fibrinolytic activity are identified, which includes *Lumbricus rubellus* [12], *Pleurotus ostreatus* [22] and *Spirodela polyrhiza* [23], Ginger (*Zingiber officinale*) [24], Garlic (*Allium sativum*) [25]. Urokinase which was derived from human urine has been broadly employed for thromboembolism therapy, but it has low specificity to fibrin and high cost [26]. The fibrinolytic enzyme prevents the formation of fibrin clot in the circulatory system. Some medicines like urokinase and streptokinase are widely used to inhibit haemostatic disorders, particularly thromboembolism [27].

Apart from the above products, Seanol from marine algae also possessing the ability to promote dissolution of intravascular blood clot via antiplasmin inhibition [28]. In our findings, concentrations of stem extract enhanced in the clot lysis are dose dependent along with the incubation time factor. Methanolic extract of *Maba buxifolia* stem also showed clot lysis ability; the process believed to be a complex and not known absolutely even today, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents. The reduced risks of cancer, cardiovascular diseases, diabetes and other degenerative diseases associated with aging can be promoted due to regular intake of antioxidants [29].

5. Conclusion

In this study, investigation of the thrombolytic activity of *Maba buxifolia* stem extract was carried out using a simple and rapid *in vitro* clot lysis model. The results indicated clearly that concentrations of stem extract enhanced the maximum 24.3% percentage of clot lysis. Some studies indicate that fibrinolytic activity is probably due to diversified phytoconstituents including rich sources of alkaloids, flavonoids, tannins and terpenoids. The total antioxidant capacity of the plant extract expressed as the µg of ascorbic acid activity equivalent is present in µg of methanol extract of *Maba buxifolia* stem.

From all these observations, it can be concluded that the Methanolic extract of *Maba buxifolia* stem consisting with high level of polyphenolic compounds showed an excellent Fibrinolytic and Antioxidant activity as assessed by *in vitro*.

6. Statistical Analysis: Values are expressed ± SD

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