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EVALUATION OF *INVITRO* ANTICOAGULANT ACTIVITY OF LEAF EXTRACTS OF *MURRAYA KOENIGII (LINN)* AND *BAUHINIA TOMENTOSA*

R.Uma*, P.T.Srinivasan, A.Arivoli, M.Vicky

PG Department of Biochemistry, D.G. Vaishnav College, Arumbakkam, Chennai- 106, Tamilnadu, India.

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Murraya koenigii, Bauhinia tomentosa, anticoagulant activity and prothrombin time

For Correspondence:

R.Uma

PG Department of Biochemistry, D.G. Vaishnav College, Arumbakkam, Chennai- 106, Tamilnadu, India.

E-mail:

umaramesh.rg@gmail.com

ABSTRACT

The present study was designed to evaluate the anticoagulant activity of leaf extracts of *Murraya koenigii and Bauhinia tomentosa.*The aqueous, methanol, acetone and ethyl acetate extracts of *Murraya koenigii and Bauhinia tomentosa* at different concentrations were tested on plasma by *invitro* prothrombin time test. The time taken for clotting were considered as the parameter to access the anticoagulant action. Heparin and saline in distilled water were used as standard and negative control respectively. The aqueous, methanol, ethyl acetate extracts of *Bauhinia tomentosa* showed potent anticoagulant activity compared to *Murraya koenigii* as evidenced by a significant increase in clotting time. The observed activity could be due to the presence of phenol compounds,flavanoids, sterols, terpenoids and cardiac glycosides. *In vitro* studies indicated that the *Bauhinia tomentosa* and *Murrya koenigii* is a significant source of natural anticoagulant, which might be helpful in preventing blood clotting disorders.

INTRODUCTION

Haemostasis is the process that retains the blood within the vascular system during period of injury. Most adult cardiovascular disorders involving hypertension,cerebral hemorrhage, arteriosclerosis and congestive heart failure are caused by problems in the blood circulatory system as blood clotting disorders which constitute a serious medical problem. Heparin is commonly used anticoagulant in various surgeries. Beside the pharmaceutical properties such as myocardial infarction, inflammatory and allergic conditions, heparin shows serious side effect like hemorrhage and it is expensive. The structure of heparin is highly complex and heterogeneous. This compound is involved in a variety of physiological and pathological infection[1]. India has history for the treatment of various diseases using medicinal plants. Indian plants show widespread bioactivity with minimum side effects [2, 3]. Plants are safer source of medicines, hence anticoagulation study of extracts from selected medicinal plants such as *Murraya koenigii and Bauhinia tomentosa.*

Figure I - *Murraya koenigii*

Figure II- *Bauhinia tomentosa*

Murraya koenigii Linn belongs to the family Rutaceae of trees. It is commonly used traditionally as a stimulant, antidysentric and in the management of Diabetes mellitus. It has been reported to possess antioxidant[4], antihelmentic[5] ,hypoglycemic[6]and hypolipidaemic [7]activities. *Bauhinia tomentosa* Linn, belongs to the family Fabaceae. It has been used traditionally for its therapeutic properties like hepatoprotective, astringent, dysentery [8], antihyperglycemic and antilipidemic[9], diuretic, tonic, antiinflammatory, antineoplastic, antiulcer[10] ,antimicrobial[11] and antioxidant scavenging activities[12].

MATERIALS AND METHODS

Collection of plant material

Murraya koenigii and Bauhinia tomentosa leaves were obtained from Thiruvallur district, Chennai, Tamilnadu in the month of January 2013 and it was authenticated by Dr. S. Jayaraman, Director of Plant Anatomy Research center, west Tambaram, Chennai, Tamilnadu. Register No: PARC/ 2012/ 1436 and PARC/ 2013/ 2188.

Preparation of extracts

The shade dried leaves of *Murraya koenigii and Bauhinia tomentosa* Linn was coarsely powdered and extracted with distilled water, methanol, acetone and ethyl acetate for 24 hrs.The liquid extract was filtered using muslin cloth, followed by Whatmann No: 1 filter paper and evaporated using a rotatory evaporator. The dried extracts were stored at 20 °C until use.

Preliminary phytochemical analysis

Qualitative phytochemical analysis of *Murraya koenigii and Bauhinia tomentosa* were carried out using standard procedures to identify the constituents as described by Harborne[13], Trease and Evans[14] and Sofowara[15].

Blood coagulation study

Study population

Blood samples obtained from healthy volunteers of age group of 20-25 years.

Collection of blood sample

The blood sample was obtained in containers containing trisodium citrate to prevent clotting process. Centrifugation was carried out at 3000 rpm for 15minutes to separate the blood cells from plasma to obtain pure platelet plasma for prothrombin test. The freshly prepared plasma was stored at 4°C until its use.

Prothrombin time test

In a test tube, 0.2ml test plasma, 0.1ml of crude extract of *Bauhinia tomentosa and Murraya koenigii* of different concentration 0.2, 0.4, 1,2,3 and 4mg/ml and 0.3ml of $CaCl_2$ were added together in a clean fusion tube and incubated at 37°C. A stopwatch was started to record the coagulation time in seconds. The tube was shaken to mix the contents and it was stopped as soon as the clot formation began. The activity is expressed in term of clotting time in seconds. The steps were repeated three times for each sample and average of the test value was noted. Normal saline was used in place of the extracts for the negative control and 50mg/ml of heparin for the positive control [15,16].

RESULTS AND DISCUSSION

Phyto chemical Analysis

Qualitative phytochemical screening of the different solvent extracts of *M.koenigi* and *B.tomentosa* revealed the presence of alkaloids, phytosterols, phenols, flavonoids, terpenes, carbohydrates and cardiac glycosides.Tannin was present in trace amounts in both the plants, where as saponin was completely absent in *B.tomentosa*.(Table I)

Anticoagulation activity

Aqueous, methanol, acetone and ethyl acetate extracts of leaves of two medicinal plants *Murraya koenigii and Bauhinia tomentosa* were tested for blood coagulation effects in normal human plasma and found to be significantly prolonged the prothrombin time of normal human plasma. In table-II aqueous, ethyl acetate, methanol, extracts of *Murraya koenigii* showed activity at higher concentration tested, with prolonged clotting time 7:30, 7:50 and 7:10 sec at 4mg/ml concentrations respectively. Whereas the acetone extracts of *Murraya koenigii* demonstrated moderate anticoagulant activity.

Similarly in *Bauhinia tomentosa* leaves extracts showed anticoagulant activity at higher concentrations. Methanol extracts of *Bauhinia tomentosa* exhibited greater activity with prolonged cloting time 23:10, 26:15, 27:20, 28:30, 30:40 and 31:10 sec at 0.2, 0.4, 1 , 2, 3, and 4mg/ml concentrations respectively followed by acetone, aqueous and ethylacetate extracts of *Bauhinia tomentosa*, whereas the ethylacetate extracts of *Bauhinia tomentosa* exhibited lower activity. *Bauhinia tomentosa* exhibited potent anticoagulant activity compared to *Murraya koenigii* at a concentration of 4mg/ml.

Table 1- Phytochemical analysis of various extracts of A: *Murraya koenigii* and B*: Bauhinia tomentosa*.

Phytochemicals	Aqueous		Methanol		Acetone		Ethyl acetate	
	A	B	A	B	A	B	A	B
Alkaloids	++	++	++	+	++	+	++	+
Flavonoids	+	++	+	++	+	+	+	++
Tannin	+	+	+	+	+	+	++	_
Phenolic compound	++	++	++	++	+	+	+	+
Steroids	+	++	+	++	++	++	+	++
Terpenoids	+++	+	++	++	++	+	++	++
Cardiac Glycoside	+	++	+	++	+	+	+	++
Saponin	_	_	+	_	+	_	_	_
Carbohydrates	+	++	+	++	+	+	+	+

Table II- Effect of various extracts of *Murraya koenigii* on prothrombin time (PT) of normal human plasma

Concentration (mg/ml)	Prothrombin Time (PT) in minutes			
	Aqueous	Methanol	Acetone	Ethyl acetate
0.2	1:10	3:10	2:40	2:00
0.4	2:20	4:30	3:00	3:20
1	3:40	5:20	3:20	4:00
2	6:10	6:00	3:40	6:00
3	6:40	6:40	4:00	7:20
4	7:30	7:00	4:30	7:50

+ve control: 2:10 min (PT) (Heparin 50mg/ml); -ve control: 1:10 min (PT) (saline)

Table III – Effect of various extracts of *Bauhinia tomentosa* on prothrombin time (PT) of normal human plasma

Concentration (mg/ml)	Prothrombin Time (PT) in minutes			
	Aqueous	Methanol	Acetone	Ethyl acetate
0.2	12:10	23:10	10:20	6:20
0.4	14:15	26.15	14:40	9:30
1	15:30	27.20	15:40	10:10
2	16:20	28:30	20:10	10:50
3	17:40	30:40	24:20	12:40
4	18:10	31:10	28:50	15:10

+ve control: 2:10 min (PT) (Heparin 50mg/ml); -ve control: 1:10 min (PT) (saline)

CONCLUSION

From the present study it is proved that methanolic extracts of *Bauhinia tomentosa* may be useful as anticoagulant. This may be due to the presence of sterols, flavanoids, terpenoids, carbohydrate and cardiac glycosides.

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